- TABLE OF CONTENTS -

A. Virology

D. Immunology

E. Immunology and Pharmacology

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A new vaccination strategy for *Mycoplasma hyopneumoniae* in pigs: Vacciplan®

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Respiratory diseases become increasingly more complex as a consequence of multiple interactions between herd management factors and pathogens. Herd management, housing and breeding conditions play an important role in not only the occurrence but also the severity of respiratory diseases. Vaccination against *Mycoplasma hyopneumoniae* (*M. hyo*) has been many times confirmed to be beneficial and is nowadays one of the basic methods used in the prevention of enzootic pneumonia (EP) and porcine respiratory disease complex (PRDC). However, in numerous cases, particularly in USA, PRDC continue to occur with a high level of intensity involving *M hyo*, even after vaccination against *M hyo*. These vaccine failures have been investigated by many researchers and it is now well accepted that the timing of vaccinations is crucial to its success. The objective of the present communication is to review the different factors that may have an influence on *M hyo* vaccination efficacy and to present a new tool aimed at helping the practitioners and the farmers to choose the best vaccination schedule.

Epidemiology of *Myoplasma hyopneumoniae* in the herd

Infection mechanism at the individual level

Mycoplasma hyopneumoniae is a pig specific, non-invasive bacterium, without cell-wall. *M hyo* is a tedious micro-organism exhibiting a slow replication both *in vivo* and *in vitro*.
At the individual level, the direct contamination occurs mainly by contact or aerosol (cough). Contamination occurs either vertically from the dam to piglet or after that, horizontally among penmates. The pathogens penetrate respiratory tract by inhalation and adhere to cilia, presumably through an adhesion protein system. They are able to slowly replicate and colonize the mucosal border in several weeks. The infection results in the destruction of the *ciliae* and an inflow of inflammatory cells in the surrounding of the air tubes. The inflammatory process due to a strong hypersensitive-like immune reaction induces the onset of characteristic but however non pathognomonic lesions of enzootic pneumonia (EP). The devastation of the mucociliary escalator as well as the immunomodulation are the main predisposing factors to super-infections. Clinical signs, essentially represented in the typical form by a dry cough enhanced when animals are moved in the pen, may vary according to super-infections. Seroconversion is commonly seen few days after infection but can be sometime delayed to several weeks after it.

S∅rensen *et al.* (1994) have showed in an experiment where 200 naïve pigs were artificially infected with *M hyo* that the onset of clinical signs took place roughly 4 weeks after contamination and pigs seroconvert from 3 to 5 days after clinical signs. Although, these periods of time may vary from farm to farm, field experience showed that the schedule determined by experimental infection is on an average not so far from what is actually seen in commercial farms.

Infection dynamics at farm level

The disease may break out after that a sufficiently large number of piglets have been infected and shed *M hyo*. Using PCR, it was demonstrated that the critical percentage of infected pigs necessary for the onset of clinical signs is estimated to be 50% (C. Pijoan, 2000). The epidemiology at the herd level depends on the overall *M hyo* infection pressure as well as the age distribution ratios of the sow population in the farm. Gilts have been shown to shed more *M hyo* than older females (C. Pijoan, 2002). Infection occurs sooner in continuous flow than in all-in, all-out management systems. Furthermore, contamination and infection are still more delayed in three-site farms (Sibila, 2004).
Usually, occurrence of EP can be classified as one of three types depending of the stage of the pig life it is appearing in:

- Early EP: mostly but not only in continuous flow farms, *M hyo* infectious pressure is high and the number of contaminated piglets is quickly increasing. Clinical enzootic pneumonia occurs at the end of the post-weaning or at the beginning of the fattening period. This was frequently the case in the small farrow-to-finish (f-to-f) farms with no all-in all-out management.

- "Conventional" EP: *M hyo* infectious pressure is average with a slow gradual increase in the number of contaminated piglets. The critical threshold for the clinical expression of enzootic pneumonia is then delayed and coughing starts by the middle of the fattening period. This is the most common situation in European countries that all-in, all-out management farms have to face in the growing (pre-fattening) period (14-18 week-old).

- "Late" EP: when *M hyo* infective challenge is weak in the farrowing pens and the number of contaminated piglets at weaning is small. It then takes time for the threshold to be reached and clinical expression is then delayed until the end of the fattening period (>19 week-old). Sometimes, coughing can hardly be heard and only EP lung lesions are seen at the slaughterhouse. This situation is more and more frequent. It is fairly common in the three site farms in the USA (C. Pijoan, 1998) but also is encountered in European countries. In the USA, this epidemiological pattern leads to what is known as porcine respiratory disease complex (PRDC).

M hyo infection can be monitored in the farm using observation of clinical signs and/or serological profiling. In order to prevent the disease and the related economic losses, the infection must remain below the threshold of infected pigs. Ten 16 week-old and ten 22 week-old pigs are blood sampled and the sera are tested for anti-*M hyo* antibodies using a qualitative ELISA test.

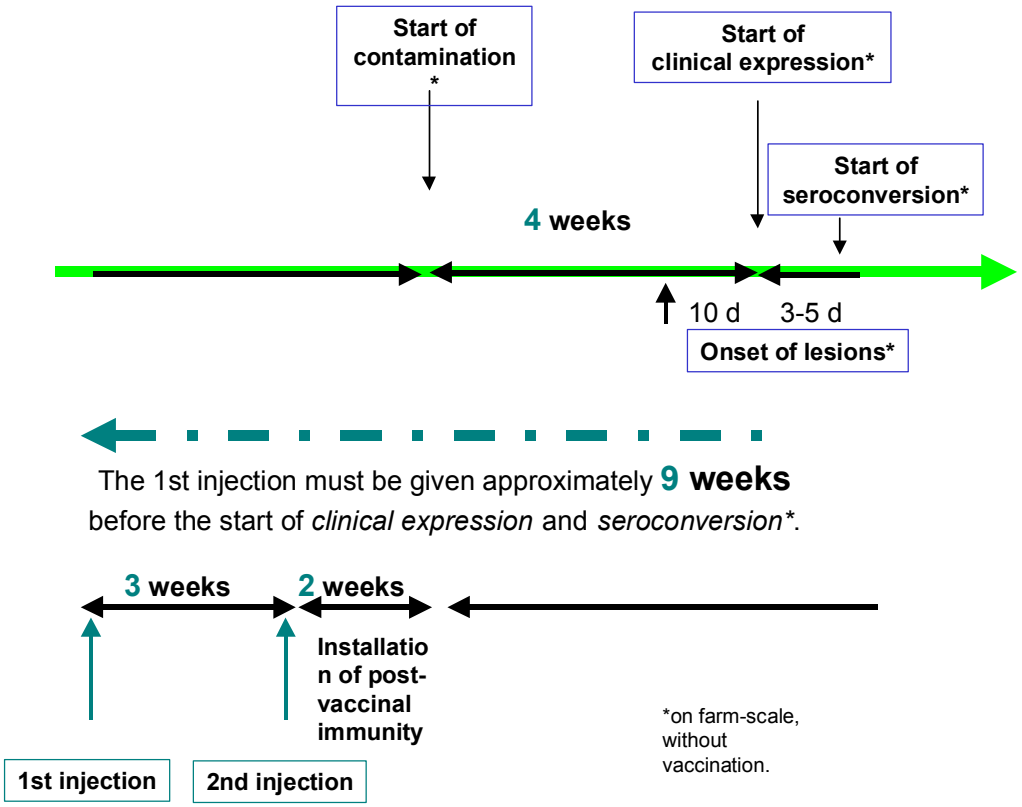

Figure 1: proposal for a retro-schedule for the optimization of *M hyo* vaccination with HYORESP®, Merial with regards to EP onset in the farm.

The Fig.1 above shows a proposed retro-schedule designed to help in the implementation of vaccination at the right time. Pigs are at the peak of their secondary immune response and then at top of the protection level few weeks after the second injection of the vaccination. To achieve this, pigs must be injected with HYORESP® for the second time 6 weeks before the clinical signs and/or seroconversion and for the first time 9 weeks before. This can constitute a basis for practitioner and breeder thinking. Results of the serological examinations may be helpful if the precise break point of other respiratory pathogens are involved in the farm.
As an additional tool easily usable in the field, a table is proposed that summarizes the different types of herd profile and the vaccination program that can be recommended accordingly.

Type of enzootic pneumonia	Clinical expression time	*M.h.* serology (% positive and suspicious)		Vaccination program
		16 weeks	22 weeks	
Early	End of post-weaning period Start of fattening period	80-100%	80-100%	1-4 weeks
Conventional	Mid-fattening	25-80 %	80-100%	4-7 weeks
Late	2nd half of fattening period	< 25 %	60-100 %	7-10 weeks
	Not visible (recent lesions at slaughter)	0 %	< 60%	Single at 10 weeks

Table 1: Guidelines for anti-*M hyo*-vaccination according to clinical observation and serological profile.

Interference with maternally derived antibodies

Passive and active immunity against *M hyo* have been also established to exert an influence over vaccination intake. Maternally derived antibodies level and persistence in piglets is highly variable. Several studies have pointed out the risks of maternal antibody interference with mycoplasma vaccination. B Thacker, (1998) showed that the amount of antibody induced by vaccination is reduced when pigs were passively immune at the time of vaccination. Numerous other workers have also warned that if sows have high serum antibody titres that are transferred to their piglets, vaccination should be postponed ((Yeske, 2001, Utrera *et al.*, 2004).
In addition, the ability of the mononucleated cells of piglets to produce antibodies increases with age. "Optimal time points for potential vaccinations against *M hyo* ought to be validated" noted P. Wallgren, 1(1998).
All these facts would suggest that too early vaccination in the piglet may not be as efficacious as wished.

Other pathogens of importance

PRRS

E. Thacker *et al.* (2000) have shown that PRRSV infection interferes with anti-*M hyo* vaccination with commercial bacterins. Her team has also showed that the immunization by other inactivated vaccines such as inactivated swine influenza virus may be jeopardized by PRRSV infection. Thus, it appears extremely important to know as precisely as possible when the PRRSV infection occurs in the pig herd. This can be monitored using by vertical serological PRRS screening across the herd: ELISA positive response with the commercial ELISA kits usually follows infection by approximately 7 days (Mieli, 2002). As for *M hyo*, this can lead to consider three different type of schedule for PRRSV infection:

- “Early” seroconversion: occurring during the postweaning leading to have the majority of the piglets positive. This indicates a high level of

PRRSV circulation in the sows which causes a high level of transmission of the virus to the suckling piglets.

- "Conventional" seroconversion: occurring at the beginning of the fattening units. This coincides with the movement of pigs, mixing and the resultant fighting and other stresses together with co-infections with other pathogens. Vaccination of coughing pigs with the same needle is also blamed for rapid contamination from a few silent virus carriers. "Late" seroconversion can then occur at any time during the fattening period according to the contamination and management of the batches.

PRRS seroconversion can be monitored by vertical sampling of the pigs. Ten, sixteen, twenty two weeks and just before slaughter are important time points for serological monitoring. The choice of the ELISA technique also influences the reliability of the interpretation (Mieli, 2002).
The period of time the pigs are hit by PRRSV is the worse time to perform a *M hyo* vaccination. PRRS vaccination with MLV is an attenuated infection but has also been shown to interfere with *M hyo* vaccination (Thacker, 2000).

PCV2
Some authors have found a clear role of Mycoplasma early vaccination in the intensity of PMWS clinical signs and losses (Allan, 2000, Kyriakis 2005, Krakowka 2005). Meanwhile, other scientists have not found such an effect. PMWS is well known to be a multifactorial disease and most probably, more than one predisposing factor is necessary to trigger significant disease. However, *M hyo* vaccination of very young piglets should be avoided when a potential risk of PMWS has been identified. On the other hand, promising results obtained in the field with sow vaccination against PCV2 may change situation.

Attempts to change vaccination programs have been made in herds affected with acute PMWS, and vaccination during the peak of the clinical signs appeared to increase mortality and to worsen the symptoms of the disease. Therefore, it seems important not to vaccinate pigs against *M hyo* during the period the disease is clinically affecting the pigs.

M hyo co-infection with PCV2 was shown to provoke clinical PMWS although PCV2 alone was not able to induce wasting in the pigs. On the other hand, the dual infection with both pathogens was responsible for an aggravation of the pneumonic lesions (Oppriesnig, 2004). This supports that *M hyo* vaccination along with combating PCV2 are pivotal elements to minimize respiratory diseases in pigs.

Optimization of the anti-*M hyo* vaccination program: Vacciplan® for piglets

The method is just based upon common sense and comprises the following steps:
1/ determine the epidemiology of *M hyo* infection and occurrence of EP. Then choose the best schedule accordingly.
2/ assess PRRSV seroconversion timing. Check and modify where needed, the vaccination program you chose at 1/.
3/ consider the PMWS problem (presence, timing of clinical occurrence)
4/ stick to the reality. Implement the program that is fitting with most of the above contingencies

5/ check on a regular basis if the vaccination program is fitting the herd situation.

Conclusion

In conclusion, as herd management changes and improves over the years, *M hyo* infection tends to develop later in the production herd. Simultaneous infections with other pathogens may increase the severity of the situation and also interfere with vaccination. Maternal antibodies interfere with early *M hyo* vaccination. Practitioners must bear in mind that every herd is a single case that has to be addressed separately. Prescribing the ideal *M hyo* vaccination program to each herd has to take into account the infection dynamics at the farm level, the risks of interference with maternal antibodies, concurrent diseases, practical and economical facts (such as manpower, for example).

M hyo vaccines that allow the largest flexibility to adapt to all vaccination programs are adding value in the fight against respiratory diseases.

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Immunostimulation and potentiation of PCV2

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Introduction and Objectives

Porcine circovirus type 2 (PCV2) infection emerged as a newly recognized viral pathogen of swine in Canada in the mid to late 90s.[1,2] Infection with PCV2 occurs in all swine-producing areas of the world and disease syndromes related to PCV2 infection are increasingly recognized as serious threats to global hog production.[3,4] The objective of this study was to determine if selected adjuvanted commercial mycoplasmal bacterins potentiate PCV2 viral replication in PCV2-infected gnotobiotic swine, and if this effect increased the severity of PCV2-specific lesions or clinical signs of PMWS in this model system.

Materials and Methods

Groups of porcine circovirus type 2 (PCV2)-infected gnotobiotic swine were vaccinated at 7 days of age with commercially mycoplasmal vaccine preparations. Each piglet was orally inoculated with 1 ml of a PCV2 tissue homogenate containing 4.3 × 106 units at 3 days of age.

As controls, groups of piglets were infected with PCV2 and not vaccinated and infected with PCV2 and immunized with KLH/ICFA. At termination, a standardized set of tissue samples were collected for virus re-isolations, histologic examinations and IHC.

Results and Discussion

Piglets with subclinical PCV2 infection were clinically asymptomatic; low levels of infectious virus was recovered from tissue homogenates and only rare single IHCpositive cells were identified in lymphoid tissues and hepatocytes. Piglets with mild PMWS were still alive and eating at the termination of the experiment and presented with mild wasting slight subcutaneous edema or ascites, mild icterus and generalized lymphadenopathy.

By IHC, viral proteins were widely distributed in lymphoid tissues and a parenchymal organ, particularly the liver and moderate amounts of infectious virus was recovered from tissue homogenates. Piglets with clinically evident PMWS were severely wasted and frequently exhibited generalized subcutaneous edema and icterus. These animals either died or were terminated when moribund before 35 days of age. In these piglets, the full spectrum of gross and histologic lesions characteristic of PMWS was seen.

Of the commercial mycoplasma vaccine products tested, only the Mycoplasma bacterin adjuvanted with mineral oil potentiated PCV2 viral replication in vaccinates; approximately one-half of the piglets in this group developed mild or overt PMWS. Tissue levels of infectious virus in piglets given the other vaccine products did not contain significantly increased amounts of infectious virus versus the PCV2 infected alone control group.

While the mechanism(s) whereby immunostimulation with either the mineral oil-containing bacterin or KLH/ICFA remain obscure, it seems that “activation” of tissue

histiocytes and macrophages by vaccination is a central event in the potentiation phenomenon.

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BREEDING ROBUST, RESILIENT AND RESISTANT PIGS

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Abstract.
Infectious diseases represent the most important source of inefficiency in pig production worldwide. Outbreaks compromise production output and often shut down whole enterprises; epidemics trigger eradication programs that destroy regional production; chronic subclinical disease causes sigificant reduction in production efficiency due to the mobilisation of resources for immune reaction. In addition, avoidance of pathogen spread is an important reason for international trade barriers. Vaccination and antibiotics are used to keep the situation under control, but are expensive and may cause pathogen resistance.

A much more effective approach would be to breed pigs that are (i) *resistant* to particular pathogens so that they do not contract the associated diseases, or (ii) *resilient* to pathogens so that they do not significantly reduce production after subclinical manifestation of the disease, or (iii) *robust* to various environmental stressors, including infectious, social, climatical and nutritional factors. The question is then if genetic techniques exist to breed such pigs.

The classical way of selecting for *resistance to a specific disease* requires complicated systems where animals are infected and the degree of morbidity or mortality is used on a per-family basis to select relatives kept in unchallenged conditions. This is an expensive approach with the disadvantage that it works for a single disease only at a time, which opens the way for *another* pathogen to become the dominant problem. So such an approach is not very useful for areas where several diseases occur at the same time. However, when a single disease really dominates the scene, breeding companies would have a strong motivation to follow this approach and increase market share.

In such cases, the development of DNA markers should be more efficient in the long term than an ongoing system of sib selection based on challenge trials. But resistance to most infectious diseases depends on several genes in the host, so the development of a successful panel of markers for resistance to a particular disease is not easy. Nevertheless, the successful development of DNA markers for resistance based on a single gene only (such as against *Escherichia coli*-F18) illustrates that this approach is technically feasible. An important question is to what extent co-evolution of the pathogen (with its fast generation turnover) would neutralise the build-up of genetic resistance in the host; of course, this issue is even more relevant in the case of medication and vaccination.

The issue of *general immunocompetence* is just as complicated. It has been shown repeatedly that increased immunocompetence does not necessarily increase resistance to specific diseases. On the other hand, when several pathogen species simultaneously cause a heavy and unstructured infectious load, genetic increase of immunocompetence should be beneficial. As far as the disease situation is chronic and subclinical, rather than in terms of clinical outbreaks, the most desirable feature of a pig population may be *resilience*, i.e. the capability to sustain a reasonable production level under chronic infectious load.

Such infectious conditions commonly trigger a re-allocation of resources towards the immune system, in terms of both energy and protein (protein requirements not so much for the synthesis of antibodies etc., but more for maintenance of body protein, including repair/replacement of tissue damaged/lost due to infection). At the same time, voluntary food intake is often depressed (anorexia), and the combination of increased nutrient requirements and reduced nutrient uptake leads to reduction of production processes. Studies have been directed to the required "upgrade" of diet composition to keep production at the desired level, and attempts have also been made to reduce the anorexia response by genetic selection.

Resilience to infection can be regarded as a special case of *robustness*. A "robust pig" can be defined as a pig that expresses its full genetic potential for production traits, independent of the effects of external stressors. Using another terminology, such a genotype is not "environmentally sensitive". Selection for increased robustness can be organised in two ways.

The first ("biological") approach requires identification of the biological factors that make the pig environmentally sensitive: general immunocompetence, upper critical temperature, excitability and social dominance patterns, specific nutrient requirements; and more general characteristics such as leg quality, cardiovascular integrity, and behaviour patterns. As far as these characteristics are heritable (there is evidence that many of them are), it would be feasible to set up a selection program. This would require (i) measurement of those traits on large numbers of pigs, (ii) statistical treatment of the data to produce BLUP values (particularly useful for traits with low heritability) and DNA markers (particularly useful for traits that are difficult to measure on large numbers of animals), and (iii) inclusion of those BLUP values and of the marker information in the selection index of the nucleus population.

The second ("statistical") approach requires (i) recording the production performance of progeny of nucleus animals in a wide variety of environmental conditions (including challenged conditions), (ii) relating these measurements back to the nucleus parents, (iii) calculating for each parent animal how sensitive the performance of its progeny is to different environmental load which would lead to BLUP values for environmental sensitivity, and (iv) including those BLUP values into the selection index of the nucleus population. These traits are again difficult to measure on large numbers of animals, so the development of DNA markers for environmental sensitivity would be desirable. This approach requires an extensive data recording system, which can be difficult to set up. But once the system runs, it produces very powerful information that is directly applicable to the resilience issue. Studies in dairy cattle and sheep have shown that there is genetic variation in the environmental sensitivity of production potential for milk production traits, growth and carcass traits, and fertility traits.

In practice, a combination of the biological and the statistical methods will be required.

PREVALENCE AND IMPACT OF PROLIFERATIVE ENTEROPATHY = ILEITIS, IN EAST ASIA

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Background Events to Ileitis in Asia

The processes of intensive pig farming are now spreading widely across Asia, with housing of specific age groups into separate units composed of breeding, nursery or fattening pigs. Other features of intensive farms are use of commercial genetics and feeds, high density of housed pigs, routine vaccinations and antibiotic usage, and movements of pigs in "batches" with full disinfection procedures. However, these changes cause complex changes in disease transmission. The limited contact between sows and their piglets leads to the reduced early transmission of some agents, which leads to susceptibility to these agents if pigs are introduced to them at a later age. For some well-adapted porcine agents, such as *Haemophilus parasuis*, *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis*, this later contact can lead to enhanced clinical signs. So we have seen an upsurge in Glasser's disease and proliferative enteropathy = ileitis, with intensive farms. For example, the acute hemorrhagic form of proliferative enteropathy generally occurs if naïve pigs older than approximately 12 weeks, contact a significant oral dosage of *L intracellularis*. In contrast, the chronic or subclinical form generally develops if younger pigs (<12 weeks-old) contact *L intracellularis*. In common with other oral-faecally transmitted enteric organisms, *L intracellularis* has a low infective dose and can persist in faeces for long periods (> 2 weeks) under optimum conditions. Despite obvious considerable opportunity, *L intracellularis* has never been identified in humans with enteric disease, even those with Crohn's or related enteric diseases, so is therefore not considered a zoonosis.

Prior to intensification, case reports of proliferative enteropathy due to *L intracellularis* were noted worldwide, including reports from China, but cases were confined to occasional cases of acute or chronic lesions in pigs on smaller non-intensive farms. On those farms, epidemic viral and parasitic diseases are common, complicating any diagnosis of chronic proliferative enteropathy and no clear laboratory confirmation was available. Because the causative agent and hence live animal tests have only recently been developed (1995), the only possible method for comparing the prevalence of proliferative enteropathy in Asia now and historically, would be by comparison of percentages of affected pigs at slaughter. The past and current percentages of pigs at slaughter with lesions of proliferative enteropathy indicates that in most global settings, a rate of less than 5 percent has been consistently present. Slaughter tests are more accurate for respiratory and reproductive tract lesions, as these tend to more long lasting. Lesions of enteric diseases such as proliferative enteropathy, swine dysentery or colibacillosis are likely to have largely healed by the time the affected pig reaches slaughter weight. Also, false results may occur due to the non-specific nature of mucosal "thickening" within the ileum. Once tests capable of detecting a specific serologic

response or antigen became routinely available in the past five years, it was evident that ileitis is an endemic disease present in post-weaned pigs on most Asian farms, but with many pigs developing only mild clinical or subclinical signs.

Current prevalence in Asia

Prevalence data has been gathered from participating diagnostic laboratories and specific published references, illustrating a high level of infection in Asia, see Table 1. Independent studies have confirmed the >90% sensitivity and specificity of the IFAT and IPMA serologic assays used - these use Lawsonia antigen coated wells, with immuno-fluorescence or immuno-peroxidase detection of bound antibody. In some studies, the faeces PCR was also used, with Lawsonia-specific primers used to detect Lawsonia bacteria in DNA extracted from the faeces of pigs, usually pigs with diarrhoea. The important difference in the tests for these surveys is that the PCR test positive indicates that the pig was a clinical case of ileitis, actively excreting the organism at the time of the test, but the serology test indicates that the pig has been exposed to positive ileitis faeces in the recent weeks before the sample was collected. Surveys of the overall prevalence of ileitis suggest that over 90 percent of European farms have some kind of on-going infection (Stege et al 2000, Chouet et al 2003, McOrist et al 2003).

***Table 1**. Estimation of percentages of porcine proliferative enteropathy prevalence in surveys of farms and pigs in different countries, 2000-2004*

Country	Positive farms %	Positive pigs %	Number of farms tested
China	85	65	45
Japan	94	60	378
Korea	100	70	100
Malaysia	100	50	10
Thailand	100	38	24
The Philippines	86	42	40
Vietnam	77	35	13
USA	96	60	405
Canada	95	60	78

There are some broad questions that arise from the data presented in Table 1. First, there is a very high level of infected farms across Asia. The widespread nature of the infection suggests that the disease is endemic on most farms, with many pigs presumably not showing obvious diarrhoea. In moderate-dose challenge-exposure studies, approximately a half of any group of challenged pigs do not go on to develop visible diarrhoea, but did suffer noticeable ileum lesions and poor growth performance. Possible carrier sites other than the lower intestine, such as the tonsils, are rarely infected, except in heavily infected pigs. This suggests that the numerous serologic-positive but apparently healthy pigs inferred from the serologic data, have in fact suffered the infection, but failed to develop beyond a localized intestinal lesion.

Ileitis in Korea

Pig farming in Korea largely consists of well-run intensive farms. Four recent specific studies of ileitis have looked at numerous pig farms in Korea. The group of Professor Chanhee Chae reported an initial study using the PCR test in faeces samples from 35 farms in 6 provinces of Korea (Kim et al, 1998). Although this test has the lowest sensitivity of any current test, they found that up to 33 % of farms were positive for ileitis (in samples from Gangwon-do). His group followed this initial study with a more detailed study using both the PCR test and the immuno-histochemistry test (IHC) on pig tissues. This study was funded by the Ministry of Agriculture, Forestry and Fisheries, Korea (Kim et al 2000). These studies were confirmed by a more comprehensive survey by the group of Professor Joong Bok Lee, using the more sensitive and specific serology test of immunofluorescence assay (IFAT). They investigated over 800 samples from 65 farms in the 6 provinces and found all 65 farms had at least one positive pig (Lee et al 2001). On each farm, usually 50 to 60 % of pigs were positive, with the highest prevalence in Gyungg-do.

Ileitis in Vietnam

Vietnam has 22 million pigs, mainly farmed in the Red River delta, which is the main pig production area in northern Vietnam. Intensive farms still only contribute around 20% of the total pork production in Vietnam, but these were the ones mainly selected for sampling in a recent study (Vu 2004), see Figure 1. Breeder farms consisted of 20 to 300 sows, with piglets usually sold to other weaner operations at 28 days-old. These operations often slaughtered pigs at only 35-40 kg for local or export markets.
PCR detected Lawsonia in 30 out of 87 (34.5%) faecal samples, originating from 4 out of 7 (56.4%) intensive farms sampled. The prevalence of infection varied from 28.57 to 90% on these positive farms. In an outbreak of acute hemorrhagic enteropathy in breeding adult pigs from Hatay province, 90% were PCR-positive. Positive DNA was also detected in two samples submitted from small household-held pigs. Positive IgG antibodies against *L. intracellularis* were detected in sera of pigs in 77 % of surveyed intensive farms. Only 3 farms had no positive serum samples at the time of testing. Within herd prevalence varied between 7 and 40%, with an average prevalence of 11.5 %. Diarrhoea in weaner pigs in commercial farms in Vietnam has been always a problem for pig producers. Tuan et al (1998) estimated that 32 - 38% of total mortality in weaners is attributed to enteric diseases. Obviously ileitis may play an important role in that situation.

Figure 1. *Farms in the Red River delta in the north of Vietnam tested by PCR and serology at the National Institute for Veterinary Research, Hanoi.*

Ileitis in Thailand, China, Philippines, Malaysia

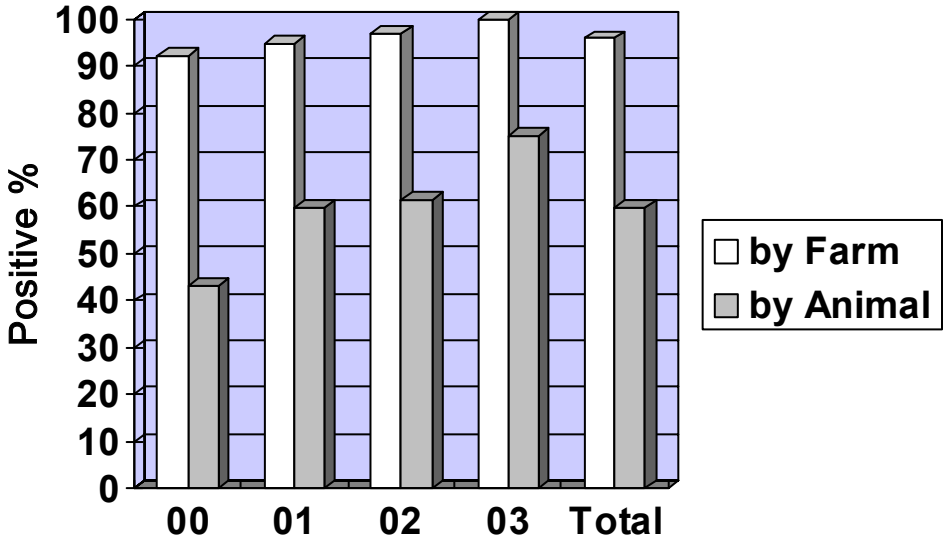
Limited local surveys have been conducted in all these countries, which are summarised in Table 1. In Thailand, this initial data originated from Dr. Worowidh at Kasetsart University. He found that 100 % of a total of 24 Thai farms tested positive for Lawsonia by serology and that an average of 38% of the pigs tested on those farms were positive. Nuntaprasert and others (2004) also tested one 2,500 sow commercial Thai farm in detail and found that the average number of positive pigs varied from 11% at 8 weeks of age (early weaners) up to 39 % at 16 weeks of age (finishers).
In mainland China, the data originated from Dr. Wei and others at the Shanghai veterinary station. They found that 76% of 17 farms sampled were PCR-positive on faeces and ileum tissue (serology was not used yet). Over 65 percent of cases of diarrhoea and deaths in 2 to 4 month old pigs were positive by their PCR, which confirmed ileitis as the most common cause of diarrhoea among pigs of this age in China. See also Chang et al (1997) for similar data from Taiwan.
Only limited initial surveys have been reported from the Philippines and Malaysian farm groups, see Table 1 (McOrist et al 2003).
Data for the important pig industries of Burma, Indonesia, Laos and Cambodia is not available, but ileitis is certainly active in these countries also. The intensive QAF-Culindo pig farms at Bulan in Indonesia-Singapore (28,000 sows) have diagnosed many cases of ileitis in occasional pathology investigations.

Ileitis in Japan

Professor Ohya and others conducted excellent studies of causative factors of ileitis in Japan in the 1980's. They established that it was an important disease in major pig herds in Japan, with the acute and chronic forms investigated by pathology and cultures (Ohya et al 1985). An initial faeces PCR survey by Dr. Takahashi and others (1998) at Aburahi indicated that over 50 % of farms were positive.

Dr. Yahara and others at Nisshin have now conducted a more extensive survey on over 370 farms using both PCR and immuno-fluorescence assays (IFAT) over a 5-year period, see Figure 2. Testing of pigs of different ages, found that the breeding herd animals (gilts and sows) were generally positive, with a steady rise in positive cases in the growing and finishing pigs – see chart 1, for a summary and discussion of the epidemiology of ileitis on this type of single-site farm, which is commonly seen in Japan.

Figure 2. *Ileitis IFAT survey in Japan, results from 2000 to 2003.*



All areas of pig farming in Japan had similar rates of positive pigs, but highest prevalence of positive farms and pigs were seen in samples collected in Hokkaido/Tohuku and Tokai/Chubu/Hokuriku, with the lowest prevalence in Kinki.

Clinical Signs - Chronic and Subclinical Ileitis

On a typical pig farm affected with ileitis, clinical observations of chronic and subclinical cases of PPE generally include two main signs: one is diarrhoea and the second is reduced weight gain or "variation" in the weights of growing pigs, 6 to 20 weeks old. In the more severe chronic form, there is usually a measurable number of runted pigs. Diarrhoea and poor weight gain are often seen together in a group of pigs, but not necessarily in the same pigs.

In affected pigs, diarrhoea is generally moderate. The stools are loose and pasty but normal in colour. In many cases, the faeces is sloppy and poorly formed, resembling cow faeces or wet cement. In more severe cases, the faeces may become more watery and have a liquid, sloppy texture. There may be some undigested feed material present. Reduced weight gain, feed efficiency and the resulting poor performance in pigs affected by either the subclinical or chronic form of PPE is an important form of economic loss. The subclinical form is merely harder to detect because poor performance is less

apparent and there may be few actual “runt” pigs. Milder chronic cases and subclinical cases can be difficult to detect but can be relatively common in the group. Therefore, groups should be carefully inspected for apparent wasting of well-grown pigs and for irregular cases of diarrhoea and runt pigs.

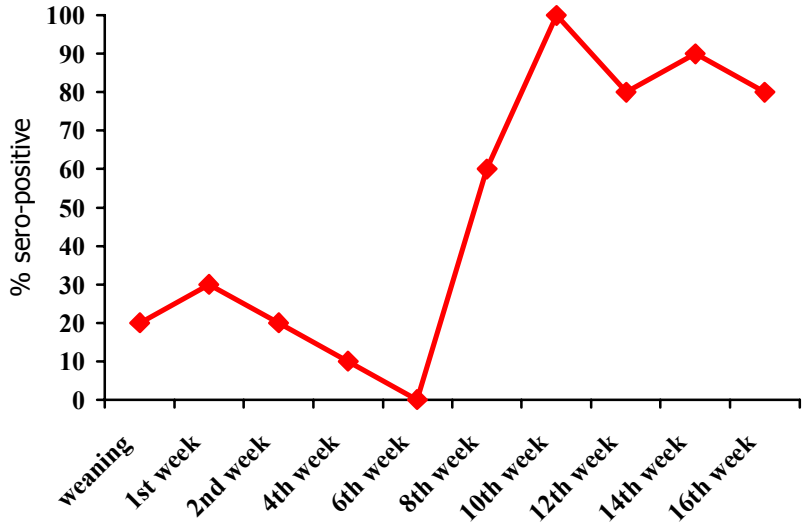
Epidemiology - the Spread of Chronic and Subclinical PPE

The chronic and subclinical forms of PPE are especially common on traditional, single-site, farrow-to-finish farms that have all farm buildings on one property. There is a simple flow of pigs and infected faeces around the farm, and the disease passes from one pig to the next. It is likely that Lawsonia can survive in pig faeces for around 2 weeks under normal farm conditions.

Infected pigs often shed *Lawsonia intracellularis* for about 2 weeks after first infection but some 10% of pigs can shed it for many weeks. Ileitis is then spread when one pig contacts the faeces of an infected pig and ingests some of the *L. intracellularis* bacteria into its mouth and gut. The bacteria enter the wall of the pig intestine, start an infection and then multiply. Once the infection develops in the intestine, *L. intracellularis* re-enters the pig faeces and moves to the next pig. Since the incubation period is 2 to 3 weeks in most individual animals, a slow build-up of disease in a group can occur over a month or more after the disease is first introduced to one pig in the nursery or grower areas. In some infected pigs, however, there can be large numbers of *L. intracellularis* in the faeces, causing rapid exposure of many pigs among a group. Once one of them becomes infected, a more obvious “outbreak” occurs. Other vectors, such as birds or rodents, appear to have little influence on *L. intracellularis* infections on infected farms.

On most farms, chronic and subclinical PPE occur after maternal antibody levels start to decline after weaning. Antibodies eventually reduce to a level where pigs become susceptible to *L. intracellularis* infection. This occurs at around 4 or 5 weeks old, and often coincides with the time that pigs are mixed into the farm nursery and in the early growing period. After the 2- to 3-week incubation period, some pigs develop disease, start to excrete the bacteria and spread it to other pigs. Infection occurs in many pigs after weaning and builds up in the grower and finisher areas. It then declines to a lower but detectable level in gilts and older breeding animals.

Chart 1 *illustrates a typical group of pigs as they age from weaning to finisher age on a traditional, single-site farm in Asia. The chart measures the percentage of pigs in the group with positive ileitis antibody levels in their blood. Maternal antibodies are still present in some pigs at weaning, but disappear in all pigs one month after weaning. Serum antibodies rapidly appear again around 8 weeks after weaning because many pigs have become infected at 4 or 5 weeks post-weaning and developed fresh antibodies to infection.*



Acute Haemorrhagic Ileitis - Epidemiology

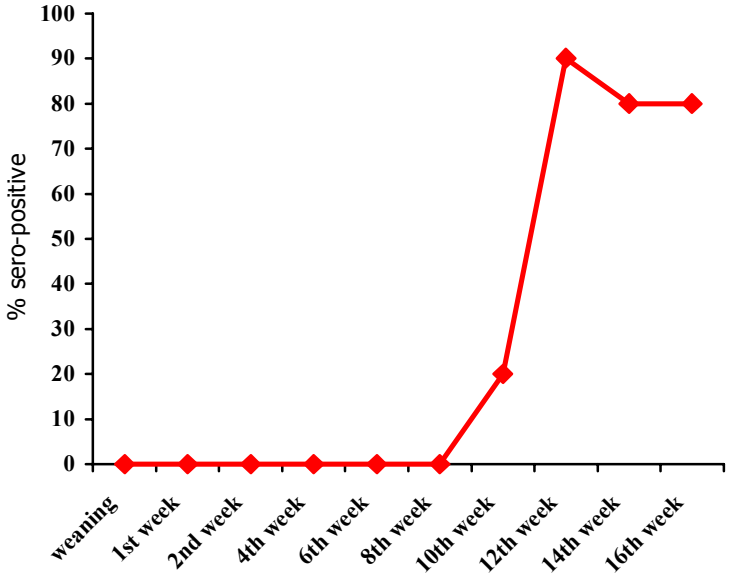
The acute haemorrhagic form of ileitis sometimes known as PHE, is a much more dramatic and severe form, with black soft faeces, pallor and sudden death. Cases are usually seen in "older" naïve pigs exposed to a relatively high oral challenge dose of bacteria. – such as the finishing or fattening period or in young adult pigs in breeding groups. Affected pigs are usually 3 to 12 months old. It is common to see a number of cases together, usually soon after some specific event in the group of pigs, such as moving them to a new building, moving them to a new pen, introduction of new breeding animals to the group, isolating the animals in testing or breeding stalls, transporting the group on trucks and so on.

Pigs can remain naïve until they are older if they receive continuous antibiotic medication that reduces early exposure to *L. intracellularis*. Natural outbreaks of acute disease, therefore, may partly reflect changes in the use of antibiotics. The other factor that may help explain an increase in acute ileitis is the increase in age separation among pigs on modern pig farms. This can have marked benefits for reducing pneumonias, but seems to affect the immunity of pigs to PPE.

On standard single site farms, usually up to 30% of the breeding herd may be sero-positive "carrier" pigs to PPE. In contrast, on some modern farms, especially the so-called "high health" and/or "multiple-site" farm operations, the breeding herd is very clean and may be well separated from the growing pigs. In many of these farms, it has been found that breeding pigs are in fact negative for PPE. An important consequence is that exposure to *L. intracellularis* among the offspring (piglets) of these clean breeding animals is delayed until later in the finishing period. The lack of exposure until these pigs are 4 or 5 months old makes them much more susceptible to the acute form of disease.
Exactly where this later infection comes from in finishers is not absolutely clear, but it is probably from infected faeces remaining in some part of the farm itself, such as the slurry pit, re-cycled water from drainage pits, faeces material on boots, clothing, pens or walkways. This would explain the consistent nature of later infection. If rodents or birds

or other vectors were involved, then the infection would be rare and more sporadic. The proper use of disinfectants and sanitation within sheds, especially all-in, all-out ones, may therefore act to reduce or remove many infections. Studies have indicated that iodine or quaternary ammonium based disinfectants are the most effective against ileitis. The question of whether sows can transmit the infection to their piglet children is not fully clear yet, but it seems likely that this happens on some occasions, though not regularly. Therefore, medication of sows to limit infection is not likely to have a major impact on PE on farms.

Chart 2 *illustrates typical blood testing results in grower and finisher pigs from a "multiple-site" farm in North America. Positive serology indicating recent exposure occurs late in the finishing pig period. These older naïve pigs are susceptible to the acute haemorrhagic form of ileitis.*



Economic impact of ileitis in Asia

Previous studies of the economic losses due to ileitis have been developed from its negative impacts on slaughter weight, feed conversion efficiency, space utilisation, and morbidity-mortality effects, totalling at US $ 0.50 to US $1.00 per affected growing pig, depending on the variable prices for pigs, building spaces and feed (McOrist et al, 1997). However, these estimates have been largely based on the effects of moderate chronic proliferative enteropathy in growing pigs. These estimates have probably underestimated the economic impact of subclinical ileitis (that is, pigs being infected and having some intestinal lesions, but without clear diarrhoea or weight loss), with sub-optimal weight gains and improper food utilisation, particularly its negative impact on digestion of in-feed amino acids. Also, breeding pigs with clinical *L intracellularis* infections can suffer lingering negative impacts on parameters such as the number of pigs born alive. This led to an estimated loss of over US $ 100 per affected breeding pig in one study, which would translate to an extra US $ 0.50 per growing pig, assuming

routine farm production factors. The total losses due to endemic ileitis on most Asian farms could therefore regularly exceed US $ 1 per affected grower-finisher pig.

Despite the short years since the elucidation of the cause of proliferative enteropathy, its endemic nature, major economic impact and variable time of onset persuasively indicate that a vaccine approach is the most logical for long lasting veterinary control. Protective vaccine immunity to other intracellular bacterial pathogens, such as *Brucella* spp or *Chlamydia* spp have only been convincingly demonstrated following delivery of whole live attenuated bacteria to the target tissue, therefore this strategy was the one adopted for the successful *Lawsonia intracellularis* vaccine (Kroll et al 2004). It is possible that successful vaccines for endemic intestinal diseases may provide innately more positive growth and feed utilisation impact than those for diseases in other body systems. The rapid progress in understanding of the epidemiology of ileitis with modern tools and a vaccine has led to an appreciation of its hitherto underestimated impact on a range of pig performance parameters.

Treatment Strategies for Ileitis: An Overview

Ileitis or PPE is a bacterial disease caused by the Gram-negative intracellular bacterium *Lawsonia intracellularis*. In actual outbreaks of PPE, it is therefore possible to use antibiotic agents that target the organism with a specific antibacterial effect. In acute PPE with high morbidity (many cases in the group) and high mortality, the use of injectable formulations of tiamulin or tylosin is usually recommended for the most "at-risk" pigs. This is usually followed with water-soluble formulations of tiamulin, lincomycin or tylosin to deliver a high dosage in an effective manner in the water supply of affected pigs. In one controlled clinical study, water medication with 60 ppm of soluble tiamulin for 5 days to groups of pigs challenged with ileitis resulted in a good clinical response and reduction of lesions during the monitoring period of 2 to 3 weeks post-medication.

The use of in-feed premix formulations is also a realistic option for managing both acute and chronic clinical ileitis. Several controlled scientific studies have confirmed the beneficial effects of tiamulin or tylosin premix formulations for control and prevention of ileitis on many farms across Asia. These drugs can be used with confidence for the treatment and control of ileitis.

The correct dose for each drug for each method of delivery must be carefully checked before it is used. It is important to realise that drugs given as a parts per million dosage in water or in feed will have different levels in the body of each pig treated because the body weight of each pig will vary with growth. It is therefore best to express the dosage of drugs on mg of drug per kg of bodyweight BW dosage. For instance, tiamulin is best used at a rate of 3 to 5mg/kg bodyweight. This usually corresponds to 100 ppm in feed in the grower phase.

Other important issues to consider when evaluating antibiotic treatments include factors such as product quality, cost, the technical and value-added support provided by the product company involved, withdrawal times and other regulatory issues. These factors can vary among different countries.

Obviously not all antibiotics are going to be effective against each disease. The several antibiotics found *not* to be fully effective for an ileitis challenge include bacitracin,

virginiamycin and salinomycin. The penicillins and fluoroquinolones likewise have proven ineffective in preliminary trials. Some of these drugs are not effective because they are aimed at Gram-positive bacteria, while others do not seem to target the correct tissues and cell location of the *L. intracellularis* bacteria.

Treatment Failures

Occasionally, use of some of the drugs mentioned, such as tylosin, are used against ileitis in farm outbreaks, but do not seem to be fully effective. Sometimes, this is thought to mean that *L. intracellularis* is becoming "resistant" to the drug. However, investigation of these situations usually reveals that either:

- Pigs were underdosed; for example, older larger pigs were only given inadequate in-feed amounts to make up their bodyweight dose. This is commonly seen in sows or finisher pigs on limited feed programs especially during hot weather, when feed intake is below normal.
- Pigs had concurrent infection with ileitis and another disease, such as swine dysentery or colitis.
- Pigs had some other disease or nutrition problem that is confused with ileitis.
- The antibiotic is given too late to be effective in preventing intestinal damage.

Another common problem occurs when antibiotics are administered to groups of "clean" pigs, which then develop acute or severe PPE after they are taken off the antibiotics, for transportation to market or breeding programs. In these situations, it is clear that the medicated pigs are not getting the chance to develop active immunity to the disease and remain naïve and susceptible. Older pigs are more likely to get acute PPE, so this strategy presents a great danger when gilts and other animals are kept on antibiotics.

Feed Additives

When added to feed, additives such as heavy metals (copper, zinc), probiotics, acids or enzymes, have not been shown to have any effect on ileitis. Although some inhibitory effect on microbial agents is likely at high levels of inclusion, these levels tend to reduce feed palatability for sensitive pigs. The removal of many in-feed antibiotics previously used at digestion enhancer levels in European pigs in the last 10 to 20 years has corresponded with a rise in the use of these feed additives, but has also coincided with a rise in the proportion of European farms positive for ileitis.

Pathogenesis and immunity

In typical oral challenge exposure studies of postweaned pigs (4 weeks-old) with a standard inoculum of 10^8 *L intracellularis* bacteria, numerous intracellular *L intracellularis* bacteria can be visualized in the developing proliferative intestines and feces one to three weeks following inoculation with a peak of infection and lesions 3 weeks after challenge. In most of these pigs, intestinal infection, proliferative lesions and excretion persists for approximately 4 weeks, but in some 5 to 10 percent of exposed pigs, excretion may persist for at least 10 weeks. *Lawsonia intracellularis* isolates

associated with one type of ileitis (acute or chronic) appear capable of initiating the range of pathological sequelae, indicating the "single strain" nature of *L. intracellularis*. Pigs of a wide age range are susceptible to oral challenge: pathogenic infections have developed following oral challenge of neonatal piglets aged 7 or 14 days and in pigs at least 18 weeks-old.

Early lesions contain very few infiltrating inflammatory cells, probably not above the normal for pig intestines. Affected epithelial cells contain a large accumulation of intracellular IgA and intestinal lavages contain a high level of *Lawsonia*-specific IgA indicating local mucosa responses are important. Macrophage ingestion of *L. intracellularis* in developing lesions probably leads to a typical Th1 type immune cell response in the lamina propria. Both cell-mediated and humoral responses occur in the blood of affected pigs. These are first detectable 2 weeks after exposure and can persist for some 3 months in acutely infected pigs (Guedes and Gebhart 2003). It is therefore likely that animals exposed to *L. intracellularis* show a specific immune response.

Acute hemorrhagic ileitis (PHE) is marked by severe bleeding into the lumen of the intestine, but with underlying lesions of chronic disease. The hemorrhage occurs concurrently with the widespread degeneration and desquamation of many epithelial cells and leakage from the capillary bed. PHE has been reproduced in older naïve pigs, challenged once with a high dose of *L. intracellularis*, indicating a host rather than bacterial effect.

Ileitis vaccine development

Once the causative agent of ileitis, *Lawsonia intracellularis* was first cultured in 1993, a start was then possible on developing a vaccine strategy. This was to take over 7 years of considerable research and development effort. For intracellular bacterial pathogens and for post-weaning enteric infections like *Lawsonia*, live attenuated bacteria delivered orally offer the most "natural" immunity and are widely considered the best form of vaccine approach (Kroll et al 2004). An attenuated live vaccine formulation of *Lawsonia intracellularis* was therefore developed (Enterisol® Ileitis, Boehringer Ingelheim). Because it is a live bacterial form that is administered orally to pigs, it is best administered in the middle of a 7-day antibiotic-free period. That way, any antibiotics that had previously been in the pig would be eliminated and would not kill any vaccine bacteria prior to its uptake. Many farms find that an antibiotic-free period can be readily developed around weaning time to allow the live vaccine use. The vaccine may be applied by adding it into the drinking water via a "Dosatron" type proportioner, or by using a simple drinking trough for each pen.

A large controlled study of on-farm immunisations of over 100,000 pigs against proliferative enteropathy caused by *Lawsonia intracellularis* has been completed in North America. These studies monitored non-medicated (or part-medicated) finisher-stage vaccinates to non-vaccinated controls, which received full "standard" antibiotic programs for ileitis during the finishing stage. The results indicate that the vaccine was indeed efficacious, with a significant reduction in lesions and reduction in the intestine colonisation of *Lawsonia intracellularis*. An interesting and consistent finding was that there was a significantly improved growth rate and reduced mortality in vaccinates compared to medicated control pigs. Along with this vaccine-enhanced growth effect due to control of the growth-retarding subclinical disease, vaccination allowed a 50-100%

reduction in antibiotics in the finisher period, allowing major cost savings. There are also clear-cut cost and welfare savings in the prevention of any occurrence of acute ileitis in groups of gilts and other breeders by this simple vaccination method.

The overall average improvement in the daily weight gain of vaccinates in these studies was 6 %, the average reduction in culls was 23%, but no significant improvement was evident in studies of feed efficiency. The reduction in culls and improved weight gains lead directly to an improvement in the overall weight grouping of finisher vaccinates at the time of marketing. It is possible that successful vaccines for endemic intestinal diseases may provide innately more positive growth and feed utilisation impact than those for diseases in other body systems. A further clear benefit of the ileitis vaccine is the possible reduction in transmissible antibiotic resistance factors being present on pig farms, due to the possible reduction of antibiotic usage.

The success of the *Lawsonia* vaccine may be partly due to actual exposure of this agent to the immune system via intestinal mucosal macrophages, with specific humoral and mucosal responses following oral infection (Guedes and Gebhart 2003). Protective immunity to infection by other intracellular bacterial pathogens (such as *Brucella* sp and *Chlamydia* sp) has only been demonstrated following delivery of whole live attenuated bacteria. In contrast, use of recombinant bacterial or killed vaccine approaches for these types of infections has not yet led to protective immunity being noted.

Additional advantages of vaccination via the water system are the elimination of animal and human stresses, time, costs and effort (including possible difficulties with full compliance and actual administrator injuries) with individual vaccinations, compared to mass vaccination methods such as oral vaccination. Further advantages include the elimination of the possible transmission of blood-borne infections such as PRRS virus via multi-use needles and the reduction of injection site reactions and needles retained in carcasses. Mass vaccination methods have been widely used on poultry farms for many years and they will probably become more widely used on pig farms. Over 50 million doses of the mass vaccination method for ileitis vaccine have now been successfully used in North America and Europe.

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European perspectives on food safety

Nigel Horrox

Europe, and Great Britain in particular, has been to the fore in food scares over the last couple of decades. This paper will briefly summarise these before going on to consider issues that are particularly pertinent to the swine/pork sector.

The current thinking of major European purchasers of meat and meat products and what they require of their suppliers will be reviewed. In particular the approach of the producer to these will be summarised. Issues such as GMP, GAP, HACCP, laboratory accreditation, drug residues, antibiotic resistance, traceability and due diligence will be considered.

David Tydliat

VACCINATION STRATEGIES FOR THE CONTROL OF CIRCOVIRAL DISEASES IN PIGS

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Porcine circovirus type 2 (PCV2) has been associated with a number of disease syndromes in pigs (Allan and Ellis 2000), including post-weaning multisystemic wasting syndrome (PMWS), proliferating and necrotizing pneumonia (PNP), abortion and reproductive problems, porcine dermatitis and nephropathy syndrome (PDNS) and porcine respiratory disease complex (PRDC).

It is now estimated that PCV2 diseases cost European producers 600 millions Euros per year. The infection is everywhere and can flare up in different areas of the world as currently in Canada, causing heavy losses and much distress.

Vaccines are among the most useful and powerful tools we have to prevent diseases and protect farm animals. This article will review the possible strategies and published efficacy studies for the vaccination against PCV2 infection and diseases.

1 - Vaccination designs against PCV2 infections and diseases

A vaccination scheme for PCV2-associated diseases that targeted gilts and sows and the passive transfer of high levels of maternally derived antibodies to PCV2 in colostrum and milk has been proposed (Charreyre *et al*. 2004) based on the following information:

- PCV2 is very stable, hardy and abundant in the environment and piglets can be exposed early in life
- In PMWS affected farms, compared to non affected farms, heavy PCV2 circulation occurs rather in the nurseries than in later stages of the pig life
- Maternal antibodies are protective against PCV2 infection and PMWS (Charreyre *et al*., 2002)

It appeared necessary to protect the piglets in their earliest days of life against a heavy PCV2 infection that can be immune suppressive and favors PMWS occurrence. This can only be achieved by vaccination of the dams to increase passive maternal antibody transfer.

New pieces of evidence still point to the vaccination of the breeder herd as a good solution against PCV2 diseases in piglets and in pigs:

- Recent vaccination challenge studies confirmed that maternal antibodies have a protective effect against PCV2 infection and associated lesions (Thomas *et al*., 2005)
- Epidemiological data confirmed that PCV2 infection earlier in life is associated with most severe PMWS problems (López-Soria *et al.*, 2005)

- Abortion and premature farrowing were obtained in sows inoculated with PCV2 three weeks before farrowing, thus emphasizing the need to protect the breeder herd in the gestational phase (Park *et al*., 2005)
- Very good preliminary results for the entire life of the pigs including the fattening phases have also been observed in field trials of gilts and sows vaccination in France and Germany.

On the other hand, vaccination of the breeder herd and passive antibodies will protect the piglets against PCV2 infection only for some time.

In isolated laboratory conditions, maternal antibodies drop below detection level by 3 to 8 weeks of age (Charreyre *et al.*, 2000; Halbur and Opriessnig, 2004; Opriessnig *et al*., 2004). For pigs with initial high levels of maternal antibodies measured by ELISA, this decay could last up to 16 weeks of age (Charreyre *et al.*, 2004)

This is reflected in field conditions, where active seroconversion is reported from 5 to 15 weeks of age (Cotrell, 1999; Larochelle *et al*., 2003; Segales and Morvan, 2004)
Several studies by different groups have demonstrated that active antibodies are protective against PMWS (Blanchard *et al*., 2004; Pogranichniy *et al*., 2004; Fenaux *et al.,* 2004). Therefore a limited natural infection with PCV2 will induce a natural protection against associated diseases.

On the other hand it seems very difficult to induce or observe PMWS when the pigs are getting older, thus suggesting that the window of opportunity for PCV2 infection to cause disease is in fact limited mostly to12-16 weeks of age and at the latest to 20 weeks of age in field conditions (grosse Beilage and Brakmann, 2004). Fattening pigs are overwhelmingly PCV2 seropositive with high titers and should be disease resistant.

Therefore the choice of a vaccination scheme will have to be evaluated in different epidemiological situations.

2 - Vaccination experiments in piglets

Early unpublished experiments in MERIAL demonstrated that two injections of experimental vaccines at 2 weeks and 5 weeks of age in conventional piglets with low level of residual maternal antibodies, could protect them against PCV2 challenge at 7 weeks of age. In vaccinates, clinical and gross lesions scores were significantly reduced and growth was improved. The detection of virus load in blood, feces, and mediastinal lymph nodes was significantly lower in vaccinates, sometimes completely suppressed. Protection was associated with a high anti-PCV2 antibody levels.

On the whole experiments done on MERIAL in the past few years demonstrated that no PMWS was observed when pigs submitted to challenge passed a certain level of anti-PCV2 ELISA levels.

These results were confirmed in independent studies by other groups. Two studies completed in France have demonstrated that vaccination with PCV2 DNA and/or recombinant proteins, with addition of the GM-CSF gene or in an experimental adjuvant, were able to protect SPF pigs from hyperthermia and growth retardation following PCV2 challenge. The best results were obtained with sub-unit proteins obtained from SF9

insect cells cultures infected with ORF1 and ORF2 recombined Baculovirus, mixed in experimental adjuvant. This experimental vaccine was able to generate an antibody response measured by ELISA and significantly reduced PCV2 circulation after challenge as assessed by PCR in various organs (Blanchard *et al*, 2004).

Classical inactivated vaccines have also been described as protective in a severe PRRSV enhanced PCV2 challenge in another US study. Mortality decreased from 70% to 20%, and vaccinated pigs continued to gain body weight at the same rate as negative controls. Surviving vaccinated pigs also had neither lesions nor PCV2 virus detected by IHC in organs at necropsy (Pogranichniy *et al*, 2004).

The largest published experimental studies on PCV2 vaccination in pigs described the performances of a chimeric PCV1-2 vaccine.

The vaccine is a live PCV2 vaccine that contains the immunogenic capsid gene of PCV2 cloned into the backbone of the non-pathogenic PCV1. Stocks of the live vaccine are obtained by transfection of PK15 cells with the infectious chimeric DNA clone (Fenaux *et al.*, 2004). Safety of use for this live genetically engineered candidate remains to be evaluated.

In a first efficacy experiment, groups of SPF pigs were vaccinated once at 9 weeks of age with the chimeric candidate as live virus by the intra-muscular route. Other groups of pigs received chimeric DNA by the intramuscular or intralymphoid routes. At 9 weeks of age, about 30 % of those SPF pigs had still some detectable maternal antibody levels with the ELISA test in use. The vaccinated pigs seroconverted in 4 to 6 weeks. Challenge was done at 15 weeks of age and did not induce clinical signs in the animals. Follow-up after challenge lasted 3 weeks. Vaccination prevented viremia, did not prevent lymph nodes swelling, reduced the viral load in lymph nodes, tonsils and spleen, and reduced the detection of histopathological lesions in lungs, liver and lymphoid tissues of the vaccinated animals. Interestingly the 3 vaccine candidates performed similarly (Fenaux *et al.*, 2004).

A second experiment was designed to evaluate the interference of maternal antibodies on the chimeric live vaccine-induced immunity against PCV2 infection (Thomas *et al*., 2005).

Different groups of 3 week-old SPF pigs were constituted with different levels of maternal antibodies as evaluated by ELISA: S/P ratio < 0.2 defined negative sera, S/P 0.2 – 0.5 defined low levels of antibody, and S/P > 0.5 defined high levels of antibodies. The different groups of pigs received either one injection of the chimeric vaccine or were submitted to a wild type virus exposure, followed by challenge or re-challenge at 9 weeks of age. Available preliminary reports indicated that the first wild virus challenge induced elevated respiratory scores in 3 week-old pigs that were seronegative of with low levels of PCV2 antibodies. These exposed pigs started to seroconvert before the next challenge. On the other hand, pigs with high levels of maternal antibodies were not clinically affected by the first exposure and did not seroconvert even after challenge.

Vaccinated pigs did not have elevated respiratory scores indicating attenuation of the chimeric vaccine. Those vaccinated pigs that were seronegative or with low levels of PCV2 antibodies at the time of vaccination appeared to have an anamnestic response to

challenge. On the other hand, pigs with high levels of maternal antibodies did not seroconvert even after challenge.

Interestingly, a first exposure to wild type virus limited by even low levels of residual maternal antibodies protected the pigs against a second challenge at 9 weeks of age. In contrast a first exposure of seronegative pigs at 3 weeks of age did not protect them against the second challenge.

After challenge, pigs of the control group had significantly more severe lymphoid depletion than pigs vaccinated when they had no or low levels of maternal antibodies. Piglets that had high levels of antibodies at the time of vaccination were not protected against challenge, indicating that the vaccine was efficacious only for piglets with S/P ratio below 0.5.

In field conditions almost all piglets are found positive for PCV2 antibodies at weaning time. A gradual decrease in antibody titers has been observed from 3 to 11 weeks of age which natural seroconversion from 5 to 15 weeks, indicating as well large variations between herds (Larochelle *et al*., 2004).

Therefore it will be necessary to evaluate the right window of opportunity for the pig vaccination in each herd, depending on the particular PCV2 circulation and associated disease in that farm.

3 - Vaccination experiments and programs in sows

3.1 – Description of two laboratory efficacy studies by MERIAL

The objective of the first study was to demonstrate the efficacy of an inactivated oil adjuvanted PCV2 vaccine (CIRCOVAC®) in a PCV2 controlled environment. Specific serological responses in vaccinated gilts and protection of their piglets after PCV2 experimental challenge at 3 weeks of age were evaluated.

The objective of the second study was to demonstrate the efficacy of this vaccine in piglets born to vaccinated gilts in the field and brought back into a PCV2 controlled environment. Protection of the piglets after PCV2 experimental challenge at about 4 weeks of age was evaluated.

Other studies demonstrated that this product presented a good safety of use in pregnant animals (Reynaud *et al*., 2004a and b).

In the first study, specific pathogen free gilts, specifically seronegative for PCV2 antibodies by ELISA were allocated to two groups. One group of 11 gilts was vaccinated at minimal antigen content via the intramuscular route 5 and 2 weeks pre-breeding and 2 weeks before farrowing. Another group of 12 gilts was not vaccinated. All the gilts were inseminated artificially at 10 months of age and 8 gilts became pregnant.

Therefore a first group of 22 piglets born to 4 vaccinated gilts and a second group of 22 piglets born to 4 control gilts were challenged intra-nasally with PCV2 at 3 weeks of age. PCV2 antibodies were measured at regular intervals in the blood of the gilts and piglets throughout the study.

After challenge, clinical signs, including rectal temperature and weight gain were monitored for four weeks. Viral load in serum and in faecal swabs was also estimated by quantitative PCR (Q-PCR). A complete necropsy assessment was carried out at the end of the follow-up. In addition, mediastinal lymph nodes were collected for PCV2 viral load by immunochemistry (IHC).

Vaccination induced a high specific seroconvertion in the vaccinated gilts, starting immediately after the first injection and further boosted by the third injection before farrowing. Vaccinated gilts had high, stable and homogeneous PCV2 antibody levels while the control gilts and their piglets remained seronegative, thus demonstrating the absence of wild PCV2 contamination in this controlled environment.

An efficient transmission and persistence of maternal antibodies following colostrum intake was demonstrated by the measurement if high and homogeneous antibody titres for 3 weeks before challenge in piglets born to vaccinated gilts.

After the challenge, a strong seroconversion was observed in piglets born to control gilts, while the level of antibodies in piglets born to vaccinated gilts continued to decrease.

Although no complete PMWS case was recorded in this experiment, clinical signs and growth impairment were observed after PCV2 challenge and the clinical scores were significantly higher in piglets born to control gilts ($p = 0.015$). Specifically, piglets born to vaccinated gilts presented a comparable growth pattern than unchallenged piglets and a better one than piglets born from control gilts.

At necropsy, the lesion scores were significantly lower in piglets born to vaccinated gilts than in piglets born to control gilts ($p<0.00001$).

The amount of PCV2 DNA in the serum of piglets born from vaccinated gilts was significantly lower than in piglets born from control gilts. The amount of PCV2 DNA in rectal swabs was significantly lower in piglets born from vaccinated gilts compared to control piglets. The extent of PCV2 viral load in mesenteric lymph nodes was also significantly lower in piglets born from vaccinated gilts than in control piglets ($p = 0.00002$).

The inactivated vaccine proved to be highly immunogenic as shown by the high and stable antibodies titres obtained in vaccinated gilts. In addition efficient transmission and persistence of maternal antibodies following colostrum intake was demonstrated. Vaccination induced a significant protection after virulent PCV2 challenge in piglets born to vaccinated gilts. The growth pattern was better in piglets born to vaccinated gilts than in piglets from control gilts. After challenge, the clinical and lesion scores were significantly reduced in piglets born to vaccinated gilts. Detection of PCV2 DNA by Q-PCR and PCV2 antigens by immunochemistry demonstrated that the replication of the virus was significantly reduced in piglets born to vaccinated gilts compared to the control piglets.

Those results demonstrated that the sow vaccination with CIRCOVAC® was beneficial in improving the piglet health and performances after PCV2 challenge in a highly controlled environment.

Sows enrolled in a field efficacy trial in a PMWS affected farm were selected as source of piglets for the second study.

The first group of 12 piglets was born to 8 control sows that were not vaccinated. The second group of 10 piglets was born to 7 sows that had been vaccinated once with CIRCOVAC® at minimal antigen content via the intramuscular route 2 weeks before farrowing. A third group of 11 SPF piglets was added to the study to monitor challenge. Piglets from the farm were brought into the challenge facility at 3 days of age at a convenient date depending on the herd management calendar. Therefore the 3 groups of piglets were subsequently submitted to intra-nasal PCV2 challenge on the same day but at somewhat different ages: group 1 from control sows were 32 days of age, group 2 from vaccinated sows were 25 days of age and SPF pigs were 47 days of age.

Throughout the study, PCV2 ELISA antibodies in blood were evaluated at regular intervals in samples from the farm sows and from all the piglets and PCV2 virus in faeces was evaluated in serial samples from the piglets.

After challenge, clinical signs, including rectal temperature and weight gain were monitored for four weeks. A complete necropsy evaluation was carried out at the end of the follow-up, targeting especially the lymph nodes. Mediastinal lymph nodes were collected to evaluate PCV2 viral load by immunochemistry (IHC).

Two weeks before farrowing, at the time of vaccination, all sows were seropositive and had similar PCV2 antibody titers. Two weeks after farrowing, and despite a possible depletion through suckling, the level of antibody had increased significantly in the vaccinated sows ($p < 0.005$). This was confirmed in the levels of antibody evaluated in piglets. Those born from vaccinated sows had more antibodies than piglets from control sows up to challenge time ($p = 0.01$).

Simultaneously 60% of piglets born from control sows were found positive for PCV2 virus detection in faeces during the first week they were brought in isolation. Moreover viral detection increased up to challenge in that group until all piglets became strongly positive. In contrast only 25 % in piglets born from vaccinated sows were found positive after arrival and they all became negative by the time of challenge.

During these first 3 to 5 weeks of age it appeared that fewer piglets born from vaccinated sows excreted less PCV2 in faeces than piglets born from control sows and this was correlated with a higher level of maternal antibodies.

In this context, the challenge did not induce very significant clinical signs in both groups of piglets born from vaccinates or from controls. The challenge was validated firstly because of the elevated clinical score of the SPF group, due mainly to higher rectal temperatures and abnormal growth patterns, secondly by the strong seroconvertion in that SPF group, and thirdly because of PCV2 excretion in faeces of all challenged piglets. Piglets born from control sows exhibited a rise in antibody levels after challenge, while antibodies continued to decay in piglets born to vaccinated sows. The absence of a booster effect in that group after challenge can be linked to the good protection conferred by maternal antibodies against subsequent PCV2 infections.

At necropsy, the piglets born from vaccinated sows displayed significant reduced lesion scores than the piglets born from control or SPF sows ($p = 0.0001$). This was especially

noticeable in mediastinal and mesenteric lymph nodes. No gross lesion was noted in the mesenteric lymph nodes of piglets born from vaccinated sows, while 70 to 80% of the piglets in the two other groups had high to very high lesion scores ($p = 0.00043$).

The virus load as determined by IHC was also lower in mediastinal lymph nodes from piglets born to vaccinated sows, although the difference was not found significant.

Those results demonstrated that the sow vaccination with CIRCOVAC® in field conditions was beneficial in reducing the natural PCV2 circulation and shedding in the first weeks of the piglet life, but also in improving the piglet health and performances after an additional PCV2 challenge. Protection against lymph nodes inflammation in young animals can be quite important as this can allow their immune system to mature and to respond better to further challenges.

3.2 – MERIAL field efficacy studies in France and Germany

Under field trial authorisation, a field efficacy study has been conducted in three PMWS affected farms in France for more than 18 months. Two farms were organized with 7 groups of about 35 sows farrowing every three weeks, and the third farm had 22 groups of about 12 sows farrowing every week.

Groups 1 and 2 out of 7, or groups 1, 3, 5, 7, 9 and 11 out of 22 were kept as control groups. The remaining groups were vaccinated over time with one injection of the minimal dose of vaccine 3 weeks before each farrowing time. The replacement gilts were obtained from outside sources and vaccinated twice in quarantine before introduction in the herds throughout the experiment. Therefore up to 70% of the animals were vaccinated over time.

Besides serological follow-up of the breeder herd, all piglets born from groups 1 to 4 and groups 1 to 12 in two successive gestations were followed up until slaughter for signs of PCV2 disease. A global comparison of all piglets born from vaccinated and from controls during an entire year was finally done.

When the experiment started, all dams were seropositive with about 12% of them being highly seropositive. After two injections, after the second farrowing time, 56% of vaccinated sows were highly seropositive versus only 7% in the control groups. Concurrently to the rise in antibody level in the breeder herds in the 3 farms, after vaccination was started PMWS cases decreased very fast, from more than 5% when the farms were selected progressively down to 1.12% in controls (n = 4,183 piglets) and 0.67% in vaccinated (n = 10,462 piglets) in about 18 months.

These satisfactory results were further confirmed in larger numbers of animals, under temporary licenses for CIRCOVAC®, that were obtained in Germany from August to December 2004 and in France in November 2004. More than 50,000 sows have been vaccinated in more than 150 farms. A very satisfactory efficacy was observed with a great reduction of losses and number of wasted pigs, more homogeneous growth rates, and reduction in the use of antibiotic treatments. Global mortality rates between weaning and the end of fattening decreased by at least 50% in the vast majority of the farms.

Conclusion

It is now confirmed that vaccination against PCV2 can provide a solution to the problems caused by PMWS and PCV2 infection. Vaccination of the piglet is efficacious in controlled laboratory conditions as long as maternal antibody levels are not too high. Vaccination of the breeder herd including pregnant animals is safe and was found efficacious in controlled laboratory conditions. This has been confirmed in field conditions in very large numbers of gilts and sows.

We can now hope that registered vaccines will soon be widely available tools to fight PMWS and PCV2 diseases.

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ON THE WAY TO 30 WEANED PIGLETS PER SOW PER YEAR ?

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Introduction

Pig production is a continually evolving process. And as modern pig production is often dictated by economic forces, the practice requires a high level of strategic control. Farmers and veterinarians are always searching for ways to improve reproductive efficiency. For both, it is essential to know when and how best to intervene in order to gain an economically optimum result. It should be clear a maximum technical result, usually not corresponds with a maximum economic result and that these figures can differ from farm to farm.

The three main parameters that influence the profitability of a pig production farm are the right number of breeding gilts, the farrowing index and the litter size (2).
The number of *weaned piglets per sow per year* is a parameter commonly used for the purposes of comparison. This figure is the product of the number of *farrowings per year* and the number of *weaned piglets per litter.* And these two parameters are comprised of various elements which are shown in the flow chart in Figure 1.

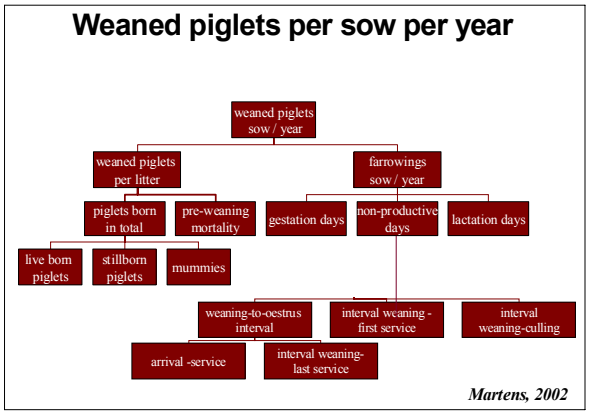


Figure 1: Parameters that influence the number of weaned piglets per sow per year

To make comparison possible, the formula of calculations should be clear. In general calculations are based on the *average number of sows present* during a defined period. Gilts should be calculated as sows from the day of first insemination onwards; sows should be considered as present until the day of culling.
In order to be able to judge and improve the fertility management of a farm it is necessary to get into the details of the flow chart. The value of each parameter can be a lead for the farm management to improvement in the different sections of the farm.

Firstly looking more into detail at the production per litter, the number of *live born piglets per litter* is crucial.
Besides the influence of breeding management, it is clear that the number of *live born piglets per litter* is influenced a great deal by the genetics of the breed

or hybrid. During the last decades genetic breeding companies achieved a continuous progress through breeding and selection research, using new techniques like quantitative genetics, genome mapping (DNA technology) and new selection traits in animal physiology (Table 1). An increase of 0.2 *live born piglets per litter* in the last decade, resulting in an increase of 0.5 *weaned piglets per sow per year*, boosted up the results in the top segment of pig farming to 26 *weaned piglets per sow per year*.

Table 1: (Achievable) genetic progress 2002-2008

	2002	**2005**	**2008**
Farrowing index	2.35	2.40	2.46
Live born piglets/litter	12.0	12.6	13.2
Weaned piglets/sow/yr	24	26	30
ADWG (gram)	850	900	950
FCR	2.7	2.5	2.4

Based on the continued improvements in the genetic merit of the nucleus sow herds we should question why these improvements are not realized in our production farms. The importance of the management of the gilt pool is often underestimated.

It is believed that the maximum achievable result will be reached with around 30 *weaned piglets per sow per year* (Table 2).

Table 2: Achievable number of weaned piglets

25 eggs recruited in both ovaria
→ 5 non-ovulated eggs
20 ovulated eggs
→ 3 unfertilized eggs
17 fertilized eggs
→ 2 embryonic death
15 vital embryos
→ 1.8 mummies/stillborn
13.2 piglets born alive
→ 1 pre-weaning mortality
12.2 weaned piglets / litter

The *farrowing index* cannot exceed 2.50 and with about 13 *live born piglets per litter* the physiological limitations of the sow will be reached.

The next challenge will be to keep alive as many *live born piglets* as possible to be able to increase the number of *weaned piglets per litter*. When management and disease control in the farrowing room and in the nursery are optimal, *pre-weaning mortality* should be limited to less than 10%.

For feed companies as well as for the veterinary services it will be a challenge to keep track with the genetic possibilities and to find out where the limitations will be.
Further research on feed intake in relation to milk production, health, growth and to carcass and meat quality will reveal the future possibilities. The influence of climate and housing on feed intake of especially lactating sows and fatteners also still needs additional investigation (6).

Secondly looking more into detail at the year production per sow, the figures per *average sow present* is a good starting point. The *gestation days* are fixed. The *lactation period* can vary. But shortening the lactation period to less than 3 weeks asks special attention for the weaned piglets in order to control the *post-weaning mortality*. A weaning age of 15 days is considered as an absolute minimum.

Reduction of the *non-productive days* per *average sow present* will have an enormous influence on the economic figures of a pig breeding farm. Also here

the management of all the different parameters involved will contribute to the optimization of the production per sow. When a reproduction problem is encountered, first look for and try to correct underlying management causes before resorting to hormone administration.

There are many opportunities to pharmacologically intervene in pig reproduction. Pharmaceutical companies offer the pig industry several products which can help to achieve better production targets. Exogenous hormones are excellent management tools in optimizing pig production, but when used inappropriately it may also result in production problems. Their use should never be allowed to substitute for good management practices. It is the responsibility of the veterinarian to ensure that any intervention is warranted.

Objective

The objective of this paper is to provide some
practical guidelines for optimization of fertility management and outlining the main indications for the use of exogenous hormonal products, from a producer's perspective.

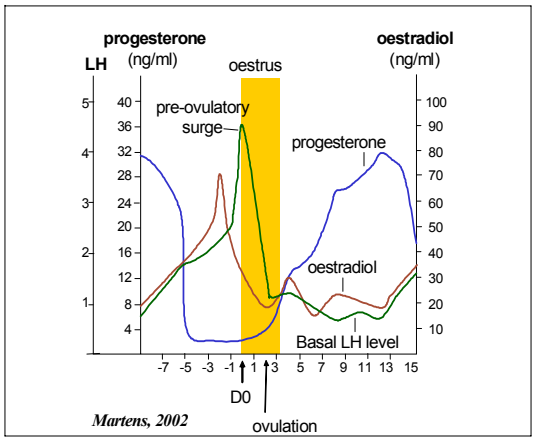
Controlling onset of oestrus

The oestrus cycle

It is essential to thoroughly understand the estrous cycle before considering intervention.

The 21-day porcine estrous cycle is composed of two phases: a 5-6 day follicular phase an approximately 16-day luteal phase (Figure 2).

Figure 2: Hormone profile of the oestrus cycle in the sow.



The follicular phase, during which the ovarian follicles form and develop, ends with oestrus. This phase is under the control of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH promotes the development of the follicles which produce oestradiol. LH stimulates ovulation and the formation of the corpora lutea, which produce progesterone.

The luteal phase corresponds to the development of the corpora lutea, which produce progesterone which blocks the secretion of gonadotrophins (FSH, LH) and so prevents the onset of oestrus. At about day 12-14 of the luteal phase, uterine (wall) production of prostaglandin F2alpha causes regression of corpora lutea and so terminates progesterone production (luteolysis). Removing the progesterone block allows resumption of appropriate secretory patterns of LH and FSH.

FSH and LH are produced in the pituitary gland, regulated by the hypothalamus via the action of gonadotrophin releasing hormone (GnRH).

Oestradiol and progesterone have a negative feedback effect on the secretion of GnRH from the hypothalamus (Figure 3).

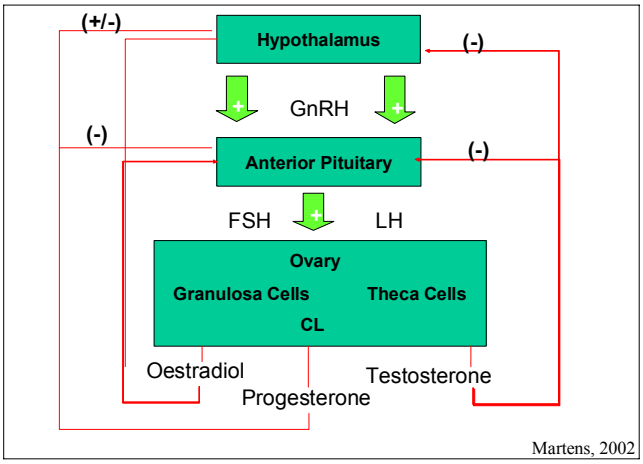


Figure 3: Hormone feedback loop

The needs of the pig producer

Induction of puberty in prepubertal gilts

Gilt management is of high importance. New breeding gilts will bring the genetic improvement of the sow herd and they have the highest percentage of farrowing. The right number of selected gilts should always be available to replace voluntary culling of sows and involuntary fall outs of sows.

Gilts are mated for the first time at various ages. Schukken et al. (The Netherlands, 1992) calculated that, from the economic perspective, the optimal time for first service was at 200-220 days of age. But, in practice, it was actually happening at 241 days of age (7).

Age at the onset of first estrus is very largely dependent on the age at which effective stimulation with boar pheromones and direct boar contact (2).

Of course, the decision on when to start is not only based on the age of the gilt, but also on her development. 180 days of age and 100 kg bodyweight are considered to be the minimum requirements, although there are differences between different breeding lines (5). The extremes of sexual maturity and growth rate in gilts can result in first oestrus gilts differing in body weight by as much as 73 kg (2).

Prepubertal gilts can best be brought into oestrus with PG 600® (Intervet), a combination of 400 I.U. Pregnant Mare Serum Gonadotrophin (PMSG, ≅FSH) and 200 I.U. Human Chorionic Gonadotrophin (HCG, ≅LH) (1). Breeding at the second or third oestrus after puberty induction will give a better life time performance (0.6-0.8 piglet per year extra). Also the feeding regime is of big influence. Usually gilts are fed restrictively from 60 kg body weight onwards. But maximum feed intake from 3 to 4 weeks prior to breeding will result in 2-3 ovulations more (2).

Oestrus synchronization in cycling gilts

A 3-week production system is widely accepted and practiced in several countries. In this system sows farrow in 7 groups. To fit into one of these groups, replacement gilts need to be synchronized.

A synthetic oral progestagen, altrenogest, (Regumate®, Intervet) can block the natural secretion of gonadotrophin.

Irrespective of the day of the cycle on which it is started, this product will lead to oestrus after 18 consecutive days of oral treatment with 20 mg altrenogest.

Gilts are managed so that the end of their treatment coincides with the weaning date of the selected sow group. Oestrus will be observed in the gilts 5-7 days after the end of the treatment, at the same time as the sows (depending on the sows' weaning to estrus interval (WOI) which averages 5-7 days).

When the precise stage of the gilts' cycle can be determined, then Regumate® can be administered over a shorter period, depending on the number of days before the intended day of mating. The product is then given

from day 14 of the cycle onwards, which prolongs the luteal phase. It is a simple and cheap system, but requires very accurate heat detection and precise treatment of the gilts.

Induction of oestrus in first litter sows

This is a big issue in the pig industry. It is very common for first litter sows to exhibit anoestrus, or an extended WOI, during periods of reduced fertility, in the higher temperatures of the summer months, for example.

Several trials have demonstrated the benefits of using PG 600® on the day of, or the day after weaning, as a preventive measure.

Numerous trials have shown the benefits of such a procedure. These show always a significant reduction of non-productive days by a shortening of the WOI (Figure 4).

In recent trials from Brazil (9) next to this also an improvement of litter size was observed in the PG 600® treated group (Figure 5). Other trials however demonstrate an overall improvement but with a reduced farrowing percentage and/or litter size (2). So, with PG 600® one can expect similar fertility results as with a natural occurring oestrus.

Figure 4: Effect of the use of PG 600® in first litter sows at weaning

PG 600® in first litter sows at weaning

	Detected in heat <10 days	WOI (days)	Duration of oestrus (in hours)	Ovulation (hrs after onset)
PG 600®	94.2%	4.3	61.8	47.0
Controls	77.8%	4.9	59.9	43.7

Vargas et al., 2001, Brazil, Abraves congres

Figure 5: Effect of the use of PG 600® in first litter sows at weaning

PG 600® in first litter sows at weaning

	Detected pregnant (%)	Farrowing (%)	Live born piglets per litter
PG 600®	92.0%	90.8%	10.5
Controls	93.8%	92.9%	9.8

Vargas et al., 2001, Brazil, Abraves congres

Induction of oestrus in anoestrous sows

Sows that do not come into heat naturally in the first 10 days after weaning, are best treated with PG 600® to induce oestrus. Most of them (those in true anoestrus) will usually come into heat, which provides a valid indication for the use of PG 600® during the so-called 'summer infertility'.

Induction of parturition

Prostaglandins have been used for many years to manipulate farrowing times in order to avoid nights, weekends and other periods when assistance is less readily available. And the synchronization of several farrowings can also bring certain advantages.

As long as farrowing is not induced too early (not before 113 days of gestation) there appears to be no disadvantages. Half the normal dose injected into the vulval submucosa has been shown to be effective.

In some countries, prostaglandins are used mainly to clean up sows after farrowing, utilizing the effect of prostaglandin on smooth muscle.

Reducing the depression of second litter production in early weaning systems

At the IPVS Congress 2000 in Melbourne, Tilton and Weigl presented

the results of a small study. It demonstrated an indication for the use of altrenogest (Regumate®, Intervet) to postpone oestrus in early-weaned gilts. After 16 days of lactation, they used Regumate® to postpone the next oestrus by 7 days. The extra seven non-productive days were more than compensated for by the higher subsequent fertility (Figure 6 and 7).

Figure 6: Effect of Regumate® treatment of first litter sows at weaning

Regumate® treatment to overcome depression of second litter production

Parameter	Control	Regumate®
Number of sows	16	21
WOI (days)	7.9 days	7.0 days
Conception rate (%)	75%	95%
Failure to cycle (%)	12.5%	4.7%

Tilton & Weigl, 2000, IPVS Melbourne

Figure 7: Effect of Regumate® treatment of first litter sows at weaning

Regumate® treatment to overcome depression of second litter production

Parameter	Control	Regumate®
Number of sows	12	18
Ovulation rate (%)	16.2% (sd 0.6)	18.2% (sd 0.8)
Normal embryos	11.9 (sd 0.9)	13.3 (sd 1.2)
Embryo survival rate (%)	73.5%	73%

Tilton & Weigl, 2000, IPVS Melbourne

This use of Regumate® needs to be confirmed in larger trials, but it looks very promising and can also be valuable for lean sows to regain some weight after (early) weaning.
Similar trials, instituting treatment after very short lactations, gave disappointing results, and 15 days lactation seems to be a minimum.

Conclusions

Careful analysis of production parameters can suggest improvement of management factors that will have a beneficial effect on reproductive performance and financial optimization of the results of a pig producing farm.
An effective gilt pool management will allow producers to introduce gilts of the right age and weight into the sow herd. This will have a major beneficial impact on breeding herd productivity.

Nowadays veterinarians and pig producers are able to utilize a variety of hormonal products to help them achieve their production targets.
Knowledge of the naturally occurring hormonal interactions is an essential prerequisite to the effective use of these products as management tools.
Many farmers and veterinarians are already using these tools and have confidence in their use.

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MYCOPLASMA HYOPNEUMONIAE: DISEASE, CONTROL AND INTERACTIONS WITH OTHER PATHOGENS

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Keywords: *Mycoplasma hyopneumoniae*, PRRSV, vaccine, PCV2

Introduction

Respiratory disease remains a significant problem to the swine industry worldwide. The most common pathogens isolated from pigs with respiratory disease presented at the Iowa State Veterinary Diagnostic Laboratory include: porcine reproductive and respiratory syndrome virus (PRRSV), *Pasteurella multocida*, swine influenza virus (SIV), porcine circovirus type 2 (PCV2), and *Mycoplasma hyopneumoniae* (MHYO). This presentation will concentrate on the diagnosis, control and MHYO disease interactions that exacerbate respiratory disease.

Mycoplasma hyopneumoniae

MHYO is a bacterium that lacks a cell wall and is ubiquitous throughout the swine world. The MHYO genome is very small and the organism colonizes the epithelium of the respiratory tract by attaching to ciliated cells. Clinically, MHYO infection is characterized by a mild non-productive cough. However, growth of the organism in the pig and in culture is slow, typically taking more than 4 weeks to replicate and induce significant pneumonia in vivo. Infection with MHYO results in ciliostasis and reduced efficacy of the mucociliary apparatus which facilitates infection by secondary bacteria such as *P. multocida* (1, 2). However, MHYO also alters or modulates the immune system in the respiratory tract. By modulating the respiratory immune system, the organism's ability to persist in the host is facilitated (10, 11). In addition, this results in increased disease from other pathogens such as PRRSV and PCV2. Currently, the virulence factors and the mechanisms by which MHYO causes disease remain unknown.

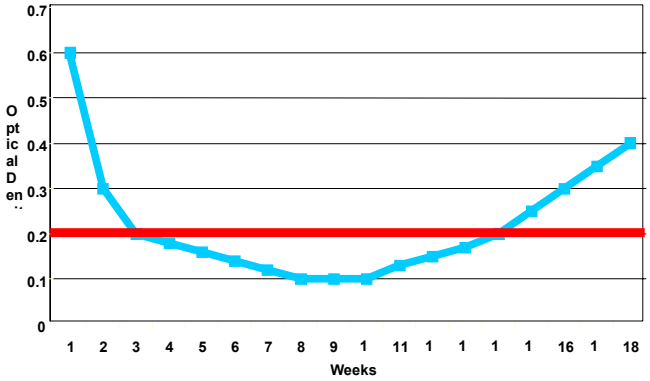
Disease Interactions

Previous research in our laboratory has demonstrated that pigs infected with both MHYO and PRRSV have significantly increased severity and duration in pneumonia (6). This interaction is independent of pathogen infection timing as well as the degree of PRRSV pathogenicity. In addition, in the presence of PRRSV, MHYO-induced pneumonia can become acute and can cause MHYO pneumonia in nursery pigs. Recent research in association with Drs. Pat Halbur and Tanja Oppressnig found similar finding when pigs were infected with both PCV2 and MHYO (4). Increased pneumonia and viral load were observed in pigs infected with both pathogens. In addition, 7 of 16 pigs had microscopic and macropscopic lesions consistent with postweaning multisystemic wasting syndrome (PMWS).

MHYO control

Long term control of MHYO-induced pneumonia is through vaccination. MHYO vaccines are bacterins made from either whole organisms or membrane preparations. In order to induce an immune response, adjuvants must be added. Use of adjuvants activates macrophages which then present the mycoplasmal antigens to lymphocytes resulting in an immune response. Induction of serum antibodies is variable according to vaccine; however there is no correlation between the presence or absence of detectable antibodies and protection against disease (7, 8). A typical profile (Figure 1) of a pig over time in a naturally infected herd includes the presence of maternally derived antibodies (MDA) which decay below the positive cut off by approximately 3-6 weeks of age.

Figure 1. Typical antibody profile of pigs in an infected herd.



Following vaccination with most 2 dose vaccines, seroconversion will occur; however, seroconversion following vaccination should not be used as a measure of vaccine compliance (7). In addition, one dose vaccines and those that are adjuvanted with aluminum hydroxide typically do not induce the antibody production. Serology can be used as a tool to assess herd stability, timing of infection and vaccination status. MHYO vaccines reduce clinical disease but do little to prevent colonization or reduce organism numbers. Timing of vaccination depends on when disease (coughing) occurs; the presence of co-infecting pathogens (PRRSV and PCV2) and what will

work within a herd. Both one and two dose MHYO vaccines can work within a system. One dose products do not work as well as two doses. One dose products work best if the herd is of high health, does not have active PRRSV circulating through the herd, all-in, all-out management strategy and a multi-site operation.

MHYO Vaccine Failure

MHYO vaccine failure can be the result of a number of factors including co-infection with PRRSV and very high MDA levels (3, 9). Research has shown that PRRSV, either pathogenic or low pathogenic isolates significantly reduce the efficacy of MHYO vaccines; however, vaccination does reduce the potentiation of PRRSV pneumonia by MHYO (9). The presence of MDAs at a level consistent with natural infection does not interfere with MHYO vaccines (5). If levels of MDA are elevated due to active sow infection or vaccination, reduced vaccine efficacy of one dose products is possible. In order to maintain overall herd stability, gilts should be well vaccinated prior to entering the sow herd in order to reduce overall organism levels within the herd that will help maintain the respiratory disease levels at an acceptable level within nursery pigs. Maintaining a stable sow herd with low levels of MHYO ensures the overall respiratory health of the entire system. With increased levels of organisms in the sow herd, MHY-induced pneumonia can occur in nursery aged pigs.

Conclusions

Respiratory disease remains a serious concern of swine producers throughout the world. While multiple pathogens contribute to respiratory disease, MHYO plays a significant role in increasing the pneumonia associated with viral and secondary bacterial infections. Control of MHYO is through vaccination; however, timing of vaccination is important for optimal results.

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SPECIFIC TOPICS
(Oral Presentations)

Virology

AN UPDATE ON PRRS PREVALENCE IN THE PHILIPPINES I. LUZON

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Introduction and Objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) prevalence has been explored as early as 1995-1996[1], showing a 64.71% seropositive status from 170 herds tested. A previous study[2] reported a 100% seropositive status on 46 farms involving 2,441 samples. Seventy six percent (76%) of the samples were seropositive. This involved farms tested from a period of January 2000 to September 2001. This paper aims to determine the seroprevalence for PRRS of farms particularly in Luzon.

Materials and Methods

A total of 9,069 serum samples from 167 farms in Luzon, the northernmost island of the Philippines were tested for PRRS ELISA. The samples included in the study were taken from March 2000 to June 2004. Sampling for majority of the herd is based on the number of animals in the farm having a 95% confidence level and an assumed prevalence of 10-20%. The proportion of samples tested that were seropositive and seronegative was determined. A seropositive farm indicates the presence of even a single animal resulting to a positive ELISA result.

Results and Discussion

Table 1. Proportion of total samples and farms seropositive for PRRS virus in Luzon

	Positive	Negative	Total
Samples Tested	62%	38%	9,069
Farms Tested	98%	2%	167

Sixty-two percent (62%) of the serum samples tested were seropositive. This means that a total of 5,623 animals from which the sample was taken had been exposed to the PRRS virus. On a per farm basis, a total of 163 are seropositive for PRRS.

A higher prevalence is seen in replacement gilts compared to sows was observed (Figure 1).

Figure 1. Percentage seropositive in Luzon breeder herds.

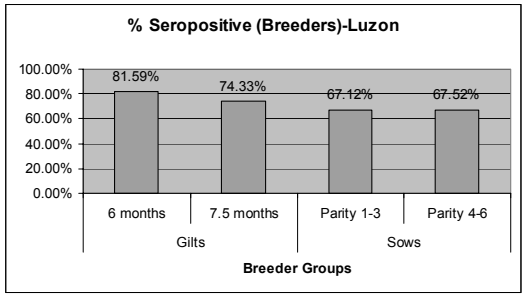
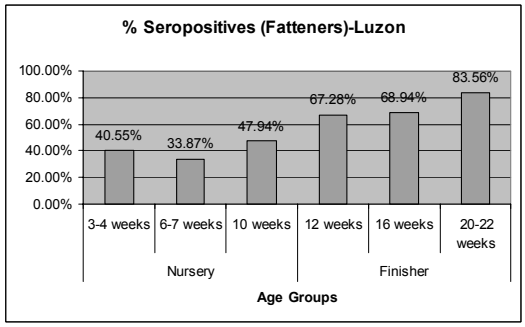


Figure 2. Percentage seropositive in Luzon fattening herds.



In line with the primary role of PRRS in Porcine Respiratory Disease Complex (PRDC) and with such a high seroprevalence of PRRS in Luzon, an effective control and preventive

measure should be implemented. Due to proximity from farm to farm, effective biosecurity measure would be difficult to implement, although possible. In a PRRS-initiated PRDC, vaccination with a modified live vaccine has been proven effective & practical in controlling the complex infection[3] .

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Development and evaluation of field tests for simple detection and quantification of antibodies to porcine reproductive and respiratory syndrome virus in swine sera

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Keywords: PRRSV, RIDEA, Gel-EIA, serology

Introduction:

Monitoring the serostatus of a swine herd is an integral part of PRRSV control and eradication. Currently, the IDEXX ELISA has become the industry standard for monitoring the serostatus of swine herds. Several serological methods including indirect immunoflourescence antibody (IFA) and serum neutralization (SN) assay are also routinely used to clarify PRRSV status and quantify antibody levels of individual animals. However, the complexity and the effects of PRRSV in many herds have created the need for an intensive detection and control measures of the virus. It created not only the need to develop efficient diagnostic assays which most of the other virological and serological assays provide. It stressed the need for on-farm virological and serological tests that will empower veterinarians to do the test in the field at the shortest time thus making necessary judgments the soonest before the infection starts or before the infection further spreads.

For this reason, the US National Pork Board has joined in an effort with the North Central 229 Multi-state PRRS Integrated Project (NC-229 PRRS) to establish a multistate collaborative model comprising the integrated activities of producers, industry and research scientists, to provide the next generation of experts with the scientific and organizational tools for controlling PRRS and other future livestock pathogens. One of the four essential problems in PRRS in the U.S. that they want to address is the development of diagnostic and monitoring tools. They are calling for the development and delivery of more effective tools for on-farm PRRS diagnosis and prevention. An on-farm serological test that can detect and quantify antibodies from serum and whole blood in individual animal will achieve the goal of determining the infection status. The development of PRRS on-farm serological tests will provide an alternative to laboratory-based serological tests that require sample collection, appropriate submission protocol, mailing time, huge diagnostic cost and lag period to obtain the results. Thus, the PRRS gel-enzyme immunoassay (gel-EIA) and PRRS radial immunodiffusion enzyme assay (RIDEA) were developed and evaluated.

Materials and Methods:

Details of the viral strain, cell culture, antigen and test plate preparation and test procedures will be presented at the congress. Experimentally-infected sera were collected for the evaluation and the comparison of serial detection of PRRSV antibody through PRRS ELISA and gel-EIA/RIDEA. In that experiment, fifty-four 6-8 week-old PRRSV-naïve pigs were divided into 4 groups. Pigs from group A were inoculated oronasally with 3ml of PRRSV VR-2332 ($10^{4.0}$ $TCID_{50}$ /ml) at day 0. At 7 day post-inoculation (dpi), 24 pigs from group B and 12 pigs from group C were vaccinated intramuscularly with 2 ml of Ingelvac® PRRS MLV. Twelve pigs from group C were vaccinated again at 37 dpi. Twelve pigs from group D were vaccinated with placebo and served as controls. Blood samples were collected on days 0, 7, 14, 37 and 67 for serial detection of PRRSV antibody through PRRS gel-EIA and/or RIDEA and ELISA. Furthermore, sera from 7 PRRS-naïve farms and sera from different endemic farms were collected. Over-all, the sensitivity and specificity evaluation of gel-EIA used 709 serum samples while the gel-EIA used a total of 366. All gel-EIA/RIDEA tests were conducted with control positive and negative sera.

Statistical Analysis: The agreement between ELISA and gel-EIA methods of serodiagnosis was assessed using percentages of agreement and kappa analysis while ELISA and RIDEA relationship was evaluated by bivariate correlation coefficient. An ROC curve was

used to plot the true positive rate against the false-positive rate to evaluate the positive cut-off value (SPSS 12 statistical software for windows).

Results

Gel-EIA- At 7 dpi, all of the infected pigs, both vaccinated and non-vaccinated remained ELISA-negative, while 3/48 were positive on gel-EIA test. At 14 dpi, only 91.7 % (44/48) became ELISA-positive, while 97.9% (47/48) were gel-EIA positive. Up to the last blood collection at 67 dpi, all inoculated pigs, both vaccinated and non-vaccinated remained ELISA and gel-EIA positive except for two pigs that became ELISA negative at 67 dpi but still remained gel-EIA positive. With the optimal incubation period of 3 hours at room temperature (25°C), sensitivity was 100% (248/248). Specificity using the PRRSV-free samples as the true negative was 99.7% (299/300) however it was reduced to 95.6% (154/161) if we considered ELISA-negative as our true negative. Kappa statistics results indicated a very high 96.9% agreement between ELISA and Gel-EIA values (n = 709, P < 0.001).

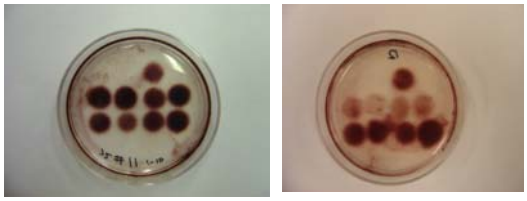

Figure 1. Gel-EIA evaluation - with only 3 hours incubation positive serum samples show distinct red zones compared to a known negative from a PRRSV-free reference sample.

Figure 2. Gel-EIA color results with known negative (from a PRRS-naïve farm) and a known ELISA and IFA positive

1. ELISA S/P= 0.308
2. ELISA S/P= 0.230
3. ELISA S/P= 0.552
4. ELISA S/P= 0.517
5. ELISA S/P= 1.23
6. ELISA S/P= 1.43
7. ELISA S/P= 2.59
8. ELISA S/P= 2.08

RIDEA- The specificity of RIDEA test was calculated to be 100% (46/46 PRRSV-naïve sera) and 96.2% (126/131 ELISA-negative sera). Sensitivity was 100% for the experimental sera (139/139) and also 100% for the laboratory-sourced sera (50/50). The ROC curve basically showed that at a chosen cut-off point of 7 mm and greater as positive, the sensitivity (true positive rate) is 100% and the specificity (false positive rate) is 99.95 %.All 46 PRRSV-naïve farm sera (100.0%) had diameters of <7.0 mm by RIDEA, while only 126 of 131 (96.2%) ELISA-negative sera had diameters of <7.0 mm. On the other hand, 189 of 189 (100.0%) ELISA-positive sera had the diameters of ≥7.0 mm in RIDEA. Bivariate correlation coefficient between ELISA S/P values and RIDEA diameters (mm) of all the samples showed a very linear relationship and high correlation (r = .803, n = 366, p < 0.01).

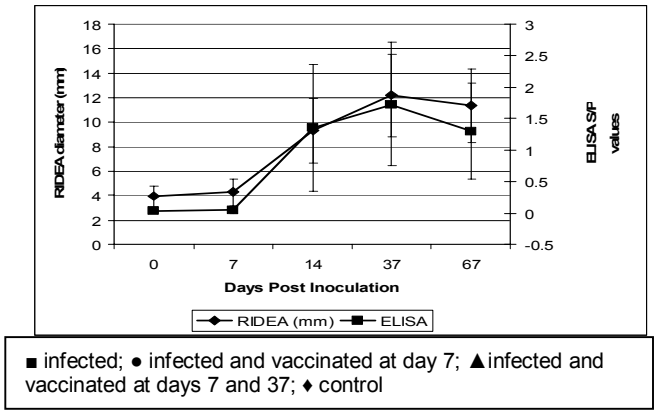

Figure 3. A plot showing the correlation of the means and the standard deviation of the PRRSV antibodies based on ELISA S/P and RIDEA (mm) values of the experimental serum samples

Discussion:

Non-infectious, detergent-extracted proteins particularly of the glycoprotein 5 (GP 5 or ORF 5) and nonglycosylated, membrane-associated proteins (ORF 6) with fair amounts of nucleocapsid proteins were the antigen coated in the PRRS gel-EIA and RIDEA test plates. The failure of the ELISA to detect antibodies in some of the infected animals causing the specificity of both gel-EIA and RIDEA tests to decrease when ELISA-negative samples were considered true negative, could be explained by a lack or under-representation of ORF 5 product in the antigen.

The ELISA most likely detects ORF 7 and 6 epitopes, which are the most abundant antigens in the virion, whereas gel-EIA and RIDEA detect majority of the antibodies to the ORF 5 product. The antibodies to ORF 5 appear after the disappearance of the viremia[2] and also persists up to 341 days[3] compared with ORF 6 and ORF 7 products[1]. This could have explained the early detection in PRRS gel-EIA and PRRS RIDEA and also caused some of the experimentally-infected samples to be ELISA-negative at 67 dpi. This could have also suggested that both gel-EIA and RIDEA are more sensitive tests than PRRS ELISA. Furthermore, because the antigen coated is a detergent-disrupted sub-unit of the PRRSV virion, infectivity is not associated with this antigen allowing use without safety concern for the spread of the infectious virus.

With the PRRS gel-EIA, veterinarians can collect the serum and within 4 hours, they can already determine the serological status of the individual pigs they are testing. Furthermore, confirmation of the increasing or decreasing antibody levels can be obtained overnight through the PRRS RIDEA. These tests have been evaluated in Fairmont Veterinary Clinic (Fairmont, Minnesota) from March, 2004 to January, 2005. Veterinarians and producers whose herds were tested agree that these on-farm serological tests will be valuable screening tools to detect PRRSV-status of replacement gilts and boar studs. These will also be valuable tools to detect if sows are producing negative-weaned pigs. A rapid, on-farm test has an increasing ramification in the current production practices and systems particularly in PRRSV acclimatization through controlled infection and animal mixing in the farms. The PRRS gel-EIA can accurately detect successful infection and the PRRS RIDEA can efficiently quantify antibody levels to measure successful acclimatization. Since the test is rapid and simple, particularly the PRRS gel-EIA, veterinarians can be decisive in mixing weaners from different sows and herds into pens if proven negative by the on-farm test. In the current system where production flow often dictates output, waiting for laboratory serological results before decision to mix or accept outside pigs may not be the best help the farms need.

Furthermore, these on-farm tests are not only developed to be rapid and accurate but are also designed to be relatively cheaper than the other serological tests. The goal is to come up with a test that will only cost around $0.25/serum or blood sample tested. For all the benefits that these tests can give to the U.S. swine industry, the simplicity, the affordability and the capacity as on-farm tests will definitely make these very valuable diagnostic tools in the developing world. With basic serological tests not readily-available, not routinely practiced or are simply expensive for most of these countries' farms, PRRS gel-EIA and PRRS RIDEA will make good alternatives and practical solution to their diagnostic problems.

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AN UPDATE ON PRRS PREVALENCE IN THE PHILIPPINES II. VISAYAS AND MINDANAO

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Introduction and Objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) prevalence has been explored as early as 1995-1996[1], showing a 64.71% seropositive status from 170 herds tested. A previous study[2] reported a 100% seropositive status on 46 farms involving 2,441 samples. Seventy six percent (76%) of the samples were seropositive. This involved farms tested from a period of January 2000 to September 2001. This paper aims to determine the seroprevalence for PRRS of farms particularly in Visayas and Mindanao (Vis-Min) regions.

Materials and Methods

A total of 3,396 serum samples from 52 farms in Visayas and Mindanao, the middle and southernmost islands of the Philippines were tested for PRRS ELISA. The samples included in the study were taken from July 2000 to May 2004. Sampling for majority of the herd is based on the number of animals in the farm having a 95% confidence level and an assumed prevalence of 10-20%. The proportion of samples tested that were seropositive and seronegative was determined. A seropositive farm indicates the presence of even a single animal resulting to a positive ELISA result.

Results and Discussion

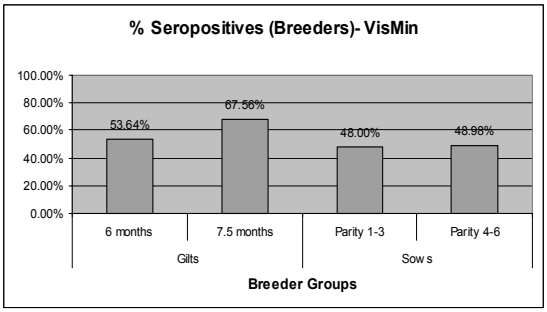
Table 1. Proportion of total samples and farms seropositive for PRRS virus in Visayas & Mindanao

	Positive	Negative	Total
Samples Tested	48%	52%	3,396
Farms Tested	65%	35%	52

Forty eight percent (48%) of the serum samples tested were seropositive. This means that a total of 1,630 animals from which the sample was taken had been exposed to the PRRS virus. On a per farm basis, a total of 34 are seropositive for PRRS.

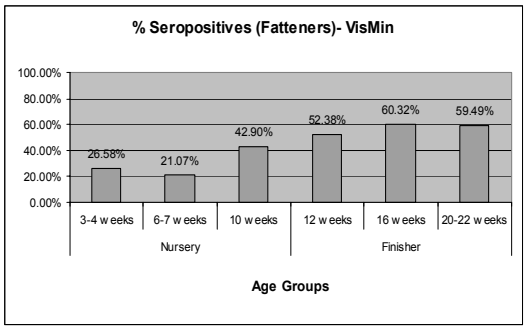
Just like in Luzon, a higher prevalence was seen in replacement gilts compared to sows (Figure 1).

Figure 1. Percentage seropositive in Vis-Min breeder herds.



Majority (>50%) of the seroconversions starts at 12 weeks of age, again progressively increasing to market age. A similar pattern in Luzon

Figure 2. Percentage seropositive in Vis-Min fattening herds.



The prevalence in Vis-Min is lower relative to Luzon. An effective biosecurity measure is more feasible. However, due to the nature of the virus and its epidemiology, PRRS still needs to be controlled and prevented.. In a PRRS-initiated PRDC, as a primary pathogen in the complex, vaccination with a modified live vaccine has been proven effective in controlling the complex infection[3] .

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Serological prevalence of porcine reproductive and respiratory syndrome virus by IFA in 2004 Korea

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Introduction

In 2003 and 2004, extensive outbreaks of Postweaning Multisystemic Wasting Syndrome (PMWS) throughout the country produced major losses in the swine industry in Korea. Especially, very pig dense areas suffered from PMWS-associated losses. As described in many publications (Kim 2003, Calsamiglia 2003, Rathjen 2004) Porcine reproductive and respiratory syndrome (PRRS) has been suggested a very important infectious co-factor of PMWS. In Korea, ELISA or IFA methods have been routinely used for serological tests of PRRSV infection. This paper has been reported the seroprevalence for PRRSV in Korea in 2004 by profiling 153 farms using IFA.

Materials and Methods

A total number of 2665 blood samples were taken in different age groups from 153 swine farms throughout Korea. Distribution of samples to age groups was determined individually based on the farm situation, pigs were selected at random. In this investigation, none of the farms previously used a PRRS vaccine, but, most of them had a history of PRDC or PMWS related problems. Samples were processed to sera, and sera were tested at the Department of Veterinary Medicine, Seoul National University using a well established IFA to PRRSV antibody.

Results and discussion

Table 1 shows the summary of seroprevalence for all farms. Despite none of the farms have been used a PRRS vaccine, but all farms were revealed seropositive.

The results by age groups resembles the previous report that was published in 1st Asian Pig Veterinarian Society Congress(APVS) in Korea 2003 (a seropositive proportion of samples tested for PRRSV, total : 61.48%, sows : 55.28%, suckling piglets : 45.28%, weaned piglets : 53.18%, growing pigs : 83.33%, finishing pigs: 78%, K Jeong APVS 2003).

In most Korean farms, piglets are weaned at 20-25 day-olds. As shown in Figure 1, seroconversion for PRRSV starts between the age of 40 and 60 days. This indicates an infection of piglets relatively early in the nursery. PMWS problems in Korea usually occurs at the age of 6-9 weeks. Thus, the infection and serconversion for PRRSV coincides with the outbreak period of PMWS.

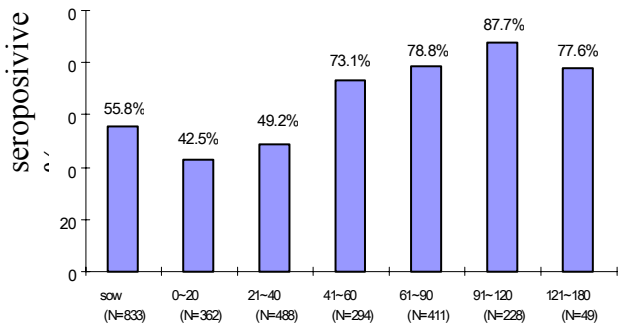
Improvement of management is the most important factor in order to control the PMWS-related problems. However, PRRS has been suggested as a major co-factor in PMWS problems in Korea, thus, a lot of farms have been successfully reduced PMWS-related problems by vaccinating against PRRSV using a US-strain based live vaccine (Ingelvac® PRRS MLV). As infection with PRRSV happens early in the nursery, the vaccination of piglets is often done at an early age (10-14 days of age). Using a modified live vaccine will induce immunity quickly and overcome the maternal antibodies that are still present at that age.

In order to compare Korean serology results for PRRSV with other publications more directly, future investigations will use a commercially available ELISA test kit.

Table 1: Proportion of total samples, sows, piglets and farms seropositive for PRRS virus in Korea

	Total	Positive(%)	Negative(%)
Samples tested	2665	1636(61.4)	1029(38.6)
Sows	833	465(55.8)	368(44.2)
Piglets	1832	1171(63.9)	661(36.1)
Farms tested	153	153(100)	0(0)

Figure 1: Proportion of seropositive sows and pigs for PRRSV



day

Reference:

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Muntinlupa city, Philippines, August 25th, 2005.

Co-infection interaction data between responsible pathogens of PRDC cases in Philippine pig farms

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Abstract

Different pathogens are involved in the already well-known PRDC (Porcine Respiratory Disease Complex). For this reason knowing the most usual pathogenic co- infections hat trigger different PRDC cases may help us to adjust vaccination protocols and treatments for aiming towards the most responsible pathogens in order to control more properly PRDC in our farms. So, in order to disclose these most usual co- infections in the Philippine pig farms we carried out the analysis of 39 (30 at the time the abstract was rendered to APVS organisation) different serum screening results and clinical observations from these 39 different Philippine farms suffering from PRDC. The pathogens included in such co- infection analysis were *PRRSV, SIV, Mycoplasma hyopneumoniae, Aujeszky virus disease, Actinobacillus pleuropneumoniae and Haemophilus parasuis and Circovirus type-2*.

Introduction

During the period in time from January 2004 to August 2005 we compiled the analysis of 39 Philippine pig farms, which suffered from respiratory problems in pigs or in other words, they suffered from what is well-known by the entire pig industry as PRDC (Porcine Respiratory Disease complex). Thus, through this analysis we wanted to disclose some quite interesting data about either PRDC most frequent appearance timings or most frequent pathogens involved in the PRDC cases or also the most frequent associations of pathogens (co- infections), which triggered such PRDC cases. The results herein may be helpful for veterinary practitioners to decide general vaccination protocol timings as well as evaluate more consistently about what pathogens require more attention in the different periods in life of commercial pigs. One of the farms, which suffered from a *HCV* case, was included in the study since it was considered wrongly at the beginning of its manifestation as another PRDC case.
Material and methods

Characteristics of the analysed pig farms: The 39 Philippine pig farms included in this study were farms that were applying the farrowing to finish production system (either breeders or pigs in the same area close to each other), in the best of the cases there was established a 2 site system where pigs were moved away from the main farm unit (breeders and piglets) at the time to set up the fattening period (11-12 weeks of age). The farm size from each farm was at least of 500 reproductive sows.

PRDC appearance timings: In order to establish a most easy way to understand and allocate the timings of appearance of PRDC cases we divided all the production life of pigs from weaning to slaughtering in four main periods; 4-7 weeks of age, 7-11 weeks of age, 11-15 weeks of age and 16 weeks of age or onwards. Certainly the decision to adapt this age periods bases on the four critical production periods in commercial pig rising. Certainly veterinary practitioners also usually use such four age periods in their cogitations to obtain a first previous diagnostic based just on field observations. So

these 4 periods of age would correspond to the early post-weaning period (4-11 weeks), the late nursery period (7-11 weeks), the early fattening or growing period (11-15 weeks) and finally the fattening or late fattening period (16 weeks and onwards).

Laboratory techniques applied:
In the 39 farms was runt a serum screening or serum profile. Serum samples from pigs from the same farm at different ages were analysed with different ELISA kits. Due to this study is based on a compilation of real PRDC cases the groups of age in every selected farm were not specifically the same but in the 39 cases the groups of age selected were involving the four main groups of age that we are considering herein for allocating each one of the PRDC cases. Aside from serological techniques we used eventually other lab techniques as well as other veterinary procedures in order to confirm or clarify in any specific case a pathogen diagnosis or identification (described below).

The most usual groups of age used in the 39 farm serum screenings were as following:

4 weeks of age, 7 weeks of age, 10-11 weeks of age, 15-16 weeks of age and 21 or more weeks of age. Only in some cases groups of age could be slightly different from ones described above but always they were delimiting the borders of the 4 main age periods in pig life herein studied. The number of serum samples per group of age in each farm serum screening fluctuated from 8 to 15 samples.

Summary of techniques and veterinary surgeon proceedings used for identify the responsible pathogens in each PRDC.

	Serology (Elisa kits)	PCR	Bacteria Isolation	Clinic signs	Necropsy findings
PRRSV[1]	X	X		X	X
H.parasuis[2]			X	X	X
Swine Influenza	X			X	X
M.hyopneumoniae[3]	X			X	X
Aujeszky virus	X	X		X	X
A.pleuropneumoniae[4]	X		X	X	X
PCV-2[5]		X		X	X
Hog Cholera virus	X			X	X

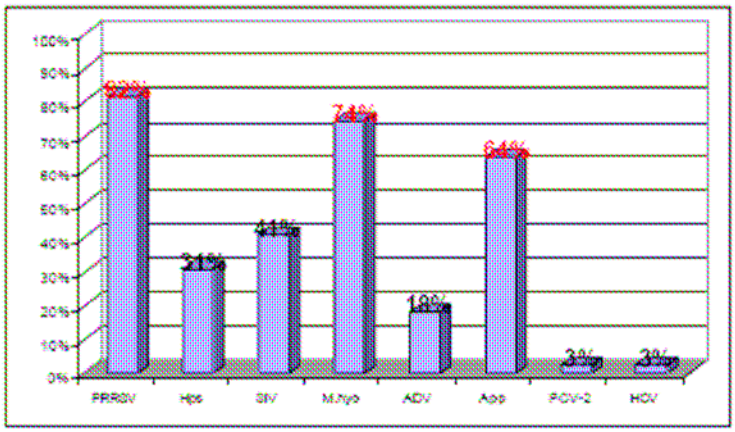
[1]Porcine reproductive respiratory syndrome virus, [2]Haemophilus parasuis, [3]Mycoplasma hyopneumoniae, [4]Acinobacillus pleuropneumoniae, [5]Porcine circovirus type 2.

Obviously, in each one of the cases the most valuable source of information in order to evaluate each PRDC case was the voice and expertise from the veterinary surgeon that was in charge.

Results

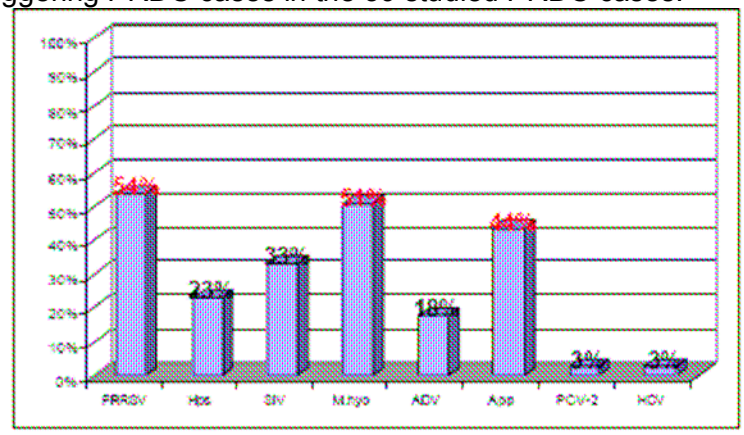
1. General results

Graphic.1.1. Prevalence of possible PRDC triggering pathogens present in the 39 monitored farms.



PRRSV: *Porcine reproductive respiratory syndrome virus*, Hps: *Haemophilus parasuis*, SIV: *Swine Influenza virus*, M.hyo: *Mycoplasma hyopneumoniae*, ADV: *Aujeszky virus*, App: *Actinobacillus pleuropneumoniae*, PCV-2: *porcine circovirus type 2*, HCV: *Hog Cholera virus*.

Graphic.1.2. Prevalence of pathogens that were considered directly responsible of triggering PRDC cases in the 39 studied PRDC cases.



PRRSV: *Porcine reproductive respiratory syndrome virus*, Hps: *Haemophilus parasuis*, SIV: *Swine Influenza virus*, M.hyo: *Mycoplasma hyopneumoniae*, ADV: *Aujeszky virus*, App: *Actinobacillus pleuropneumoniae*, PCV-2: *porcine circovirus type 2*, HCV: *Hog Cholera virus*.

As it is observed in these results the identification of a pathogen as a real responsible of a PRDC case or just a mere habitant of the farm is essential in PRDC in order to have a clue about against what pathogen we should do any effort. Therefore, just pathogen detection in the farm is not enough to determine the main approach to be applied to fix any PRDC case. In fact, the most of the times some pathogens are almost always present in the farm but they could be doing none role at all in the moment of main respiratory distress.

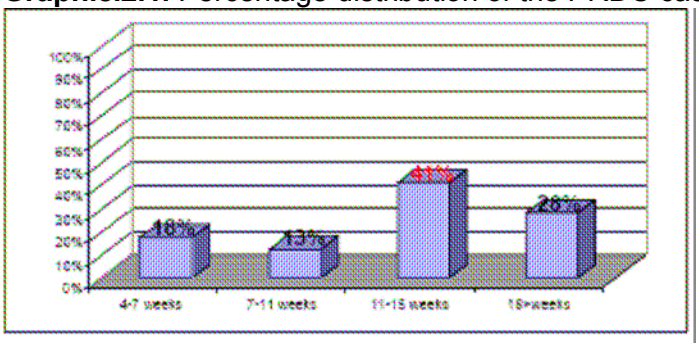
So, it is obvious also that serum screening may help us in each case to determine which pathogens are decisive. For instances in table 1.1 we can observe that PRRSV is present in the 82% of the studied farms but is really being a triggering PRDC factor only

in the 54% of the PRDC cases. This is mainly interesting to take into account at the time to decide which treating approach should be aimed. Obviously doing anything against a non-active PRDC pathogen will not improve probably that specific PRDC case.

Table 1.2. showed also clearly that the most frequent pathogens related to PRDC cases were *PRRSV*, *M.hyo, and App*. Anyway, although in third position, the quite high prevalence of *SIV* should be highlighted. Undoubtedly it is a pathogen that seems to be taking relevancy in Philippines as well as in other Asian pig producer countries lately.

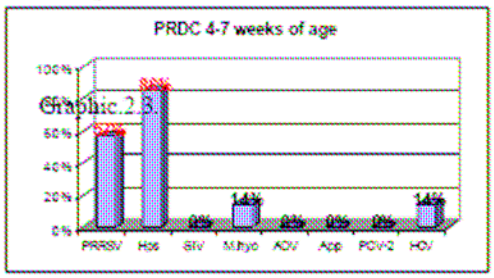
2. Results in each age period.

Graphic.2.1. Percentage distribution of the PRDC cases in the four age periods

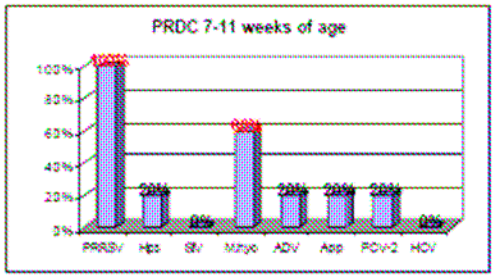


Graphics. 2.2; 2.3; 2.4; 2.5. Prevalence of pathogens involved in the PRDC cases occurred during each one of the delimited four age periods.

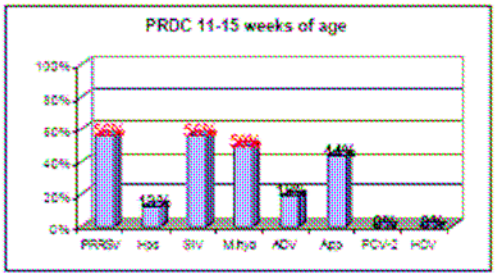
Graphic.2.2.



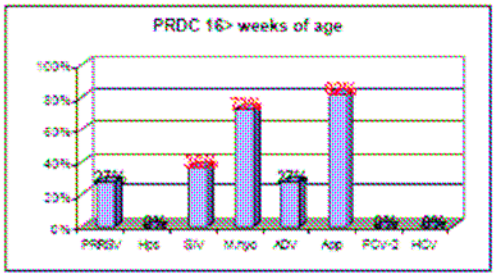
Graphic.2.3.



Graphic.2.4.



Graphic.2.5.



The most of the PRDC cases occurred in the age period from 11 to 15 weeks of age (41%). It is not a very unexpected result since almost the most of the farms were quite old farms, which used Farrowing to finish system in such away that breeder population was already highly immunized against the most of the pathogens. This factor induces the synthesis of colostrums plenty of antibodies. Obviously these antibodies can protect during the most of the time in the nursery period but they are totally waned at the time

the animals are moved to fattening units (at around 10-12 weeks of age). At that precise moment pigs are more usually exposed and challenged by the most of the respiratory pathogens. Anyway when results are analyzed according to each age period there are very interesting results to highlight.

In the first period (4-7 weeks, Graphic.2.2.), the most frequent responsible pathogens are clearly two; *PRRSV* and *H.parasuis. H.parasuis* was found in the most of the cases at this first period and it was not always accompanied by *PRRSV*. So, *H.parasuis* it is not at all always playing a secondary infection role. Anyway it is also true that the coinfection

PRRSV with H.parasuis is the most frequent co- infection in this period and the most frequent co-infection within all the co- infections encountered in all the age periods. The only unexpected case was one involving *HCV*, as we mentioned before it was considered wrongly a respiratory problem at the beginning whereby it was included in this field study.

In the second period (7-11 weeks, Graphic.2.3.), permanent presence of *PRRSV* is remarkable since *PRRSV* is present in 100% of the PRDC cases. But more interesting result is the prevalence of PRDC cases where *Mycoplasma hyopneumoniae* was involved in this stage (60%). It means that very early *M. hyopneumoniae* infections may be occurring quite frequently whereby early vaccination programmes (7 and 21 days of age) are probably a more safe policy in order to protect already at these still quite early ages. During this period we observed the only PRDC case that associated *PRRSV* and *PCV-2* at the same time it was also the only case where *PCV-2* was taking part in a PRDC.

In the third period (11-15 weeks, Graphic.2.4.), *Swine influenza SIV* is getting the relevancy that *PRRSV* is losing. Certainly *SIV* is herein a PRDC component in the 50% of the cases. Simultaneity *M.hyopneumoniae* increases also its prevalence at this period. In this period there is an increasing relevancy of *A.pleuropneumoniae as well* and quite frequently *A. pleuropneumoniae* combines its action with *SIV*.

During the last period (16 weeks onwards) typical diseases from fattening units get more importance. Thus, in this period *A.pleuropneumoniae* is the pathogen more frequently involved in PRDC cases. *M.hyopneumoniae* is in second position and still very relevant *SIV* in 36% of the cases. The role of *Aujeszky virus (ADV)* is increasing in this period but it is present only in the 27% of the cases. Although through all the age periods *ADV* participation seems less important it doesn't mean surveillance against this virus should be relaxed. In fact in the entire population of farms were field *ADV* was detected this virus was always taking part of a PRDC case. Thus, this 100% of pathogenic efficiency respect to the PRDC should be considered.

3. Results. Association of pathogens (co-infections)

Table.3.1. Pathogen associations encountered in PRDC cases in four different periods.

Co-infections	4-7 w	7-11 w	11-15 w	16 or >	Total
ADV + APP				1	1
ADV + App + M.hyo				2	2
ADV + M.hyo			1		1
ADV + M.hyo + PRRSV		1	1		2
ADV + SIV			1		1
App + M.hyo			1	2	3
App + M.hyo + PRRSV			1	1	2
App + M.hyo + SIV			1		1
App + PRRSV		1	1		2
App +PRRSV + SIV			1	1	2
App + SIV			2	1	3
HCV[1]	1				1
H.parasuis	2				2
H.parasuis + PRRSV	3		1		4
H.parasuis + PRRSV + SIV			1		1
M.hyo + H.parasuis + PRRSV	1	1			2
M.hyo + PRRSV		1	1	1	3
M.hyo + PRRSV + SIV			2		2
M.hyo + SIV				2	2
PCV-2 + PRRSV		1			1
SIV*			1		1
Nº co-infections = 19[1]			Nº PRDC cases = 39		

*SIV was found the only strictly respiratory pathogen that was considered the only one responsible from a PRDC case. [1]The suspected PRDC case provoked by HCV or the one provoked by SIV alone are not added as co-infections.

As we can see in this table above the most of the co- infections have at least a virus component (*ADV, PRRSV, and SIV*). In fact the only association of pathogens responsible of PRDC without a virus component was the association *APP* + *M.hyo* (in the last two age periods). So viruses seem to be almost essential to trigger a PRDC. The co-infections that globally are repeated more frequently are *M.hyo-PRRSV, H.parasuis-PRRSV, App+SIV and App+M.hyo.* Basically *H.parasuis+PRRSV* association is only occurring during the first age period meanwhile the combination *M.hyo+PRRSV* seems to have a less preferable timing. In the case of the associations *App+SIV and App+M.hyo* seem to show preferable appearance timings. Certainly, it seems that these two co- infections would occur only during the last two age periods but not before. As we already mentioned *SIV* seems to have more relevancy in the third age period. Besides this, *SIV* shows an increasing tendency to joint *App* alone or accompanied by another pathogen. Maybe this could be the source of under diagnosed cases of *SIV*, which could be confused as merely *App* cases. In fact, a previous *SIV* serological survey in Philippines (Bruguera, S.D., and Torres, M.I. in the PVMA convention 2005) disclosed that *SIV* was both probably under diagnosed and dismissed as a frequent respiratory trouble maker in the growing- fattening units. This same study found that 64% of the fattening units from a specific population of Philippine farms (farms size of at least 500 reproductive sows) had been in contact with SIV at anytime during the fattening period.

References:
References and questions are available from the author on request.

NOTE: Most of the samples were analysed in the facilities of Laboratory Diagnos©, HIPRA Philippines, INC (Philippines). But some few samples were analysed for the first time or confirmed in Laboratory Diagnos©, Laboratorios HIPRA, S.A. (Spain).

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Foot and Mouth Disease Control in Southeast Asia

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ABSTRACT

The OIE Southeast Asia Foot and Mouth Disease (SEAFMD) Campaign coordinates the regional control of Foot and Mouth Disease in Southeast Asia. This paper describes the eight components of the SEAFMD Campaign and the strategies adopted to control this disease, and the achievements made in implementing progressive zoning in the Malaysia-Thailand-Myanmar Peninsula, Lower and Upper Mekong Zones. It also describes the transition arrangements to ASEAN in January 2006, to harness greater support from member countries and ensure long-term sustainability of the program.

The current status of FMD in the region is also presented. The FMD serotypes present in the region are types O, A and Asia 1. Type C has not been reported since 1995. Type O strains have been the most common and there are several topo-types present. These include the Southeast Asia and the Pan-Asia topo-types reported mainly in Vietnam, Thailand, Myanmar, Malaysia, Lao and Cambodia and the Cathay (pig adapted strain) topo-type has been reported from the Philippines and Vietnam. Type A has been reported in several countries and there has been a recent upsurge in outbreaks due to type A in Thailand and Malaysia. Lao PDR reported its first case of type A in 2003 but was immediately put under control by January 2004. Vietnam reported its first case of type A last August 2004 and continued up to present. Type Asia 1 has never been reported in the region for more than 3 years.

The temporal and spatial distribution of FMD virus Types from 2001 to 2004 is presented. The risk associated with the spread of FMD virus to animal movement in the region is also presented. The outbreaks of FMD Type A in Lao PDR and Vietnam is most likely brought by infected cattle.

THE IMPORTANCE OF EARLY GROWTH

APVS CONGRESS
MANILA, THE PHILIPPINES

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The young growing farm animal has very specific nutritional needs during the critical early days and this applies to lambs, calves, piglets and even chicks.

There are specific needs during the early days of life in terms of energy, protein and amino acids that are essential if the young growing animal is to get over this hurdle. Moreover, the vitamin and mineral requirements are crucial to establish a strong skeletal framework from which to allow the rapid development of lean tissue later on.

What has become obvious from work at a variety of research centres around the world is that when the young growing animal loses early growth because of under-nutrition or due to sub-optimal health status, in many situations, **it never gets this growth back.**

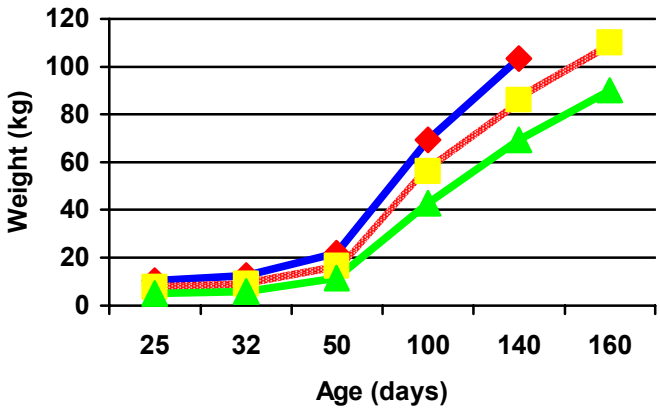
Young ruminant animals such as calves and lambs can show compensatory growth to an extent which allows them to get back to their true genetic potential but even ruminants, if subjected to a severe loss of growth, will ever after remain on a lower growth curve. All of this means the number of days to slaughter is extended significantly.

Pigs are particularly adversely affected by loss of growth in the period immediately after birth and also in the period after weaning. This latter phase when they have to make the difficult transition from sows' milk to a dry pelleted diet with different protein sources, is especially pivotal in growth terms.

It is therefore important in production terms to ensure a smooth transition and provide the quality of nutrition that keeps feed intake and growth moving on up. SCA Nutrition using their Green Hill Farm unit in Northern England have investigated growth curves in 5000 young weaned piglets fed and weighed these pigs individually all the way through to slaughter.

The results from this study are shown in Figure 1 below.

Figure 1



Size effects from weaning to slaughter

Green Hill Farm
The range in weaning weights in the Green Hill Farm study were from 3.5 kg to 12 kg. At these extremes of size at weaning, the difference in days from birth to slaughter was about 40 days. For a 1 kg increase in weaning weight on average, another 100 g/d increase in post-weaning daily liveweight gain was seen and this reduced the age at slaughter by 10-12 days.

Clearly, size and post-weaning growth were very important determinants of ultimate growth potential to slaughter. The bigger the pigs at birth as a result of better sow feeding produced a bigger pig at weaning and this was helped by an appropriate creep feeding programme. If this is then followed through with a high quality post-weaning diet programme, then this is the most appropriate way to capitalise on the genetic potential of modern pigs. A quality diet programme in the early days after weaning then represents a real investment to the business for the future growth and efficiency of the finishing pigs.

This therefore epitomises the SCA nutritional philosophy – the provision of high quality nutritional expertise and products to sustain profitable animal production.

Foot and Mouth Disease Eradication Program
Victor C. Atienza

The FMD Campaign in the country is showing signs of success with the first sign of absence of FMD reports after 10 years. The last FMD outbreak reported in the country was last 27 April 2005 in a slaughterhouse. Outbreak level as of 8 August 2005 remains at 37 compared to 80 of the same period last year. The effort to an FMD-free status is a multi sector effort. Support from the livestock industry players, local chief executives, media practitioners and other government offices such as Philippine National Police (PNP) and Philippine Information Agency (PIA) have been crucial including the financial support from the Australian Government.

The National Foot and Mouth Disease Task Force is intensifying its campaign against FMD even with the apparent FMD-freedom running on its fourth month. Among the intensified activities include monitoring and surveillance; public awareness activities; animal movement management by maintaining animal quarantine checkpoints to safeguard FMD Protected and Free Areas; strategic ring vaccination in endemic areas; and the progressive zoning approach. The latter would be applied in Regions I, the Cordillera Administrative Region and other provinces in the regions classified as surveillance zones. Surveillance zones are areas that report FMD cases on sporadic basis with some areas already free from the disease for more than a year already.

These activities area aimed at totally eliminating clinical cases this year. It is foreseen that, most of the regions in Luzon would be locally declared as FMD-free regions or provinces. Regions II, IV-B and V have been locally declared FMD-free last June 2004. If FMD-freedom in the country is sustained in two years, Luzon could prepare for an international recognition of its FMD-freedom without vaccination.

HERD-LEVEL SEROPREVALENCE OF SWINE INFLUENZA H1N1 VIRUS IN LUZON, PHILIPPINES

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Introduction and Objectives

Commercial pig production in the Philippines is practiced in large scale farrow-to-finish operations with capacities of up to 10,000 sows, and in off-site fattening units. Seventy percent (2.5 million) of the commercial pig population in the country is concentrated in Luzon, the largest island in the Philippine archipelago. In commercial farms, pigs are kept indoors in half-open buildings with natural ventilation.

The presence of several viruses has been reported in pig farms in the Philippines (3). Classical swine fever virus is widespread and endemic in most farms (1, 2), but there is limited information on other viruses such as swine influenza (SI) viruses. In a small study conducted in 1992 in two provinces, infection with the SI H1N1 subtype was detected in two out of five farms tested (3). In the present study, the survey was updated and expanded by examining pigs from 40 farms from seven provinces in Luzon.

Materials and Methods

General features of the study population: The study was conducted in the provinces of Bulacan, Batangas, Rizal, Laguna, Tarlac, Pampanga and Quezon where 40, 24, 15, 4, 3, 2 and 1 percent, respectively, of the commercial pig population in Luzon are located. The first 3 provinces are high pig density regions while the remaining provinces are considered low pig density regions (Fig. 1).

Selection of farms: A list of commercial pig farms from each province was obtained. Subsequently, letters introducing the seroprevalence study were sent, or farm-owners/veterinarians were contacted by phone. The study was restricted to farrow-to-finish herds with more than 100 sows and were not vaccinating against SI. In each province, the number of herds selected for the study was proportional to the total number of farms in the province. The sample size of the study was limited to 40 farms. From each farm, blood samples from 10 fattening pigs of 20 to 24 weeks of age were collected. The study was done from December, 2003 to June, 2004.

Antibody detection: A total of 400 serum samples were examined for SI H1N1 antibodies using a commercial ELISA kit (Idexx Laboratories, Westbrook, ME) following the manufacturer's instructions. Samples with S/P ratios equal to or greater than 0.4 were considered positive. A farm was considered seropositive if at least 2 sampled animals were positive.

Results and Discussion

Sixty-three percent (25/40) of the farms tested were seropositive for SI H1N1 antibodies (Table 1). Seropositive herds were detected in all high pig density provinces (Bulacan, Batangas and Rizal), while 2 (Laguna and Pampanga) out of the 4 low pig density provinces, seropositive farms were not observed. In general, high pig density regions had a greater percentage of positive farms (60-75%) compared to low density regions.

Table 1. Herd-level seroprevalence of swine influenza HINI virus in seven provinces in Luzon, Philippines, 2003-2004.

Province	No. farms tested	No. seropositive herds	Percentage (%)
Bulacan	11	6	55
Batangas	11	9	82
Rizal	8	6	75
Laguna	0	4	0
Tarlac	3	3	100
Pampanga	0	2	0
Quezon	1	2	50
	40	25	63

* Herds are considered positive if at least 2 sampled animals are positive

The percentage of seropositive herds was highest (81%) in large farms (>601 sows), while percentages in medium (301-600 sows) and small (100-300 sows) farms were comparable (Table. 2).

Table 2. Percentage of swine influenza HINI seropositive herds in Luzon, Philippines (2003-2004), based on herd size.

Number of sows	No. farms	No. seropositive herds	Percentage (%)
>601	11	9	81
301-600	6	3	50
100-300	23	13	57

This study is the first extensive survey for SI H1N1 antibodies in commercial pig farms in the Philippines. The present findings indicate that farms seropositive for SI H1N1 are widespread in Luzon, Philippines.

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Secretion Expression of the Gene Encoding Classical Swine Fever Virus E2 B/C Antigenic Domain in Pichia Pastoris and Identification of the Protein

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Abstract:

Based on the fact that the envelope glycoprotein E2 protecting pigs from classical swine fever virus (CSFV) had two structural antigenic units B/C and A/D, we amplified the specific gene encoding B/C antigenic domain of E2 protein of CSFV, This PCR product of 261bp was inserted into secretory pPICZαC Pichia pastoris expression vector with AOX1 promoter and α-factor secretion signal sequence. After being linearized by digestion, the vector was transformed into Pichia pastoris X33 by electroporation to integrate with the genome-five transformants with high copies were acquired by screening with ZeocinTM and were induced with methonl.SDS—PAGE-Western blot and ELISA analysis showed that the supernatant of the induced P. pastoris culture contained protein E2 which had good reacting ability. Our findings provided a basis to develop sub-unit vaccine and diagnostic antigen.

Key words: CSFV; B/C antigenic domain; *Pichia pastoris*；Expression

PSEUDORABIES PREVALENCE IN THE PHILIPPINES II. VISAYAS AND MINDANAO

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Introduction and Objectives

The first, and most important part of the control of Pseudorabies is awareness. The use of the Enzyme- Linked Immunosorbent Assay (ELISA), particularly the gene deleted (gE type), have shown the capability of the test in identifying the infected animals [1,2]. Although the disease is being, or has been eradicated in some countries,
Seroprevalence studies from countries in Eastern Europe[3], Latin America[4] and Asia[5] have shown that the disease is still present. This paper attempts to discuss the awareness in terms of seroprevalence of Aujeszky's Disease in the Philippines particularly . Visayas and Mindanao (Vis-Min).

Materials and Methods

The study involved 52 farms from Vis-Min from the period of July 2000 to September 2004 with a total serum sample of 2,204 tested. Breeders (gilts and sows) as well as 12 week old fatteners to market pigs were tested in the study. A differential (gE which later became gI) ELISA test (Idexx Laboratories) was used for this purpose. The proportion of samples tested that were seropositive and seronegative was determined. A seropositive farm indicates the presence of even a single animal resulting to a positive ELISA result.

Results and Discussion

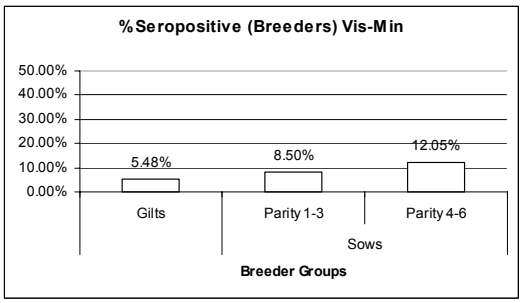
Table 1. Proportion of total samples and farms seropositive for Pseudorabies virus in Vis-Min

	Positive	Negative	Total
Samples Tested	32%	68%	2,204
Farms Tested	23.08%	76.92%	52

Thirty two percent (32%) of the samples tested and 23% of the farms tested in Vis-Min registered positive for the test. Out of 52 farms tested, 22 were considered positive for exposure to the field virus.

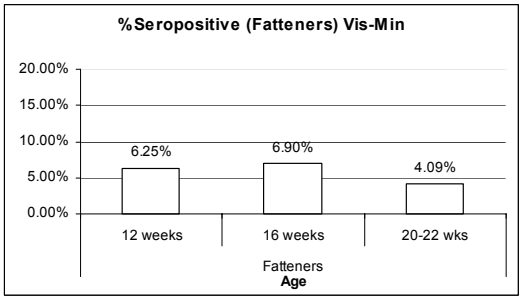
Similar to Luzon, the seroprevalence was relatively higher in the older sows (Parity 4-6) compared to young sows and gilts

Figure 1. PRV prevalence in the Vis-Min breeder herds.



The trend of seroprevalence in fattening, as in Luzon, is also decreasing with market age.

Figure 2. PRV prevalence in Vis-Min fattening herds.



In the Vis-Min region where prevalence is lower, relative to Luzon, preventing the entry of the virus through latenly infected pigs is cruicial. Coupled with a vaccination program[6], eradication can be achieved

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EFFECT OF A 20 kDa INTERFERON-STIMULATED GENE ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION

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Porcine reproductive and respiratory syndrome causes huge economic losses in swineherds worldwide. As such, much effort is geared towards finding ways to control or totally eliminate this disease. In particular, understanding the role of the host immune system is essential to PRRSV control and clearance. An ideal anti-PRRSV response is one that specifically targets the virus without adversely affecting the host. To this end, a recently discovered human interferon-stimulated protein, ISG20, was shown to have RNAse activity specific for single-stranded RNA viruses. Human ISG20 is made up of 181 amino acids and possesses three exonuclease domains. It is distantly related to RNAse T and the proofreading domain of bacterial DNA polymerase III. The purpose of this research is to determine if pigs express a functional ISG20 which will be useful for developing molecular-based strategies to control PRRSV and other viral infections in pigs. RT-PCR, using primers derived from conserved regions of human and mouse ISG20, were used to amplify ISG20 mRNA from porcine alveolar macrophages and porcine kidney (PK15) cells. The resulting cDNA was cloned and sequenced. Porcine ISG20 shows 96% nucleotide and 95% amino acid homology with human ISG20. Based on peptide sequence analysis, pISG20 possesses the same conserved domains as human ISG20 and should be totally functional. Current work is directed at expressing pISG20 in cell lines and determining its ability to inhibit PRRS viral replication.

A SEROLOGICAL SURVEY ON THE INCIDENCE OF SWINE INFLUENZA IN THE PHILIPPINES THRU ELISA H1N1

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Introduction
Swine Influenza is one of the many causes of swine respiratory disease complex which primarily due to various bacteria and viruses. It is caused by a number of closely related influenza A viruses that are noted for their ability to change their antigenic structure and create new strains. The only difference is that SI disease is very short, as little as 12-48 hours. SI can be introduced by infected people, carrier pigs and probably on the wind although this has not been proved.

Each serotype is identified by surface proteins referred to as H and N. There are three common strains that are known to affect pigs are described and they are H1N1 which is the most common, H1N2 and H3N2.

Clinical Signs:
When the virus first enters the herd two or three animals may be observed sick from the first two days, followed by a rapid explosive outbreak of inappetence and clinically very ill pigs. The side effects on the reproductive system follow the sudden onset of a rapid spreading respiratory disease with coughing, pneumonia, fever and inappetence. Acute respiratory distress persists over a period of 7-10 days. There are three important periods when infection causes infertility. First if sows are ill in the first 21 days post service that result to return to heat. Second, if infection occurs in the first five weeks of pregnancy, it may results to embryo mortality and adsorption and sows become pseudo pregnant. The third major is its effect in boars where high body temperatures affects semen and depress fertility for a 405 weeks period.

The Survey:
At the time of this study only H1N1 Elisa test is made available by IDEXX so getting to know its incidence in the Philippines was the primary objective especially that only few farms then were vaccinating. It was made sure that all farms subjected for the survey were not giving any swine influenza vaccine

Objective of the Study:
To determine the incidence of swine influenza H1N1 among 19 different location farms in the Philippines thru IDEXX Elisa.

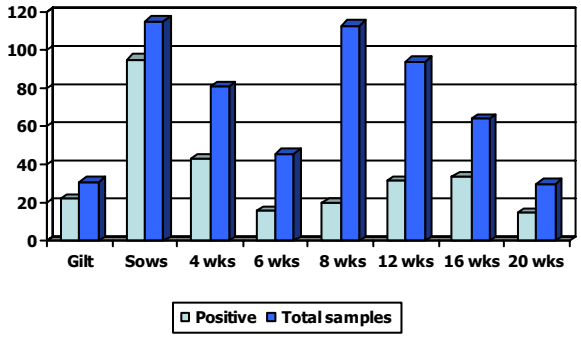
Materials and Methods:
In September 2003, the technical services group of Schering-Plough started to identify farms that would be willing to undergo survey thru blood collection of various ages. For equal representation we agreed to get samples from Gilts, Sows/ boars, 4 weeks, 8 weeks, 12 weeks, 16 weeks and 20 weeks old pigs. The study ends up last October 2004. Farms identified were having 300 sow populations and the highest was 4,000 sows and they were located in North Luzon, South Luzon, Visayas and Mindanao. An average of 25-35 samples was taken from each farm representing various ages for total of 574

samples. Processed serums were brought to Lipa Quality Control Center (LQCC) for Elisa test.

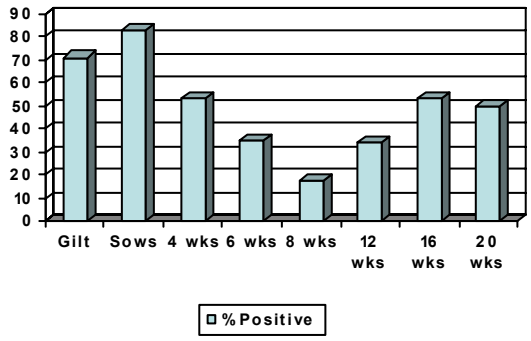
Interpretation data:
Elisa comes out with the following interpretation; S/P ratio below 0.4 is considered negative to Swine Influenza H1N1 antibody while above 0.4 is considered positive to SI antibody.

Results and Discussion:

1. Survey of the 19 farms tested -
   Result: 48% of the blood samples were positive to
   H1N1



2. Survey of 19 farms tested by stage affected – Results of positive cases: Gilts 71%, Sows/Boars -83%, 16-20 wks -50%



Conclusions:

1. There are close to 50% of positive cases seen of the 574 samples tested.
2. Most stage highly affected are gilts, boars and sows and grower-finisher stages
3. The main island of Luzon gets the higher % positive cases at 56%

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PSEUDORABIES PREVALENCE IN THE PHILIPPINES I. LUZON

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Introduction and Objectives

The first and most important part of the control of Pseudorabies is awareness. The use of the Enzyme- Linked Immunosorbent Assay (ELISA), particularly the gene deleted (gE type), have shown the capability of the test in identifying the infected animals [1,2]. Although the disease is being, or has been eradicated in some countries, Seroprevalence studies from countries in Eastern Europe[3], Latin America[4] and Asia[5] have shown that the disease is still present. This paper attempts to discuss the awareness in terms of seroprevalence of Aujeszky's Disease in the Philippines particularly Luzon.

Materials and Methods

The study involved 167 farms from Luzon, the northernmost part of the Philippines from the period of March 2000 to June 2004 with a total serum sample of 6,438 tested. Breeders (gilts and sows) as well as 12 week old fatteners to market pigs were tested in the study. A differential (gE which later became gI) ELISA test (Idexx Laboratories) was used for this purpose. The proportion of samples tested that were seropositive and seronegative was determined. A seropositive farm indicates the presence of even a single animal resulting to a positive ELISA result.

Results and Discussion

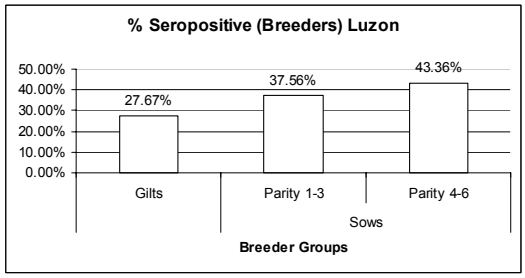
Table 1 shows the prevalence in the samples and farms tested for Luzon. One thousand eight hundred and two (1,802) which accounts for 28% of the total samples registered positive for the test. Out of 167 farms tested, 97 were considered positive for exposure to the field virus.

The seroprevalence was relatively higher in the older sows (Parity 4-6) compared to young sows and gilts

Table 1. Proportion of total samples and farms seropositive for Pseudorabies virus in Luzon

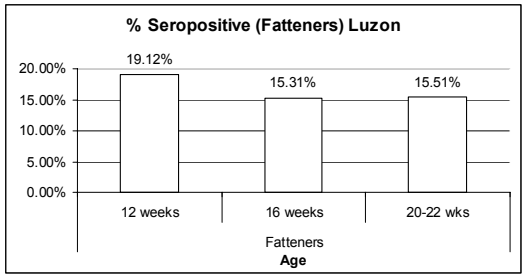
	Positive	Negative	Total
Samples Tested	28%	72%	6,438
Farms Tested	58.08%	41.92%	167

Figure 1. PRV prevalence in the Luzon breeder herds.



The reduction in seropositive replacement animals can be appreciated more with the fact that more finishers (figure 2), from which gilts mostly come from, also have a low seroprevalence.

Figure 2. PRV prevalence in Luzon fattening herds.



Inasmuch as eradication has been done in other countries, in Philippine farms, this has also been achieved on a herd basis[6]. Assuring PRV-free replacement animals and vaccination to reduce latent infection would be key.

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Production and Pharmacology

Agalactia syndrome during the second week of lactation

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Introduction

Parturition constitutes a critical phase in the sow due to physical, physiological and psychological reasons. The parturition of the sow is frequently affected pathological events which may affect both the sow and the piglets. The following symptoms may present: hyperthermia, anorexia, vaginal discharges, mastitis, agalaxia, or hypogalaxia in the sow; and diarrhoea in the piglets during the first days, always accompanied by fever in the sow (1, 2, 3). Agalactia syndrome in primiparous and multiparous sows remains to be of economic importance in breeding herd. The primary clinical importance of this syndrome is related to the affected sows and impaired performance of weaning piglets, e.g. increased piglet mortality, elevated sensitivity to infections and significant reduction of piglet growth (4). Multi-risk factors, such as infectious diseases, mycotoxin, *environmental stress and poor prevention programme for MMA* have been suggested to increase number of sow with agalactia syndrome in farrowing unit. Although, complete agalactia is the final and most severe consequence of this lactation failure, early detection and adequate treatment of the disease often allows colostrums and milk production to be maintained on a level sufficient to eliminate the risk of subsequent starvation and high susceptibility to infections in the piglets (5). The aim of this study was to investigate the risk factors of agalactia syndrome, subsequent sows and piglets performances as well as an appropriate prevention programme in the particular farm.

Materials and Methods

Study population

The pig population was constituted of Large White and Landrace crossbred sows. The farm is located in the western part of Thailand, had 2,700 sows, divided into 3 units, 900 sows in each unit; 80 sows were carefully observed during lactation period. The sows were fed with a commercial type of diet, formulating for lactating sows. During lactation, the daily feed allowance was increased to a maximum of 4 kg + 0.2 kg per piglets within 14 days. Water was *ad libitum*. The average weaning age was 24 days. This study was carried out from the middle of May 2004 to the middle of July 2004 (8 wk).

During this period, the farrowing house was modifying from open system into evaporating system. The sows were always present in the farrowing house throughout the period of construction. Induce farrowing of all the sows were made by administration of PGF2 alpha (10mg, i.m.) and Oxytocin (20 IU, i.m.). The prevention programme for MMA was done by administration (i.m.) of Amoxycillin (L.A.) on the day of farrowing. The gilt replacement rate was above 40 percent. The S/P ratio of PRRS indicated that gilts had high possibility (S/P ratio ≥ 2) to shedding virus to the other pigs. Using PCR technique, the results showed that the strains of PRRSV in this farm were both European and US strains. All the pigs were not vaccinated against PRRSV.

Data collection and analysis

The total of 80 sows were examined for agalactia syndrome (complete agalactia in more than 2 udders). The parity of the affected sows was recorded. The date

from the farrowing day was also recorded in order to determine the onset of agalactia syndrome. Clinical examinations such as measuring of rectal temperature (the temperature of ≤ 39.5 C was designated as normal), palpation of the udder for the signs of inflammation (swelling, warm, reddening), vaginal discharge and appetite of the sows, and diarrhoea of piglets were made. The weaning weight of the piglets during the period of study was also recorded. The protocol for the preparing of farrowing house was evaluated, concerning the means of cleaning and disinfectant as well as downtime (empty period) of the farrowing pens. In addition, the method of handling the sows before parturition was carefully studied, especially the possible troubles during the transfer from gestation house to farrowing house (method of handling, feeding and environmental changes).

Time schedule

The work was undertaken in 2 steps. The profiles of 80 sows with respect to the risk factors were determined in the first and action plan to reduce agalactia syndrome was implemented in the second, based on the correction of the failing parameters.

In this case, the risk factors of agalactia were thought to be involved with the large number of gilts with high S/P ratio (PRRS positive) in the farrowing house, the construction of the farrowing house (changing from open house into evaporating system, i.e. environmental stress), the downtime of farrowing pens and poor prevention programme of MMA. For the action plan, the farmer was recommended to: (1) increase downtime of the farrowing pen from 2-3 days to 5-7 days, (2) changes the prevention programme of MMA by administration (i.m.) of Amoxycillin (L.A.) 3 days before farrowing date. However, if the sows still not farrow in the next 3 days, the second injection should be made.

The prevalence of agalactia syndrome and the weaning weight of piglets have been evaluated for 2 weeks after the action plan was made.

Results

The present investigation found that 64 out of 80 sows (a prevalence of 80%) in the farrowing house were affected with agalactia syndrome, indicating by milk production failure of the udder. For the clinical examination, the rectal temperature in all sows was normal. There are no clinical signs of mastitis (firmed, swelled, redden and sore mammary glands) and Metritis (vaginal discharge). The sows were normal in appetite. The piglets with diarrhoea were not observed in the present investigation. The downtime for the farrowing pens was found to be too short, i.e. 2-3 days. The administration of antibiotic in order to prevent MMA was found to be too late, i.e. on the day of farrowing. For the performance of piglets, the average weaning weight 2 weeks before the incidence of agalactia syndrome was 7.27 kg. During the outbreak of agalactia syndrome, the weaning weight was 6.75 kg. Three weeks following the action plan (i.e. the corrective programmes), the weaning weight was improved to be 6.96 kg. In addition, the decreased in the prevalence of agalactia syndrome (from 80% to 75% within a week) was observed.

Discussion

Many studies have been undertaken on the MMA syndrome (agalactia post partum syndrome, APP). Most studies provide information on etiology agents (infectious agents, e.g. *E. coli*) (6) and other factors like hormone profiles, feed and watering conditions, genetic predisposing, handling methods and urinary tract infections. The wide variety

of names attributed to farrowing disorders expresses their complexity. A wide range of measure is also suggested to cope with the disease. Most of these are treatment.

The results we obtained from this investigation showed that agalactia syndrome in primiparous and multiparous sows remains to be of economic importance in breeding herd. The primary clinical importance of this syndrome is associated to the affected sows (lactation failure or complete agalactia) and reduction of piglet growth (4). In the present investigation, the three primary risk factors are the environmental changes (i.e. construction), poor prevention programme for MMA which is in agreement with the previous study (2) and also high S/P ratio (≥ 2) of PRRS in the replacement gilts.

During the construction period, sows may face with the stress conditions. It has been suggested (7) that stressors activate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of corticotrophin-releasing hormone (CRH). CRH causes adrenocorticotropin hormone (ACTH) release from the anterior pituitary which, in turn, stimulates glucocorticoid (cortisol) secretion from adrenal glands. It is generally accepted that high level of cortisol may inhibited the releasing of prolactin hormone from anterior pituitary gland. Therefore, complete agalactia (lack of milk production) found in the present investigation may be due to an elevation of cortisol level as corresponding to the stressor (construction). Pigs that are exposed to environmental stressors have been shown to have altered immune function in term of peripheral blood neutrophils and lymphocytes (8, 9, 10). This may increased in the susceptibility to PRRSV infections in the farrowing pens. Another possible reason may be that the high S/P ratio of PRRS found in replacement gilts, which indicate the ability to shedding virus to the other pigs in the farrowing pens, subsequently cause agalactia in sows. However, these risk factors can be solved by using combination of management changes (i.e. create stable herd for PRRS, reduce stressors and proper prevention programme for MMA). Nevertheless, further investigation is needed, concerning the seroprofiles of PRRS in sows at the first and third week of lactation in order to clarify whether the stressor (i.e. construction) lead to the increased in PRRSV challenge in the farrowing unit.

Conclusion

The present investigation showed that stressors such as construction of the farrowing house (i.e. while the sow is present) together with poor management to prevent MMA, and high S/P ratio of PRRS in gilts, could have negative effect on sows and piglets performances in the breeding herd. An appropriate programme should immediately be implemented in order to correct the failing points.

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THE INFLUENCE OF R-CLOPROSTENOL (PRELOBAN®) AND OXYTOCIN ON PARTURITION

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Introduction

Induced farrowing is a practical management aid to allow for increased supervision and to improve utilization of labor. The administration of prostaglandin F_2-alpha (PGF_2-alpha) or an analogue like R-cloprostenol, within 2 days of normal delivery, is effective in inducing parturition in sows. PGF_2-alpha is a luteolytic hormone produced by the sow's uterus. Prepartum release causes lysis of the corpus luteum, which removes the progesterone block that maintains pregnancy. When oxytocin is administered 20-24 hours after PGF_2-alpha administration, farrowing is initiated in most sows within 6 hours.

The objective of this study was to investigate the difference in perinatal mortality between induced farrowing and natural delivery and to investigate the difference between two PGF_2-alpha-oxytocin intervals.

Materials and methods

Two trials were carried out on a 2,000 sow commercial pig-breeding farm in Suphunburi province in Thailand.
In trial 1, 63 pregnant sows with the same predicted farrowing date were assigned at random to the treatment group and the control group. At noon at day 115 of pregnancy the sows in the treatment group received an intramuscular (IM) injection with 75 ug R-cloprostenol (1 ml Preloban®, Intervet), 20 hours later followed by an IM injection of 20 IU of oxytocin (10 IU per ml) (Table 1).

Table 1: Trial 1, Preloban® - oxytocin interval of 20 hours

Group	Product	Time	Dose	n
T	Preloban® Oxytocin (generic)	12.00 8.00	1 ml 2 ml	22
C	-	-	-	41

In trial 2, 23 pregnant sows with the same predicted farrowing date were assigned at random to the treatment group and the control group. At 8.00 at day 115 of pregnancy the sows in the treatment group received an IM injection with 75 ug R-cloprostenol (1 ml Preloban®, Intervet), 24 hours later followed by an IM injection of 20 IU of oxytocin (2 ml Intertocin-S®, Intervet) (Table 2).

Investigation of live and dead piglets per litter took place within two hours after the last piglet was born. From sows that farrowed outside normal working hours the piglets were counted early morning.

Table 2: Trial 2, Preloban® - oxytocin interval of 24 hours

Group	Product	Time	Dose	n
T	Preloban® Intertocin-S®	8.00 8.00	1 ml 2 ml	13
C	-	-	-	10

Results and Discussion

A summary of the results is shown in Table 3. Although two different oxytocin products were used, it is unlikely this influenced the results.

Table 3: Results

	Trial 1		Trial 2	
	Treatment	**Control**	**Treatment**	**Control**
N	22	41	13	10
total born	230	452	151	104
total born/litter	10.45	11.02	11.62	10.40
perinatal mortality/group	22	45	3	9
% perinatal mortality/ group	9.57%	9.96%	1.99%	8.65%
total live piglets	208	407	148	95
live piglets/litter	9.45	9.93	11.38	9.50
% live/total born per litter	90.43%	90.10%	97.90%	91.30%

In trial 1 there was no difference between the groups in the percentage of perinatal mortality (Figure 1), while in trial 2 there was a difference (Figure 2). As the numbers of sows in trial 2 was small, the results can only be evaluated as a tendency. A trial with a larger number of animals treated according to this scheme could bring more clarity.

Figure 1: Trial 1, perinatal mortality

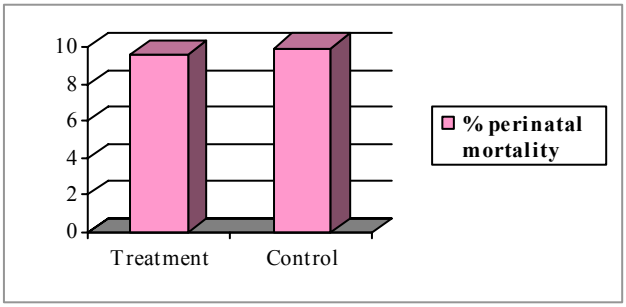
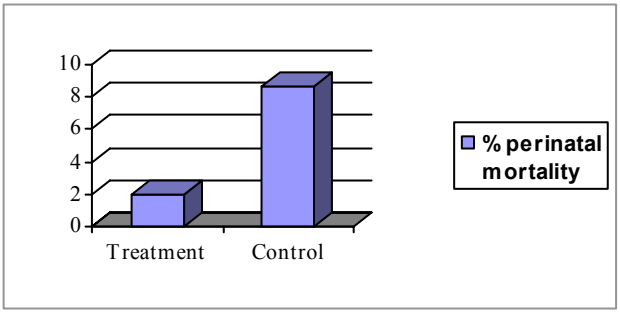


Figure 2: Trial 2, perinatal mortality



The average gestation period on a farm has to be known, before the described way of induction of parturition can be considered. Too early induction of parturition can have a negative influence on the health of the newborn piglets.

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DISTRIBUTION OF SPERMATOZOA IN THE FEMALE REPRODUCTIVE TRACT AFTER INTRAUTERINE INSEMINATION IN PIG

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Keywords: Pig, Reproduction, Artificial insemination, Intra-uterine insemination

Introduction

Artificial insemination (AI) in pig improved genetics faster than natural mating. The present technique for conventional AI (intra-cervix insemination) required 2-5 billion spermatozoa per insemination. Studies demonstrated that over 90% of the sperm loss before approaching the fertilization area in the oviduct by semen backflow and phagocytosis in the uterus (Mburu *et al.*, 1996; Steverink *et al.*, 1998). Recently, a new procedure for intrauterine insemination (IUI) in sows has been developed (Martinez *et al.*, 2002). The procedure consists of a specially designed flexible catheter that allows passage through the cervix and deposit semen in the body of the uterus. Using this technique in sows, the number of spermatozoa per insemination could be reduced up to 1 billion spermatozoa in 50 mL. Studies have shown that, under farm conditions, farrowing rate and litter size after IUI were not significantly difference from conventional AI (Watson and Behan, 2002; Rozeboom *et al.*, 2004). To apply IUI technique under farm condition, more studies on the sperm transport around ovulation times using IUI technique compared with conventional AI still need to be investigated.

Objectives

The purpose of the present study was to compare the number of spermatozoa obtained from the different part of the female reproductive tract at about 24 h after intrauterine insemination (IUI) and conventional AI.

Materials and Methods

Twelve crossbred (Landrace x Yorkshire) multiparous sows were used. All sows were examined for estrus every 6 h using back pressure test with the present of a mature boar. Transrectal ultrasonography were used to detect the time of ovulation. The sows that showed oestrus symptoms within 6 days after weaning and ovulated normally were included in the experiment. During the second estrus after weaning, the sows were inseminated once at 6-8 h before the expected ovulation using diluted fresh semen from a mature boar. The sows were divided into two groups: group I (n=6) sows were inseminated using conventional AI method with $3x10^9$ spermatozoa in 100 mL and group II (n=6) sows were inseminated using IUI method with $1x10^9$ spermatozoa in 50 mL. All sows were ovario-hysterectomized at about 24 h after insemination and the female reproductive tracts were removed and divided into 7 parts: ampulla, cranial isthmus, caudal isthmus, utero-tubal junction (UTJ), cranial uterine horns, middle uterine horn and caudal uterine horn. The spermatozoa within each part of the reproductive tracts were flushed using phosphate buffer saline (PBS) solution and the number of spermatozoa were counted using hemocytometer. Number of spermatozoa in all parts of the uterine horns was pooled for the statistical analyses. The number of spermatozoa and number of ovulation on the left and right side of the reproductive tract within animal were compared using paired *t*-test. The distribution of the spermatozoa in different part of the reproductive tracts was compared using analysis of variance (ANOVA). Due to the lack of normality, the number of spermatozoa was log transformed before being analyzed. The least-square means were obtained from each class of the variables and were compared using student's *t*-test. Number of spermatozoa in each part of the reproductive tracts between groups was compared using student *t*-test. The differences with $P<0.05$ were regarded as statistical significance.

Results and Discussion

The number of ovulation of the sows in AI-group and IUI-group did not differ significantly (18.3 versus 20.3, *P*=0.44). Ovulation took place on the left side more than the right side of the ovaries in both group (10.6 versus 8.8; *P*=0.02). In both groups, the number of spermatozoa was found in both sides of the reproductive tracts. Sperm counted on the left and right side of the reproductive tracts was not differ significantly in both groups (*P*>0.05). The numbers of spermatozoa in each part of the reproductive tracts were not differ significantly between conventional AI and IUI-group (*P*=0.49). The numbers of flushed spermatozoa in each part of the reproductive tracts are presented in Table 1. In both groups, the number of spermatozoa in the UTJ and the uterine horns were higher than those in the ampulla, cranial isthmus and caudal isthmus (*P*<0.001).

The present study demonstrated that the number of spermatozoa in all parts of the female reproductive tracts after IUI with 3-fold reduction in the number of spermatozoa and 2-fold reduction in the volume were not differ significantly from those inseminated with conventional AI. These data support the previous field studies that farrowing rate and litter size after IUI did not differ significantly from conventional AI (Watson and Behan, 2002; Rozeboom *et al.*, 2004). Application of IUI technique under farm conditions would allow a more efficient use of semen from superior genetic boar. Further studies on environmental factors affecting the fertility rate and the difficulty of the insemination in gilts and young sows should be considered.

Acknowledgement

This study is granted by the Research and Development Center for Livestock Production Technology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. The authors wishes to thanks Phillips International Co. Ltd. for providing IUI catheter (Deep-golden pig®) for the experiment.

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Table 1 Number of sperm counted in different parts of reproductive tracts in sows after intra-uterine insemination (IUI) compared with conventional artificial insemination (AI)

Reproductive tract	N	Conventional AI			IUI			*P*-value
		Mean ± SEM	Range		Mean ± SEM	Range		
Ampullae	6	87.2 ± 17.3[a]	40-150		85.3±24.3[a]	32-195		0.95
Cr. isthmus	6	343.5±115.9[b]	118-900		296.8±68.9[a]	90-594		0.74
Ca. isthmus	6	1411.2±277.2[c]	810-2600		1280.3±211.4[a]	800-2200		0.72
UTJ ($x10^3$)	6	142.5±20.6[d]	95-205		131.2±22.3[d]	70-185		0.72
Uterus ($x10^3$)	6	204.2±34.5[d]	115-330		193.4±48.7[d]	103-395		0.86

[abcd] means with different letters within column differ significantly (*P*<0.05)

SPERM TRANSPORT AFTER DEEP INTRA UTERINE INSEMINATION COMPARED WITH CONVENTIONAL ARTIFICIAL INSEMINATION IN PIG

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Keywords: Pig, Reproduction, Artificial insemination, Spermatozoa

Introduction

Artificial insemination (AI) in pig has been developed since 1926 and nowadays it is widely used in the pig industry all over the world (Johnson *et al*., 2000; Tummaruk *et al*., 2000; Tummaruk *et al.,* 2004). Practically, about $3x10^9$ motile spermatozoa in 80-100 mL of volume are inseminated to the sow for 2-4 times during the standing oestrus. The AI catheter is inserted through the vagina and deposit in the cervix of the sows, and the semen is released at the distal part of cervix. The fertilization takes place at the ampullatory-isthmic junction in the oviduct of the sows soon after ovulation. Less than 5% of sperm are discovered at the fertilization site during peri-and post-ovulation period (Mburu *et al*., 1996). Krueger *et al.* (1999) found that 10 million spermatozoa per insemination are sufficient for successful surgically insemination into the tip of the uterine horns in gilts. Researchers are then moving forward to investigate more advance technology to increase the efficacy of AI technique in pig. Deep intra-uterine insemination (DIUI) has recently been developed for non-surgical insemination using a special designed catheter (Vazquez *et al.,* 2005). The catheter could be inserted through the proximal third of the uterine horn where the semen was deposited. Using this technique a 20-fold reduction in the number of spermatozoa per insemination could be used without any significant effect on farrowing rate and litter size (Martinez *et al.*, 2002). The success of DIUI technique under farm conditions would allow a more efficient use of semen from superior genetic boar. The technique is also applicable for some advance biotechnology such as frozen-thaw semen, sex sorted sperm and embryo transfer (Roca *et al*., 2003; Vazquez *et al.,* 2003; Martinez *et al.*, 2004). Investigation on sperm distribution and fertilization after DIUI technique are important to be investigated to know more about the mechanism on the successful of DIUI in pig.

Objectives

The present study was performed to investigate number of spermatozoa in the female reproductive tract at 24 h after insemination using DIUI with a low number of spermatozoa per dose compared with conventional AI.

Materials and Methods

Twelve multiparous sows were used in the experiment. The sows were randomly assigned into 2 groups, Group I and II. The sows in both groups were detected for standing oestrus twice a day after weaning. Transrectal ultrasonography was used for detection of ovulation. Sows returned to oestrus within 6 day after weaning and ovulated normally were included in the experiment. During the second oestrus after weaning, the sows were inseminated once at about 6-8 h before expected ovulation using diluted fresh semen from one proven sire with an individual motility of ≥70%. Group I (n=6) sows were inseminated using conventional AI with $3x10^9$ motile sperm in 80 mL of volume and group II (n=5) sows were inseminated using DIUI with $0.15x10^9$ motile sperm in 7.5 mL of volume. The sows were generally anesthetized and the ovario-hysterectomy was performed at about 24 h after insemination. Sows that did not ovulate were excluded from the study. The reproductive organs were removed and divided into 7 parts on each side: ampulla, cranial isthmus, caudal isthmus, utero-tubal junction (UTJ), cranial

uterine horn, middle uterine horn and caudal uterine horn. The spermatozoa within each part of the reproductive tracts were flushed using phosphate buffer saline (PBS) solution and the number of spermatozoa were counted using hemocytometer. Number of spermatozoa in all parts of the uterine horns was pooled for the statistical analyses. The number of spermatozoa and number of ovulation on the left and right side of the reproductive tract within animal were compared using paired *t*-test. The distribution of the spermatozoa in different part of the reproductive tracts was compared using analysis of variance (ANOVA). Due to the lack of normality, the number of spermatozoa was log transformed before being analyzed. The least-square means were obtained from each class of the variables and were compared using student's *t*-test. The differences with $P<0.05$ were regarded as statistical significance.

Results and Discussion

The number of ovulation of the sows in AI-group and DIUI-group did not differ significantly (18.3 versus 17.2, $P=0.63$). Ovulation occurred on the left side more than the right side of the ovaries in both group (+1.8, $P=0.08$). For the conventional AI-group, the spermatozoa were found in both side of the reproductive tract in all sows (6/6) (Table 1). The number of spermatozoa between the left and the right side in the conventional AI-group were not differ significantly ($P>0.05$). The spermatozoa were found in only one side of the reproductive tract in the DIUI-group (3/5 in the left and 2/5 in the right side) (Table 2). On average, number of spermatozoa in AI-group were higher than DIUI-group ($P<0.001$). In both groups, the number of spermatozoa in the UTJ and the uterine horns were higher than those in the ampulla, cranial isthmus and caudal isthmus ($P<0.001$). Sperm number in the ampulla, cranial isthmus, caudal isthmus, UTJ and uterine horns in the AI-group were significantly higher than those in the DIUI-group ($P<0.001$).

The present study demonstrated that using DIUI, the spermatozoa distributed to only one side of the uterine horn during the first 24 h after insemination. One side of the sperm reservoir (caudal isthmus) was free of spermatozoa in all sows. Martinez *et al.* (2002) demonstrated that the embryo was found in both side of the uterine horn on day 2 after insemination. Therefore, there is likelihood that the spermatozoa were transported from one to another horn of the uterus sometime between 24-48 h after insemination. These spermatozoa were likely to distribute from another side of the sperm reservoir. The mechanism for the sperm transport may need further investigation. Transperitoneal migration of the spermatozoa has also been report in heifer (Larsson, 1986).

In conclusions, DIUI in multiparous sows resulted in a significantly lower number of spermatozoa in the female's reproductive tract during a 24 after insemination compared with conventional AI and the spermatozoa were found in only one side of the sperm reservoir.

Acknowledgement

This study is granted by the Research and Development Center for Livestock Production Technology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. The authors greatly appreciated Prof. Juan M. Vazquez for providing DIUI catheter for the experiment.

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Table 1 Number of sperm counted at different part of reproductive tract at 24 h after conventional artificial insemination with $3x10^9$ motile spermatozoa in 6 multiparous sows

Reproductive tract	1		2		3		4		5		6	
	L	R	L	R	L	R	L	R	L	R	L	R
Ampulla	12	28	50	70	35	18	100	50	14	51	80	15
Cr. isthmus	50	120	110	150	50	68	120	150	750	150	220	123
Ca. isthmus	710	520	390	420	610	525	1000	800	1600	1000	350	542
UTJ ($x10^3$)	55	50	130	70	80	70	60	40	145	60	40	55
Uterus ($x10^3$)	110	105	200	130	110	75	60	56	149	115	60	55

L=Left; R=Right; Cr.=Cranial; Ca.=Caudal; UTJ=Uterotubal junction

Table 2 Number of sperm counted at different part of reproductive tract at 24 h after deep intra-uterine insemination with $0.15x10^9$ motile spermatozoa in 6 multiparous sows

Reproductive tract	1		2		3		4		5	
	L	R	L	R	L	R	L	R	L	R
Ampulla	22	0	35	0	0	44	0	15	11	0
Cr. isthmus	150	0	60	0	0	80	0	60	30	0
Ca. isthmus	420	0	250	0	0	250	0	360	140	0
UTJ ($x10^3$)	22	0	15.5	0	0	25	0	35	20	0
Uterus ($x10^3$)	29	0	46	0	0	28	0	41	13	0

L=Left; R=Right; Cr.=Cranial; Ca.=Caudal; UTJ=Uterotubal junction

Efficiency of Moxidectin pour-on in the treatment of Sarcoptic mange in swine

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Introduction

Sarcoptic mange is often a severe disease. Intense, constant pruritus results in annoyance, irritability, decreased feed intake, anemia, decreased weight gain or weight loss, difficulties in estrus detection, lowered conception as well as reproductive problems.

Definitive diagnosis is base on history, physical examination, skin scraping, skin biopsy, and response to therapy. Mites are often very difficult to find in scraping. In swine, crusts and cerumen gathered from ear canal and pinna are the most consistent sources of mites (1).

Moxidectin pour-on was approved to control the endoparasites and ectoparasites in cattle (2) and also has an efficiency to against swine nematodes (3). The aim of the present study was to determine efficiency of moxidectin pour-on in treatment sarcoptic mange in swine.

Materials and methods

A farm was selected in which clinical signs of mange were evident: encrusted lesion in ears, dermatitis, and pruritus. This was confirmed by positive ear scrapings.

Thirty pigs were naturally infected and divided into 2 main groups; control group (5 pigs) and treatment group (25 pigs): control group were treated with normal saline, and treatment group were subdivided into five-dose of moxidectin 0.5% pour-on with five pigs per group. The doses were: moxidectin 0.5, 0.75, 1.00, 1.25, and 1.5 mg/kg body weight. Moxidectin was given twice with a 14-days interval (d0 and d 14, respectively). Each animal in the groups was monitored for clinical signs such as scratching index, average dermatitis score, and mite score on day 0, 7, 14, 21, and 28 after treatment. Ear scrapings were taken from the inside of the pinna of the ear an area of approximately 2 cm^2 with a curette. The material collected from each ear was examined microscopically for the presence of live and dead mites.

The scraping were collected into centrifuge tube and digested in 10 ml of the 10% potassium hydroxide solution overnight. On the following day mixed with vertex and centrifugation at 2,500 rpm for 15 minutes. The suspended was calculated the mite including adults, nymphs and larvae, and mite egg from day 0 to day 28 post-treatment were calculated from the means.

On the same days when scraping were collected, pigs were observed for 15 minutes for signs of rubbing and scratching. The severity of skin lesion and pruritus were scored from 0 to 3. The scheme used for scoring was as following – for skin lesion: 0= no active lesion, 1= slight lesion of encrustation, scaling or erythema, 2= a few localized moderately thick dry crusty lesions, 3= large, thick, grayish, rough and dry crusts with coalescence of lesion. Chi-square was used to statistically interpret the results.

Results and discussion

Table 1. The amount of pig was remained the live mite on day 7, 14, 21, and 28 after treatment.

Groups	days after treatment			
	d7	d14	d21	d28
Control	4/5	4/5	3/5	1/5
1	3/5	3/5	2/5	2/5
2	2/5	2/5	2/5	1/5
3	5/5	3/5	0/5	0/5
4	1/5	1/5	0/5	0/5
5	1/5	0/5	0/5	1/5

All of treatment group has shown the reduction rate of live mite on Day 28 was significantly ($p<0.005$) (Table 1). After secondary treatment, were begin decrease the skin lesion in the

treatment group (2, 4, and 5 subgroups). On the day 28 after treatment, 3, 4, 4, 2, 2,and 3 pigs were not found skin lesion in group control, 1, 2,3 4, and 5 respectively. There was no significant difference between control and treatment groups in the data of scratching index.

2 pigs in group 5 had skin irritation after treatment on day 16.

Discussion

The field trial of moxidectin pour-on has shown the high efficacy against sarcoptic mange in treatment group (3, 4, and 5 subgroups). But the effective dose and not irritated skin is 1.00-1.25 mg/kg and give two times interval 14 days. After secondary treatment, were begin decrease the skin lesion in the treatment group (2, 4, and 5 subgroups). Scratching index is not completely specific for mange, other cause of the pruritus observed in this study could account for the lack of a significant difference in scratching index (4).

Clinical observations, scratching observations, average dermatitis scores, and examination of ear scrapings have their limitations as indicators of sarcoptic mange infestation. However, their combined use allows an evaluation of the efficiency moxidectin pour-on for treated sarcoptic mange in swine by the results of the present study.

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IN VITRO EFFICACY OF AN ORGANIC DISINFECTANT CITREX ON SELECTED SWINE VIRUSES

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Keywords: PRRSV, biosecurity, aerosol, organic, citrex

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), Classical swine fever virus (SFV) and pseudorabies virus (PRV) are economically significant pathogens in major swine raising countries including Thailand. Several prevention and control strategies including vaccines, quarantine regulation and good management have been practiced. Biosecurity using cleaning, disinfection and aerosol spray will help to reduce contaminated pathogens and break the cycle of diseases. Disinfectants can be classified into various groups such as phenol, chlorine-releasing, acid, alkalis etc (1). The objective of this study is to evaluate the *in vitro* efficacy of Citrex an organic, non toxic, non corrosive, non mutagenic disinfectant recommended in biosecurity programs against PRRSV, SFV and PRV for surfaces and aerosol spray in swine farms.

Materials and methods

Disinfectant: Citrex is the active ingredient of a commercial organic disinfectant (Bioclean™, Citroclean™ from Citrex Inc, USA) made from the physical activation of vitamin C.

Viruses and cells: SFV (ALD) and PRV (Tongsong) were prepared in PK-15 cell line (2,3). PRRSV (SVI 275) was prepared in MARC-145 with 5% fetal bovine serum(FBS) in growth medium incubate in 5%CO_2 at 37°C for 48 hours (5).

Virucidal activity: Citrex was prepared in a 2× concentration and mixed with the equal volume of the virus stock to yield concentration of 100, 200, 400, 800, 1000 and 1200 ppm. The mixtures were then titrated in a 10 – fold dilution and incubated at room temperature for 0, 5, 10, 15, 30 and 60 minutes before inoculating in to 96 – well microtiter plates incubated in 5%CO_2 at 37°C for 1 hour.

The mixtures were removed and replaced with 2 %FCS maintenance medium incubate in 5%CO_2 at 37C for 48 hours.

Fig. 1: Positive staining of SFV- infected PK-15 using IPMA.
Fig. 2: Positive staining of PRRSV- infected MARC-145
using IPMA.
Fig. 3: Cytopathic effects of PRV - infected PK-15.
Fig. 4: Monolayer of PK-15 cell line (control).

Indirect immunoperoxidase monolayer assay (IPMA) was performed for SFV and PRRSV to determine the infection (Fig.1 and 2). PRV infected cell was determined by evidence of cytopathic effect (CPE) (Fig.3) compared with the control (Fig. 4). Virus titer was calculated by Reed and Muench method (4). The titers were expressed as $TCID_{50}$/ml.

Results and Discussion PRRSV

The efficacy of Citrex against PRRSV, SFV and PRV is shown in Table 1. PRV was completely killed at 200 ppm for 15 minutes, 400 ppm for 5 minutes or 800 ppm for 5 minutes. SFV was completely killed at 200 ppm for 30 minutes or 800 ppm for 5 minutes. PRRSV was completely killed at 200 ppm for 10 minutes or 400 ppm for 5 minutes.

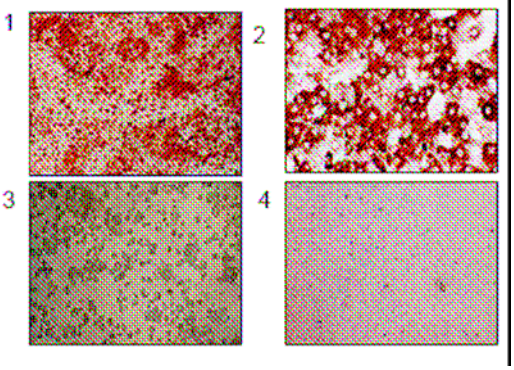
PRV, SFV and PRRSV are enveloped swine viruses. These swine viruses are highly resistant in the environment but are easily destroyed by lipophilic agent and very sensitive to acid or alkalis. Citrex, an organic disinfectant is able to kill PRRSV, SFV and PRV at different concentration depending on the viral susceptibility. Citrex may be used as an alternative for disinfecting the environment in biosecurity programs in swine farms.

Table 1:
In vitro efficacy of Citrex on virus titers at different times and concentrations.

Times (min)	Concentration of Citrex (ppm)												
	100			200			400			500-1200	control		
	A	B	C	A	B	C	A	B	C	A,B,C	A	B	C
0	5[a]	3.23	4.68	4.75	3	3	2.83	2.75	0	0	6	4.5	4.5
5	5	3.67	3.25	2.1	3	2.75	0	2.25	0	0	6	4	5
10	5	3	3.25	2.7	3.67	0	0	2.75	0	0	6	4.5	5
15	4.5	4.3	3.5	0	3.88	0	0	2.5	0	0	6	5	5
30	4.5	3.75	3.95	0	3.75	0	0	0	0	0	5.5	4	4.5
60	4.25	3.5	3	0	3	0	0	0	0	0	5	3.5	4

[a] log 10; A = PRV, B = SFV, C = PRRSV

Figueres 1, 2, 3, 4



Acknowledgements

We would like to thank Dr. Eileen Thacker for providing cells and antibodies and Department of Veterinary Pathology, Chulalongkorn University and Innovet Corporation Thailand for funding this study

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Origanum Essential Oil as Growth Promoter and PWDS Control in Weaning Pigs in a Farm

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Introduction and Objectives

At the time of weaning, young pigs are submitted to many stressors such as the separation from the sow, mixing with other pigs, end of the maternal immunity and changes in housing and diet (1). So weaning pigs usually show non-infectious diarrhea and infectious diarrhea from opportunistic pathogens particularly enterotoxigenic *Escherichia coli* strains (ETEC) and cause the post weaning diarrhea syndrome (PWDS) (2).

Antimicrobial were used for control that problem but EU restricts the use of antimicrobial feed additives. The essential oils from plant Origanum reduce diarrhea and act growth promoter. The present study sought to investigate the MIC of Origanum essentail oils (Oregano®) to protect to *E.coli* from field strain and was designed to determine the possibility of Oregano® as growth promoter and PWDS control in weaning pigs.

Materials and methods

A total of 190 isolates of *E.Coli* were obtained from fecal of averages age 28 ± 3 d weaning pigs which showed diarrhea in a farm in Chiang Mai – Lamphun provinces, Thailand. We performed MIC_{50} and MIC_{90} of Oregano® against of *E.coli* isolated agar dilution test follow to National Committee for Clinical Laboratory Standard (NCCLS).

The trial study was carried out in a commercial pig farm (300 sows) and had previous history of PWDS caused mainly by ETEC. A total of 20 healthy piglets (24±3 of age) with the same genetic background (Doroc×Large White×Landlace), 10 male pigs and 10 female pigs were selected. A completely randomized design was used with 2 treatments of 10 piglets each with similar average liveweight, weight range ($p<0.05$) and equal sex ratio (5 males + 5 females) per group. The total trial period was 28 d. The samples were divided into 2 groups: a control group (feed with free antimicrobial drug and growth promoter) and a treatment group (feed with free antimicrobial drug but added 1 ml of Oregano® solution per oral at day 7 and day 14 of the trial.

All piglets were maintained in the same conditions during this trial work. Piglets were daily observed to monitor health status including diarrhea and mortality. Liveweight, diarrhea scores, average daily gain (ADG), feed conversion ratio(FCR) and average daily feed intake(ADFI) were also noted. Results of ADG, FCR and diarrhea score were analyzed by t-test using the SPSS program 10.0 for windows and other parameters into consideration were descriptive analysis.

Results and Discussion

MIC was a good indicator of effectivness in *vivo*. The aim is to determine the concentration of active substance that can inhibit the growth of the bacteria from field strains (Table 1).

Table 1: Dosage MIC_{50} and MIC_{90} of Oregano®

Herb extract	E.coli No. Of Isolation	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)
Oregano®	190	1800	5000

Results of ADG, FCR and diarrhea score (Table 2) among both groups were not significantly difference ($p>0.05$). However, the ADG between the control group and the treatment group was significantly difference ($p<0.05$) in the last peroid of the study (Table 3).

Table 2: Averages of growth performance data

Parameters	Control group	Treatment group
start weight (Kg/piglet)	6.74	6.72
end weight (Kg/piglet)	16.81	16.78
ADG (g/d, 28 d)	356.6^a	357.5^a
FCR (28 d)	1.28^a	1.14^a
ADFI (g/d, 28d)	460.7^a	428.5^a
Diarrhea score	28^a	20^a

Table 3: ADG in each week

ADG (g/d)	Control group	Treatment group
Day 1-7	194.2^a	144.2^a
Day 8-14	331.4^a	332.8^a
Day 15-21	405.7^a	317.1^a
Day 21-28	507.1^a	635.7^b

Remake: The different superscript indicate the ADG differed significantly ($p<0.05$)

Oregano® solution had two main ones of which are the phenol carvacrol and thymol which have revealed the bactericidal activity (3).

Oregano® was trend to develope growth performance of piglet in the last period. The number of pigs and the extended trial period (finishing period) should be added in the farther experiment. The effectiveness in diarrhea control was probably better than the control group.

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Bacteriology

In Vitro Sensitivity of Pathogenic *Escherichia coli* and Non-Pathogenic *Escherichia coli* against 16 antimicrobial agents.

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Introduction

Swine enteric diseases cause severe economic losses in pig production throughout the world including Thailand. Enteric colibacillosis and edema disease, causing by toxin producing *Escherichia coli*, affect pig ages between 4 to 12 weeks (1). *Escherichia coli* are gram negative, peritrichous flagellated rods (2). The organisms may be the normal flora of gastrointestinal tract and some may cause a broad variety of intestinal and extraintestinal diseases in pigs. The diseases are endemic where fecal contamination and inadequate management of animal waste are involved. Moreover, E. coli-induced diseases are also of significant concerns in public health (1).

Controlling of diarrhea using antimicrobials is normally practice in the pig industrial. However, excessive use of antimicrobials in the veterinary medicine is often criticized since it may result in emerging of drug-resistance bacteria. In Thailand, laboratory detection for pathogenic *E. coli* is routinely interpreted by producing the hemolytic zone on blood agar, even the zone can not represent in all pathogenic isolates. Therefore, minimal inhibitory concentration (MIC) values or other susceptibility interpretation yielded from the non-pathogenic *E. coli* may not reflect a real representative value.

This study was to determine the MIC values of *E. coli* isolated from piglets. All isolates were characterized and classified into 2 groups of pathogenic and non-pathogenic *E. coli* using approved digoxigenin probes.

Materials and Methods

Bacteria used. *E. coli* were isolated from piglets with endemic colibacillosis and routinely use antimicrobials especially colistin in 7 farms in Thailand. A total of 116 *E. coli* were isolated and confirmed with routinely biochemical tests (hemolysin assay, indole test, methyle red, eosin methylene blue agar, citrate utilization and Voges-Proskauer(VP) test). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* 27853 were used as the references.

Bacterial cultures. *E. coli* field isolates were cultured on trypticase soy agar at 37^{o}C for 24 hours for preparation of inoculum and grew on Muller-Hinton agar for MIC determination (4).

Pathogenic *E. coli* detection. Genomic DNA encoding virulent factor of *E. coli*; LT, heat labile toxin; STh, human heat stable toxin; STp, porcine heat stable toxin; SLT-I, shigella-like toxin type-I; SLT-II, shigella-like toxin type-II; EIEC, enteroinvasive *E. coli*; BfpA, Bundle-forming protien A gene; EAF, enteroadherent factor gene, EAE enteroeffacing factor gene; EAG, enteroaggregative gene, were detected using standard operating procedure of digoxigenin probes. Briefly, the bacterial colonies were spotted and cultured on N+ nylon membrane on dry TSA plates (Amercham, Germany).

The cells were lysed with lysing solution containing 10%SDS before the DNAs were hybridized for the apprppriated probes. The detection was performed with the colorimetric detection reagents, NBT and X-phosphate (BCIP) with Genius system (9,11).

Antimicrobial agents. Halquinol (Big Chemical, Thailand), berberine (Sigma, U.S.A.), Colistin (Sigma, U.S.A.) (breakpiont ≥8 μg/ml), Streptomycin (Sigma, U.S.A.) (breakpiont ≥16 μg/ml), Penicillin (Sigma, U.S.A.) (breakpiont ≥16 μg/ml), Amoxycillin (Sigma, U.S.A. (breakpiont ≥16 μg/ml), Enrofloxacin (Sigma, U.S.A.), Nalidixic acid (Sigma, U.S.A.) (breakpiont ≥32μg/ml), Lincomycin (Sigma, U.S.A), Erythromycin (Sigma, U.S.A.), Tylosin (Sigma, U.S.A.), Tiamulin (Sigma, U.S.A.), (breakpiont ≥16μg/ml), Doxycycline (Sigma, U.S.A.) (breakpiont ≥16μg/ml), Chlortetracycline (Sigma, U.S.A.) and Sulfamethoxazol-Trimetroprim) (Sigma, U.S.A.) (breakpiont ≥4/76μg/ml) were used in this study and were diluted according to the standard method of the National Committee for Clinical Laboratory Standards (NCCLS). All suspensions were kept at 4°C and used within 72 hours (4).

***In vitro* susceptibility test**. The agar dilution test was carried out for detection of MIC values among 116 isolats against 16 antimicrobials as the recommendation of NCCLS. The antimicrobial agents were diluted from 1.25 to 1280 μg/ml and mixed in Mueller-Hinton Agar (Difco, USA) with the final concentration of 0.12 to 128 μg/ml. The inoculum size was 0.5 MacFarland concentration. ($1.5x10^8$ CFU/ml) (4,7). After inoculation, all plates were incubated at 37 °C for 18-24 hour. The minimal concentration of the antimicrobials inhibited visible growth of 50% and 90% of *E. coli* isolates on the agar was interpreted as the MIC50 and MIC90, respectively (4).

Results

About 25% (29/116) of the field isolates contained DNAs encoding virulent factors and were identified as pathogenic *E. coli*. The toxins were heat labile toxin (6/116), porcine heat stable toxin (16/116), and shigella-like toxin type-II (17/116). There were 10 isolates contained both exotoxin, STp and SLT II.

The overall MIC values of 116 isolates against 16 antimicrobial agents were shown in **Table 1**. Colistin and haquinol were significantly more effective than other antimicrobials. Over 90% of PEC and non-PEC were resistant to amoxicillin, berberine, chlortetracycline, tetracycline, lincomycin, nalidixic acid, penicillins, tiamulin, tylosin and Sulfamethoxazole/ Trimethoprim.

The MIC values of streptomycin, doxycycline and streptomycin to 90% of non-PEC were ranged in intermediate level from 0.5-16 μg/ml whereas those of PEC were 1 to 5 folds higher. In addition, the strains of PEC also had 3 times and 64 times higher MIC_{90} than those of non-PEC for halquinol and colistin, respectively.

Discussion

So far routine antimicrobial susceptibility tests of field *E. coli* isolated from post weaning diarrhea (PWD) did not show any evidence of enterotoxigenic potency. *E. coli* producing hemolysin strains may be virulent because of a toxic effect on various cells but pathogenesis of intestine is involved by development of exotoxin triggers secreted into the gut lumen. Many virulent factors are examined and mainly are plasmid and certain particular regions of chromosomal located (2,5). As the results, 10% of exotoxin producing strains did not show hemolytic zone and

30% of hemolytic strains did not have pathogenic effects. It should be noted that hemolytic phenotype might not be a specific tool for determining the pathogenic *E. coli*.

MIC90 of PEC was obviously higher than those of non-PEC and resistant levels of *E. coli* both PEC and non-PEC were quite high to all antimicrobials tested. Especially, tiamulin, tylosin, sulfamethoxazole/ trimethoprim, lincomycin, doxycyclin, chlortetracycline, that were demonstrated to be effective for respiratory pathogens, but did not exhibite efficacy against Enterotoxegenic *E. coli* (ETEC), Enterohaemorrhagic *E. coli* (EHEC) strains or other enteric pathogens (3,8). Therefore, the administration of antibiotics to non-enteric target may indirectly pledge a selective pressure for enteric organism. With regard to enteric pathogens, a possible explanation for the reduced sensitivity of *E. coli* to multiple antimicrobials is that the organisms are involved in a high rate of plasmid exchange. The gut is colonized with abundant bacterial species in close proximity, resulting in an increased probability of interspecies conjugation such as plasmid specified-Mdt (A) protein functioning of energy-dependent efflux systems in bacteria or plamid encoding exotoxin (1,10). None of these properties actually confers increased virulence to the bacterium. However, they provide the opportunity for PEC to proliferate and produce other virulence factors in pigs being treated with an inappropriate antibiotic in endemic colibacillosis areas. The results allow us to select adequate treatment and reliable use of the antibiotics, avoiding their indiscriminate use and the risk of increasing medication costs due to the difficulty of selecting the appropriate chemotherapeutic products as well as resistance phenomenon.

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Table 1 Antimicrobial susceptibilities of 116 *E. coli* divided as PEC and Non-PEC isolates to the 16 agents

Antimicrobials	**MIC_{50}**(μg/ml)		**MIC_{90}**(μg/ml)		MIC Range Gr1	MIC Range Gr2
	Gr1 (n=29)	Gr2 (n=87)	Gr1 (n=29)	Gr2 (n=87)		
Amoxicillin	>512	>128	>512	>128	>512	128->512
Berberine	>512	>128	>512	>128	>512	>128->512
Colistin	8	1	16	0.25	1-32	0.25-32
Chlortetracycline	>512	>128	>512	>128	>512	64->512
Doxycycline	32	32	64	16	4-128	0.125->128
Erythromycin	32	64	128	16	16-128	0.125->512
Enrofloxacin	16	16	32	0.5	0.25-128	0.5-128
Halquinol	32	8	32	8	8-32	8-32
Lincomycin	>512	>128	>512	>128	>512	128->512
Nalidixic	>512	>128	>512	128	>512	16->512
Penicillin	>512	>128	>512	>128	>512	128->512
Streptomycin	128	64	256	8	16-256	0.25-256
Tetracycline	128	>128	128	64	64-128	4->512
Tiamulin	128	>128	>128	64	64-256	64->128
Tylosin	>512	>128	>512	>128	>512	128->512
Sulfamethoxazole / Trimethoprim	>8/152	>8/152	>8/152	>8/125	4/76->8/152	0.06/1.19->8/152

MIC50: minimal inhibitory concentration value of the agents inhibited 50 % the number of isolates.
MIC90: minimal inhibitory concentration value of the agents inhibited 90 % the number of isolates.
Gr1 = *E. coli* positive with DNA probe detections **Gr2** = *E. coli* negative with DNA probe detections.
Bold letters indicates effective agents against *E. coli* isolates.

THE PREVALENCE OF *Lawsonia intracellularis* IN EUROPE

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Introduction

Although it is well established that Ileitis due to *L. intracelluaris* infections is an important enteric disease, there is a surprisingly lack of comprehensive and comparable epidemiological data. The development of an easy to use and cost effective Ileitis ELISA [1] opened a realistic way for the broad investigation of the prevalence of *L. intracellularis* infection in Europe.

Material and Methods

The study is based on the cross-sectional serological screening of *L. intracellularis* across Europe during the year 2004. The farms were selected according "typical in size and production system in the respective country". In total 15.997 single blood samples from 342 farms in 12 European countries were analyzed by using a blocking ELISA for the detection of antibodies against *L. intracellularis* [1]. To ensure that all age groups are covered the applied profiling scheme included 50 blood samples per farm (5 in sows < 1st litter; 5 in sows > 1st litter; 5 in piglets 3rd/4th week; 5 in pigs 8th to 10th week, 10 in fatteners 13th, 18th and 24th week of age each).

Results and Discussion

The average European seroprofile shows the highest percentage of positive serum samples in gilts and sows (79% and 85%, respectively). The lowest prevalence was found in piglets at the start and at the end of the nursery (15% and 10%, respectively)

(Figure 1)

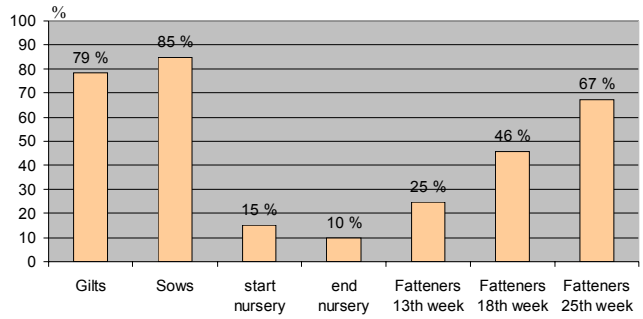

Fig. 1: Average percentage of positive **pigs** in different age groups on 342 European farms

Piglets at the start of the nursery (3rd/4th week of live) showed consistently more positive serum samples than the ones at the end of the nursery. It is reasonable to assume that a part of these antibodies at the start of the nursery are of maternal origin. With the start of the fattening period a clear increase in the prevalence from 10 % to 25 % is found in the average farm seroprofile. The seroconversion typically starts between the end of the nursery and the 13th week of life. Considering about 2 to 3 weeks from infection to seroconversion the pigs are infected in the nursery or short after introduction into the fattening units in the majority of European farms. In the 13th week of life, each 4th pig in Europe is already infected with *L. intracellularis*. Thereafter, a nearly linear increase in the percentage of positive samples towards the end of the fattening is observed. Close to marketing, as much as two out of three animals are infected. Any signs of suboptimal growth, compromised feed conversion and increased weight variation between the 13th week of live and slaughter significantly affect the economic results of pig production in Europe.

Compared to the average percentage of positive pigs within a farm, the average percentage of positive farms is much higher. In 93 % and 97% of all farms in Europe at least one fattener and/or breeding animals was diagnosed as *L. intracellularis* positive

(Figure 2).

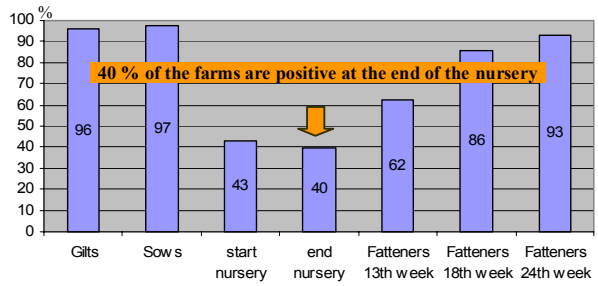

Fig. 2: Average percentage of positive **farms** in different age groups on 342 European farms

It is important to note that at the end of the nursery (8th to 10th week) already 40 % of all European herds are infected with *L. intracellularis*. Compared to the European situation, the average herd in North America seems to reach this point much later. In the United States a herd prevalence of 40 % is found between the 15th and the 18th week of live [2]. This difference in the prevalence pattern may be partly influenced by the applied antibiotic feeding regimes or/and by differences in production system (higher number of multi site in the US compared to single site production systems in the EU).

Within the ongoing survey a number of farms in different Asian countries are about to be serologically screened by the ELISA. The expected profiles from Asia will help to investigate the factors influencing the Lawsonia infection and the time of seroconversion. This information will provide veterinarians with the knowledge for fighting this economically important disease efficiently.

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A BLOCKING ELISA FOR THE DETECTION OF ANTIBODIES AGAINST LAWSONIA INTRACELLULARIS

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Introduction and Objectives

For the detection of serum antibodies against *Lawsonia intracellularis* so far only IFATs (1,2) are available. These tests are interpreted on an individual and subjective judgment of microscopic examination. Now a new blocking ELISA (bELISA) becomes available (3). This test uses two different monoclonal antibodies directed against antibody epitopes of *L. intracellularis* to compete with serum antibodies against *L. intracellularis.* The results are measured in an ELISA reader and evaluated in comparison to a negative and positive reference serum.

High reproducibility, repeatability and comparative analyses can easily be performed with this bELISA. In this study, the first comparative examination with the new bELISA and an monolayer IFAT with samples from Asian swine farms are presented.

Material and Methods

The bELISA was performed as described previously (3) according to the manufacturers instructions. Results were calculated as percent inhibition compared to that of the negative control serum. IFAT was performed as previously described (2). The serum samples were taken from two farrow to finish farms in the Philippines and from one Japanese farm. Farm A (2,700 breeding sows) had a diagnosed Ileitis in the nursery and fatteners within the last 12 months prior to sampling. Feed antibiotics effective against *L. intracellularis* were used in this farm in the nursery (4th to 6th week of age) and in the fattening unit (9th to 23rd week of age). A cross-sectional screening with 100 samples covering all age groups was performed in this farm. Farm B (10,000 breeding sows) did not report Ileitis within the last 12 month before the samples were taken. The farm selects the piglets into three performance groups according to their weight at weaning. Category A covers the best performing piglets and category C the runting piglets. From this farm 70 samples obtained from gilts, sows and weaning pigs were tested. In the Japanese farm, that reported clinical Ileitis, 10 samples were obtained from pigs at 12 and 20 weeks of age, respectively (Farm C).

Results

The results of the cross-sectional screening of farm A and that of farm C are summarized in table 1.

	Farm A			Farm C		
	n	IFAT	bELISA	n	IFAT	bELISA
gilts	10	10	10			
sows	30	23	30			
weaners	10	1	4			
mid nursery	10	1	4			
end nursery	10	0	5			
12 weeks of age	10	2	3	10	0	1
16 weeks of age	10	0	2			
20-22 weeks of age	10	2	4	10	3	10

Table 1: Results obtained in the IFAT and bELISA for farms A and C The data indicate the number of positive results in IFAT and bELISA, respectively for each of the tested age groups.

All gilts were tested positive in both assays, whereas 7 out of 30 tested sows reacted negative in the IFAT, but positive in the bELISA. In all tested age groups from the nursery and the fattening the bELISA detected more samples positive than the IFAT. In the 16 and 22 weeks old pigs the bELISA scored only 1 and 2 samples clearly negative, respectively. In farm C the bELISA detected 100 % positive animals in the group of 20 – 22 weeks old pigs, whereas in the IFAT only 30 % of these pigs reacted positive.

	category	n	IFAT	bELISA
gilts	---	10	3	10
sows	---	30	19	30
3 - 4 weeks of age	a	10	3	7
3 - 4 weeks of age	b	10	3	6
3 - 4 weeks of age	c	10	3	4

Table 2: Results of the IFAT and bELISA from farm B. The number of samples from sows, gilts and weaners that were tested positive in IFAT and bELISA are summarized for each category.

In farm B (table 2) all samples from gilts and sows were tested positive by the bELISA, whereas only 30 % of the gilts and 63 % of the sows reacted positive in the IFAT. Maternal antibodies were detected with 30 % of the weaners by the IFAT, but with up to 70 % in category A piglets by the bELISA. The mean PI value (bELISA) of the piglets from category A was substantially higher than the mean PI values in category B and C (figure 1).

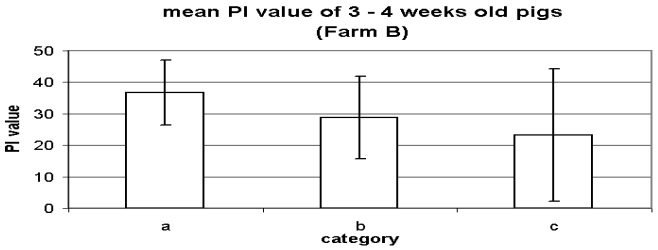

Figure 1: The graph shows the mean PI values including the standard deviation for the piglets in farm B after selection into categories A, B and C according to their weight at weaning. PI values > 30 were scored positive.

Discussion

This is the first report of examinations with the new Ileitis bELISA in Asia. Recent presentations already demonstrated higher sensitivity of the bELISA compared to the IFAT and its suitability in herd profiling (3,4). This new tool for fast and sensitive screening of large amounts of samples now proved its performance

in Asian farms. In all three Asian farms the bELISA had a superior sensitivity compared to the IFAT. As antibiotic treatment could negatively influence the serological response to an infection with *L. intracellularis* in pigs, the higher sensitivity of the bELISA clearly enhances the reliability of diagnostic screenings.

In addition the bELISA allows a semi-quantitative interpretation of the ELISA values. As shown in the piglets from farm B the mean PI value and the number of positive samples correlated with the growth performance of the piglets. The antibodies detected in weaned piglets from farm B derived from colostrum uptake, indicating a higher antibody level in the sow or a larger colostrum uptake by the piglet.

In conclusion the new bELISA provides a powerful tool for serological diagnosis of Ileitis. This test is suitable for routine testing of even large sample numbers and will improve and broaden laboratory diagnosis of Ileitis as well as awareness on ileitis and its influence on growth performance in pig production.

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Prevalence of Virulent Factors among Hemolytic and Non-hemolytic *E. coli* isolated from post weaning piglets in Thailand.

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Introduction

Escherichia coli is the most common opportunistic pathogen associated with extraintestinal infections and diarrhoea in humans and animals. *E. coli* isolates associated with enteric diseases have been classified on the basis of their distinct virulence properties into major categories; enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), and enteroaggregative (EAEC). Among these, ETEC predominantly causes neonatal and postweaning diarrhea in piglets by elaborating heat labile (LT) or heat-stable (ST) enterotoxins (3). Several studies have shown that □-hemolytic property is a frequent and important virulence factor of the pathogenic strains. Hemolytic strains are more lethal than non-hemolytic strains to chicken embryos and mice or rats in models of extraintestinal infection, causing hemorrhagic lung lesions and hemoglobinuria in addition to increased mortality (6). However, enterotoxin producing *E. coli* caused colibacillosis and edema disease in pig by secreted exotoxin into lumen of gut resulting an imbalance of luminal electrolytes malabsorption (3). In addition, pathogenic associated proteins such as invasive-associated protein, fimbrial adhesin, bundle-forming protein, also promote virulence *in vivo* (5, 7, 8).

In this study, we examined the prevalence of pathogenic *E. coli* among hemolytic and non-hemolytic strains and evaluated whether the hemolytic production related to its pathogenicity.

Materials and Methods

Bacterial used. 152 fecal samples were collected from 4-8 week-old piglets in seven farms in Thailand with endemic colibacillosis and routinely used antimicrobial agents. Samples from diarrheal pigs were obtained from Veterinary diagnosis laboratory, Farm animal hospital, Faculty of Veterinary Science, Chulalongkorn University. 152 *E. coli* strains were isolated from the fecal content. 117 out of 152 *E. coli* strains isolated from healthy piglets and the 35 isolates were isolated from piglets with diarrhea. All isolates were confirmed with routine biochemical tests (hemolysin assay, indole test, methyle red, eosin methylene blue agar, citrate utilization and Voges-Proskauer (VP) test). Seven *Escherichia coli* strains; pMAR-22 (EAF), No.2 (EIEC), 933-J (SLT-I), 933-W (SLT-II), XAC (negative with LT, ST), AS-04-1 (EAE), PMSD 207 (BfpA) were included as the controls for the non-ETEC and three strains; 299(LT), pDAS-100 (STP) and pDAS-101 (STH) were included as the controls of Enterotoxegenic *E. coli* (ETEC).

Bacterial cultures. *E. coli* isolates were cultured on trypticase soy agar at 37°C for 24 hour for preparation of inoculum. Pure culture of *E. coli* were spotted on Hybond N+nylon membrane (Amercham, Germany) and incubated for 16 hour at 37 °C before lysis of bacteria.

Probes preparation. Ten DIG-labeled probes for detection of LT, heat labile toxin; STh, human heat stable toxin; STp, porcine heat stable toxin; SLT-I, shigella-like toxin type-I; SLT-II, shigella-like toxin type-II; EIEC, enteroinvasive *E. coli*; BfpA, Bundle-forming protien A gene; EAF, enteroadherent factor gene, EAE enteroeffacing factor gene; EAG, enteroaggregative gene, were provided by Department of Enteric diseases, The Armed Force Research Institute of Medical Science, Bangkok, Thailand (9,12).

Hybridization for DIG-labeled probes. After cell lysis and fixation of DNA samples on the membrane by UV cross-linked at 312 nm for 2 min, DNA samples were prehybridized with standard prehybridization buffer. Then, 10-20 ng/ul/ml of the probes in hybridization buffer were

reacted with the DNA samples at 65 °C overnight (1,12). With Genius system, positive spots were visualized by the colorimetric detection reagents using Genius buffer 1-4, NBT and X-phosphate (BCIP) according to the recommendation of manufacturer (10,11,12).

Statistic Analysis. The correlation of hemolytic activity and enteric virulent factor was analyzed by Chi-square test using Fisher's exact test.

Results

All 152 isolates used were characterized and confirmed as *E. coli* using routine biochemical tests. All isolats from diseased pigs (35 isolates) exhibited □-hemolytic zone on 5% sheep blood agar whereas 2 out of 117 (1.7%) isolated recovered from healthy pigs exhibited the zone.

The prevalence of virulent factors in 152 *E. coli* isolates was shown in **Table 1**. Three groups of exotoxin; LT, STp, and SLT-II, were detected in 20 of 37 hemolytic *E. coli* (HEC) (54 %) and STp and SLT-II were detected in 3 of 115 non-hemolytic *E. coli* (NHEC) (2.6 %). The numbers of exotoxin-positive in HEC group were significantly higher than that of NHEC group ($P<0.05$). 16/35, 45% of pigs with diarrhea were positive for SLT-II gene. There were 10 out of 35 HEC isolates possessed both of STp and SLT-II (28.5%).

All LT-positive bacteria exhibited hemolysin, whereas STp and STL-II-positive isolates could also be detected in NHEC isolates at 1.7 and 0.9%, respectively. On the other hand, specific DNAs encoding virulent proteins; EIEC, BfpA, EAF, EAE, EAG, were not found in any isolates.

Table 1. Comparison of the exotoxin gene detected and between hemolytic *E. coli* (HEC) and non-hemolytic *E. coli*. (NHEC).

Toxin	Number of Samples	
	HEC = 37	N-HEC = 115
LT	6 (16.2)	0
STp	14 (37.8)	2 (1.7)
STh	0	0
SLT-I	0	0
SLT-II	16 (43.2)	1 (0.8)
EIEC	0	0
STp+SLT-II	10 (27)	0

Numbers in parentheses indicate percentage.

Discussion

In the present study, we compared the number of *E. coli* possessing enterotoxin genes and other virulent factors and its relationship to the hemolytic activity was examined. The results indicated that approximately 50% of isolates from diarrheal pigs were not found the evidence of virulent factors. Therefore, checking for and ruling out viral or parasitic causes and herd history; past treatments, feed medication, incidence of scour should also be taken into consideration. The collection of the sample is critical and requires a rectal swab from a recently infected (ideally untreated piglet) or if the problem is severe the submission of live recently ill pigs provides the best material (3).

E. coli isolates from diarrheal pigs showed a markedly higher proportion of hemolytic *E. coli.* Hemolytic *E. coli* strains may be virulent because of a toxic effect on various cells, hemolysin is a pore-forming cytolysin causing the cell to swell and finally burst (10). This effect may be strengthened because the bacterial growth enhanced by iron available by lysis of the erythrocytes. From the previous study, most of *Escherichia coli* strains (serogroups O138, O139, and O141) isolated from post-weaning diarrhea of pigs possessed hemolytic activities and harbored the genes encoding for Shiga Toxin-II and ST enterotoxins (8). However, the results from this study indicated that 46% of HEC did not possess DNAs encoding exotoxin, whereas 2.6% of NHEC isolates possessed the DNAs. Generally, virulent factors cause enteric disease by triggering secret ion into gut lumen (2). However, pathogenesis may be exacerbated by co-operation of the virulent factors. Therefore it has been suggested that routine diagnostic observing hemolytic property may not be a good virulence marker of pathogenic isolates. More recently, hemolytic *E. coli* could be divided into two categories on the basis of the ability to produce cytotoxic necrotizing factor 1 (CNF1) and that $CNF1^+$ hemolytic *E. coli* is more virulent *in vivo* than those strains that produce only hemolysin (13,14).

Using DIG-labeled probes, it was clearly demonstrated that *E. coli* strains possessing genes for enterotoxin type (classified as ETEC; LT, STp and EHEC; SLT-II) could be observed the cause of post-weaning diarrhea in

approximately 50% of pigs with diarrhea. In Poland, most isolates (57.3%) from pigs with diarrhea were positive for LTI, or LTI in combination of STp or SLT-II toxin genes (7), whereas exotoxin gene detection of SLT-II or its combination with STp was a major cause of colibacillosis in Thailand.

On the other hand, a few NHEC strains harbored exotoxin genes especially the verotoxin (SLT-II) were detected in healthy piglets. The result was consistent to the evidence of non-hemolytic strains in Chili that showed some antigenic properties (11/18) (O64:K 'V142'; O157:K 'V17', O149:K91, K88, K99, and K89) (15). In addition, non hemolytic *E. coli* strains identified as enterotoxigenic *E. coli* (ETEC) could be found and caused enteric colibacillosis in other mammals such as dogs and experimental rabbits (2,4). It is possible to conclude that both hemolytic and non-hemolytic *E. coli* strains could be pathogenic. Therefore, the evidence of exotoxin positive NHEC from healthy pigs should be aware for possible contamination from healthy looking animals. In conclusion, a number of exotoxin genes (ETEC and EHEC) can be detected among hemolytic *E. coli* strains. However, routine diagnostic observing hemolytic property may not be a good virulence marker of pathogenic isolates.

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DIAGNOSTIC TOOLS FOR THE DETECTION OF ILEITIS INFECTION IN SWINE DUE TO LAWSONIA INTRACELLULARIS

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Introduction and Objective

Ileitis, also known as Porcine Proliferative Enteropathy (PPE), is a common enteric disease problem affecting pigs worldwide. The causative agent is *Lawsonia intracellularis*, an obligate intracellular Gram (-) bacterium. The subclinical form of the disease causes more severe economic losses to the farmer due to higher cost of production coming primarily from decreased weight gain, poor feed conversion, lower slaughter weights, some diarrhea, and dramatic weight and growth variation towards the end of the fattening period. The acute form causes severe hemorrhaging--black-red tarry feces, which leads to anemia and eventual sudden death mostly in finishers and/or young adult pigs. The chronic form is mainly characterized by diarrhea, growth retardation, and weight variation among pigs of the same age thus, making Ileitis a very important disease entity to contend with.

This paper aims to present a general insight on the existing diagnostic tools and techniques currently available for the diagnosis of PPE, either for ante-mortem and/or post-mortem studies, namely:

- Tissue Staining techniques, such as,
  - Haematoxylin and Eosin (HE)
  - Warthin-Starry (WS) silver staining
- Immunohistochemistry (IHC)
- Polymerase Chain Reaction (PCR)
- Indirect Immunofluorescence Assay (IFA)
- Enzyme-Linked Immunosorbent Assay (ELISA)

Discussion

Visual diagnosis of PPE is quite difficult owing to its non-specific, or sometimes even lacking, clinical presentation. In addition, with 4-6 weeks post-infection, the lesions would have healed[1,2]. Certain diagnostic methods and approaches must therefore be applied in order to effectively treat, control, and prevent Ileitis, particularly its sub-clinical form. Table 1 and 2 shows these different tools for diagnosis.

Table 1. Different Post-Mortem Tests and their Characteristics

TEST	SAMPLE	REMARKS
HE Staining	Tissues (ileum, cecum, colon)	Non-specific, demonstrates crypt epithelial changes only
WS Silver Stain	Tissues (ileum, cecum, colon)	Non-specific, demonstrates both intra and extra-cellular pathogen
IHC	Tissues (ileum, cecum, colon)	*Specific, but depends on sample quality
PCR	Tissues (ileum, cecum, colon)	*Specific, but depends on sample quality

***** Timing, quality and quantity of sample collection is very crucial and therefore dictates the outcome of the test result.

Table 2. Different Ante-Mortem Tests and their Characteristics

TEST	SAMPLE	REMARKS
PCR	Feces	*Specific, but depends on sample quality
IFA	Blood serum	Specific, but can become quite subjective depending on human skills
ELISA	Blood serum	Specific, semi-quantitative, machine-based, hence highly objective and repeatable test results

***** Timing, quality and quantity of sample collection is very crucial and therefore dictates the outcome of the test result.

Because *L. intracellularis* does not grow in conventional cell-free media, bacterial culture is uncommonly employed for diagnostic testing.

Since PCR detects the organism only when the concentration is at least 10^3 per gram of feces, not all infected pigs can be diagnosed[3]. Furthermore, the use of antibiotics will interfere with serological and virological tests, involving fecal PCR test[3,4], as well as Immunofluorescence Antibody test (IFA)[5].

In comparison to the other tests, the blocking Ileitis ELISA provides a more sensitive tool for faster and more reliable screening of large amounts of samples with the least risk of contamination and workload. The data can be measured and evaluated on a more objective basis, there are no unspecific fluorescence concerns thereby improving reproducibility, repeatability, and comparative analyses[6]. Other diagnostic tests are done only for a few pigs from a herd or rely on samples from dead ones. The blocking ELISA serological test, on the other hand, focuses on the dynamics of the disease through antibody values derived from live pigs--in relation to *Lawsonia intracellularis* field infection. It provides a powerful tool for serological diagnosis of Ileitis suitable for routine testing of large sample sizes and cross-sectional herd screenings[7].

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SALMONELLOSIS IN SUSPECTED CLASSICAL SWINE FEVER CASES IN THE PHILIPPINES

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Introduction and Objectives

Salmonella sp. infection is one of the most overlooked diseases worldwide. Its historical discovery was only in association with Classical Swine Fever (Hog Cholera). The identification and eradication of the viral etiology of the latter relegated Salmonellosis to an opportunistic pathogen in swine. It was only in the 1980's in North America that the pathogenic potential of *S. cholerasuis* was fully realized[2]. The purpose of this study is to determine the prevalence of Salmonellosis in 22 cases from commercial swine farms in the Philippines.

Materials and Methods

The study involved 22 cases suspected of Classical Swine Fever from Philippine commercial farms. Clinical signs and pathognomonic lesions served as bases for suspicion. From the period of January 2002 to November 2004, a total of 330 tissue samples (e.g. spleen, lungs, kidneys, stomach and lymph nodes) were tested using *Salmonella* PCR.

In order to rule out, most of these farms experienced high mortalities in the nursery and early growing phase. A positive case indicates the presence of even a single sample positive result.

Results and Discussion

Twenty-five percent (25%) of the samples tested and 68% of the cases tested registered positive for Salmonellosis. Out of 22 cases, 15 were positive to the test.

This indicates that in almost 7 out of 10 cases, Salmonellosis was also confirmed in farms with suspected Hog Cholera cases.

Table 1. Prevalence of Salmonellosis in Suspect-ed Hog Cholera cases.

	n	% Seropositive
Sample	**157**	**25%**
Cases	**22**	**68%**

In farms that have decided to control the problem directed towards Hog Cholera, variable results - from improvement to persistence of the problem - were noted. Proper and complete diagnosis should be done before final decisions on control measures are taken.

In most of these cases, however, no confirmatory test was conducted for Hog Cholera. Future studies should therefore take this in consideration.

This has a tremendous impact in possible zoonotic and epidemiologic implications. Reduction of the impact of infection is possible and should be implemented in farms confirmed for the said disease[3,4].

In the light of the ban using antimicrobials as growth promotants and the consciousness for "clean meat", reduction of *Salmonella* contamination and infection becomes an even more urgent and important objective in the industry.

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DETECTING THE PRESENCE OF *LAWSONIA INTRACELLULARIS* IN 17 SUSPECTED PPE-INFECTED PIG HERDS

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Introduction

The presence of Porcine Proliferative Enteropathy (PPE) has been confirmed in many countries worldwide since the successful isolation and identification of *Lawsonia intracellularis* from diseased pigs in 1993. Very few laboratories can test the *Lawsonia intracellularis* at present by PCR. Despite tedious and cumbersome in vitro culturing of *L. intracellularis*, China has made some initial studies. This article describes the study done on the detection of *L. intracellularis* in 17 suspected PPE infected pig herds in China, from January to October of 2004.

Materials and Methods

Suspected PPE cases were selected based on the following criteria: (a) sporadic outbreak of diarrhea in 2-4 month old swine; (b) cutaneous pallor/pale skin indicating anemia due to blood loss; (c) acute/sudden death among replacement gilts or growing-finishing swine;

Collection of test samples: feces from diarrhea cases and sections of ileal tissues were collected for PCR amplification.

The PCR reaction system: a pair of *Lawsonia intracellularis* specific primers was designed on the basis of published *Lawsonia intracellularis* sequences.
These were primer A: 5/ TATGGCTGTCAACACTCCG3/ and primer B: 5/TGAAGGTATTGGTATTCTCC3/, which amplifies a length of 318bp product. PCR was performed in a 25µl volume, containing 20× buffer 2µl, 2.5mM dNTP 2µl, 5U/50µL Taq DNA polymerase 0.5µL, 25mM $MgCl_2$ 2µl, primer A 1µl, primer B 1µl, total DNA template 7µl, DDW 10µl.

The circling parameters consisted of predenaturation at 95^0C for 15 min, then denaturation at 94^0C for 30 sec. Annealing at 50^0C for 2min, and extension at 72^0C for 30s, totaling to 35cycles, and finally by a terminal extension at 72^0C for 5 min. DNA purification kits and DNA amplification kits were supplied by Huamei biotechnology Co., Ltd.

Results

Results showed 12/17 positive reactions among the 17 suspected PPE infected pig herds, indicating a positive rate of more than 71%.

In the fecal and ileum tissue samples, 30/52 and 35/52 turned out positive, respectively, indicating an >85% correlation between fecal samples and ileum tissue samples.

Among the replacement gilts in herd 6, sudden death was observed, while diarrhea was the more common symptom seen after 2-3 days in other herds. Moreover in herd 10, sudden

death was observed in some pigs preceded by bouts of diarrhea.

Table 1. PCR test results of fecal vs. ileum samples from 17 suspected PPE infected pig herds:

Herds	Clinical signs		The number of samples		The results of PCR	
	diarrhea	acute death	Feces	ileum	feces	Ileum
1	+	-	3	3	0	0
2	+	-	3	3	2	2
3	+	-	4	4	2	3
4	+	-	3	3	3	3
5	+	-	3	3	0	0
6	+	+	2	2	2	2
7	+	-	3	3	0	0
8	-	-	3	3	3	3
9	+	-	3	3	2	3
10	+	+	3	3	2	3
11	+	-	4	4	3	3
12	+	-	3	3	2	3
13	+	-	3	3	0	0
14	+	+	2	2	0	0
15	+	-	3	3	3	3
16	+	-	3	3	2	3
17	+	-	4	4	4	4
total			52	52	30	35

Discussion

Results showed that *Lawsonia intracellularis* is the main culprit causing diarrhea among 2-4 month old pigs. PCR was the test of choice for this particular study, considering the difficulty of bacterial isolation via in vitro culturing of *Lawsonia intracellularis* organism. Positive and negative control should be included as a consequence of the relatively high sensitivity of PCR method and likewise, to avoid accidental false-positive results. Likewise, enough attention must be paid in order to avoid cross-contamination during sample collection, treatment, amplification and electrophoresis. Because of the detected high correlation between fecal shedding and ileum tissue antigen PCR detection, feces from diarrheic pigs may be collected for the early diagnosis of ileitis due to *Lawsonia intracellularis* in order to mitigate further economic losses.

Tiamulin given via the drinking water was used to treat animals which tested positive via PCR for *L. intracellularis*. Recovery after a 3-5 day medication using the said regimen further corroborated the PCR detection results as shown in this study.

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Immunology

FIELD EXPERIENCES WITH INGELVAC® PRRS MLV IN CONTROL OF PRRS IN JAPAN

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Introduction and Objectives

Control of Porcine Reproductive and Respiratory Syndrome (PRRS) has been topic for discussion for many years. Successful control strategies involve stabilizing the breeding herd which has been consistently achieved globally by mass vaccination with PRRSV modified live vaccines in combination with sound animal management.

This article discusses three PRRS cases in Japan.

Case 1:

In a farrow to finish farm (two-site system) sows were routinely vaccinated against PRRS with Ingelvac® PRRS MLV with the 6-60 program, gilts which were recruited internally twice at 180 and 210 days of age (dd) however, no piglet vaccination was performed. In June 2003 nursery mortality went up by 5% without any remarkable clinical signs in sows. However, PRRS ELISA titres strongly varied

After a thorough examination of the situation the vaccination program was modified as follows:

- Internal replacements were vaccinated at 180dd, 210dd and 240dd in the isolation barn with Ingelvac® PRRS MLV
- Sows were vaccinated in a whole herd mass vaccination scheme with Ingelvac® PRRS MLV four times per year
- Piglets were vaccinated with Ingelvac® PRRS MLV at two weeks of age

Results:

Nine months after modifying the program titres stabilised and nursery mortality decreased to 2%.

Case 2:

In a 1600 sows 3-site system mass vaccination was applied in sows 4 times per year, including a double vaccination of replacement gilts. However, although the sow herd seemed to be stable, nursery mortality went up to 22% in June 2003. Following serological testing, it became obvious that the field PRRSV was circulating in the nursery. Inadequate pig flow management appeared to be responsible for impaired performance as weaned pigs with different immune status were mixed in the nursery; however the production system did not allow major modifications on the pig flow. Following consultation with the vet and pig farmer it was decided to implement additionally vaccination of piglets at 14 d. In October 2003 nursery mortality declined to 2.5% and is stable since this time. Piglet vaccination distinctly distributed to the health performance of the herd and ELISA profiling was the major tool to fully understand the situation in the herd.

Case 3:

In a 700 sow farrow-to-finish herd with continuous flow management sows were exposed to PRRS field virus by feeding placenta of infected sows. Gilts were acclimatised by natural exposure to PRRS field virus at the finishing site and piglets were not exposed to PRRSV at all. In the course of this "control" program, farrowing rate decreased from 94% to 73% and the abortion rate of 1st parity sows increased distinctly. Serological investigation provided evidence that the breeding herd was very instable and in order to recover the farrowing rate it was decided to stabilize the immune status by an adequate gilt acclimatization program. Accordingly, the

uncontrolled field virus exposure by feeding placenta of infected sows was ceased and mass vaccination with Ingelvac® PRRS MLV (4 times/ year) was implemented. Replacement gilts were kept in an isolation barn for a minimum of 2 month (from 6 to 8 months of age) prior to introducing into the breeding herd. One year after the implementation of mass vaccination of sows and a proper isolation period of internal replacement gilts, the farrowing rate went back to 84%. It also seemed to be crucial that isolation of internal gilts helped to contribute to increasing the overall reproductive performance. However, it took up to 12 months until pre-break reproductive performance were achieved again.

Discussion

In conclusion, this report suggested that mass vaccination with Ingelvac® PRRS MLV in combination with adequate acclimatisation of replacement gilts, piglet vaccination and appropriate pig flow management proved to be a successful tool in control PRRS in Japan. In these reported cases, it was experienced that Ingelvac® PRRS MLV mass vaccination provided sufficient results in terms of efficacy and safety, compared to the exposure of the animals to the PRRS field viruses in the way they used to practice at these farms. It should be also noted that an adequate pig flow, a basic hygiene management, and appropriate diagnostic/monitoring examinations are essential to maximize the success of PRRS control using Ingelvac® PRRS MLV.

Aluminum Hydroxide Adjuvant Inactivated Vaccine to Porcine Parvovirus

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Abstract: Porcine Parvovirus(PPV)Z strains isolated from aborted piglets in ZheJiang province were used to develop the Aluminum Hydroxide Adjuvant inactivated Vaccine to Porcine Parvovirus, PPV were propagated in porcine kidney cell culture and inactivated by formaldehyde, it were mixed Aluminum Hydroxide Adjuvant at normal rate to make the vaccine, a little dose vaccine inoculation could induce high titer antibodies in adult pig and guinea pig,HI titers were over 1:320(1:640~1:1280 in general) when Guinea pigs were immunized twice,HI titers could reach the rang from1:20~1:320 when adult pigs were vaccinated twice with dose of 0.2ml、0.5ml、1.0ml respectively or once with dose of 2.0ml,immunization efficacy test of the vaccine showed that over 98.7% fetal piglets were protected. The result of lab tests and field trails indicated that the vaccine has the advantage of long period of immunity and safety.

Key words: Porcine Parvovirus; Vaccine

ARE ONE-SHOT MYCOPLASMA HYOPNEUMONIAE VACCINES ALIKE? – RESULTS FROM A FIELD INVESTIGATION IN AUSTRIA

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Introduction and Objectives

Vaccination against Mycoplasma hyopneumoniae (M.hyo) has become a major tool in controlling the clinical signs and economical impact of enzootic pneumonia (1, 2). Recently so-called one-shot products have become available and subsequently gain market share all over the world. The objective of this study was to investigate the efficacy of two commercially available one-shot products (i.e. Ingelvac® M.hyo and Stellamune® One) under field conditions in Austria.

Materials and Methods

The trial was carried out in a commercial farrow-to-finish farm in Austria with confirmed M.hyo circulation. A total of 380 piglets of two different sources were randomly allocated to two vaccination groups receiving either Ingelvac® M.hyo at 3 weeks of age or Stellamune® One one-shot vaccine at 8 days of age. In order to eliminate possible time effects the study was run in two phases. The primary parameter for efficacy was the lung lesion score; which was evaluated in all animals from each of the vaccinated groups sent to slaughter. The lungs were assessed for M.hyo typical lesions according to Madec and Kobisch (3). Animals of both vaccinated groups had identical fattening periods. Furthermore, production parameters such as slaughter weight and lean meat were measured. The available data were analysed with SAS, version 8e. The data were evaluated by analyses of variance procedures (ANOVA) and a model was fitted which accounted for the vaccine used and the source of origin of the animals as interaction term. Differences were analysed applying the Tukey-Kramer Test. Results were considered significant if $p \leq 0.05$.

Results and Discussion

A total of 187 pigs were vaccinated with Ingelvac® M.hyo, 193 pigs were vaccinated with Stellamune® One.
Table 1 summarizes the results of the Tukey-Kramer Test between the two vaccinated groups.
As evident from this table, vaccination with Ingelvac® M.hyo resulted in significantly lower lung lesion scores as compared to Stellamune® One with a mean lung lesion score of 2.22 for the Ingelvac® M.hyo group and a mean score of 5.71 for the Stellamune® One group.

Table 1: Results of the Tukey-Kramer Test

Variable	Ingelvac® M.hyo LSmeans	Stellamune® One LSmeans	Probability p (Tukey-Kramer)
Weaning weight (kg)	8.28	8.09	0.20
Slaughter weight (kg)	89.18	86.90	0.15
Lean meat %	57.54	56.91	0.76
Total pneumonia score	2.22	5.71	**< 0.0001**

The significantly total lower lung lesion score of the Ingelvac® M.hyo group was a result of a general significant lung lesion score of each of the seven lung lobes, reflecting a general superior lung

health status of the Ingelvac® M.hyo group. Due to the fact that due to the slaughter house procedure the slaughter weight of only 176 animals could be recorded in total, the numeric but not statistical advantage of 2.28 kg of the Ingelvac® M.hyo group over the Stellamune® One group can be surely attributed to the too low sample size.
In summary, vaccination with Ingelvac® M.hyo proved to be superior over vaccination with Stellamune® One with regard to the lung health status.

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The production and primarily application while diagnosing of pig pseudorabies virus high immune serum

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Abstract: This study produced the pseudorabies virus (PrV) high immune serum by multi-incubated the pseudorabies antibody negative healthy pig with strong pseudorabies vaccine strain S with different doses at certain intervals. And primarily identified the high immune serum by neutralization test, ELISA and immuogold labeling test strip. The results approved that the PRV high immune serum produced by this method is of high specification, the titer is over 256 times, completely meet the needs of the diagnostic requirements.

Key words: pseudorabies virus；high immune serum；ELISA；neutralization test；immuogold colloid

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No. 1 factory of Heilongjiang province biological product

A CASE OF PRRS AND PCV 2 CONTROL IN THE PHILIPPINES

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Introduction and Objectives

Co-infection of PRRS and PCV-2 have been reported to be high, as much as 76%[1,2]. Previous works from other authors showed vaccination with a modified live PRRS vaccine have resulted to at least 6% decrease in pre-weaning and at least 3% in nursery and at least 1% in fattener mortalities[3]. This case report discusses an experience of such a co-infection with a commercial swine farm in the Philippines.

Materials and Methods

The Farm is a 600 sow level operation, continuous farrow-finish. High mortalities mainly in the nursery (average of 6.71%) and growing finishing (average of 6.63%) was observed from July 2002 to January 2003. Total mortalities went as high as 34.87% in September of 2002 (manifested as App-dominated PRDC lesion). In the months of October-November, 2002, confirmed cases of Colibacillosis and Coccidiosis were also observed.

Based on clinical signs, post-mortem lesions and serological evaluation (PRRS ELISA, IDEXX®) as seen in figure 1 as well as Fluorescent Antibody Test (FAT) for PCV-2, the farm was diagnosed to have PRRS and PCV-2 as coinfection between December 2002 to January 2003 although PMWS signs were seen as early as November.

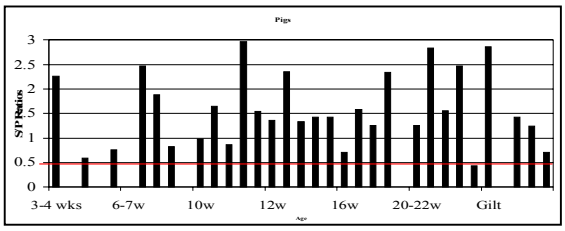


Figure 1. Serological Profile of the farm before control

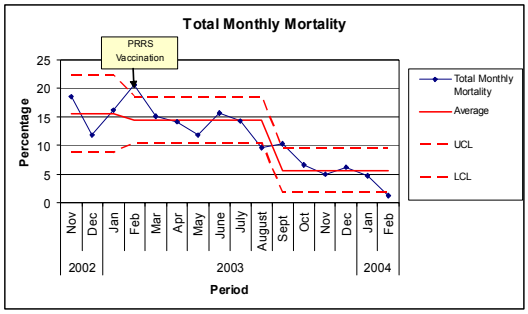
In February 2003, an initial mass vaccination of breeders and pigs up to end of nursery (10-11 weeks old) was done, followed by a maintenance program where sows are vaccinated 1 week post-farrow and pigs 1 week pre-weaning with a Modified Live Vaccine (Ingelvac® PRRS MLV). The production data before and after vaccination were then compared using averages and Statistical Process Control (SPC).

Results and Discussion

Before vaccination, total mortality (birth to market) was at 15.53%. Seven months into the intervention, the mortality dropped slightly to 14.45%, and then to 5.65%.

Figure 2 shows the progression of the mortalities before intervention and the continuous reduction thereafter. Both the averages and variability, as set by the control limits, also improved.

Figure 2. Total Mortality (Nov 2002-Feb 2004)



The signs & lesions indicative of PCV-2 infection (both PMWS and PDNS form) subsequently subsided in the middle of March, less than a month after vaccinating with PRRS.

This report coincided with the report of the previous author on the control of PCV-2 lesions through the control of PRRS.

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A META-ANALYSIS ON LUNG LESIONS IN PIGS AT SLAUGHTER IN BRITTANY (FRANCE)
RESULTS OBTAINED WITH DIFFERENT MYCOPLASMA VACCINES

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Introduction and Objectives

Mycoplasma hyopneumoniae (M.hyo) is the causative agent of enzootic pneumonia (EP) in pigs. Vaccination against EP has become a major tool in controlling the clinical signs and economical impact of this disease (Kyriakis, 2002). For years, vaccinating pigs with two doses has been shown to distinctly reduce *Mycoplasma hyopneumoniae* induced lung lesions. A paradigm shaft occurred when in mid 2002 the first one-shot vaccines were introduced Europe-wide. For this purpose a meta-analysis was performed based on a broad field setting which was carried out from October to December 2003 in Brittany, France, involving almost 73.000 lungs.

Material and methods

The French Institute for Animal Production and agricultural food products (ISPAIA) trained seven technicians thoroughly the lung scoring technique described by Madec and Kobisch and allocated them subsequently to different slaughter plants in Brittany, France.

The scoring service was offered to swine vets for elucidating efficacy of the applied *Mycoplasma hyopneumoniae* vaccine in their client farms.

Macroscopic lesions were quantified using a lung lesion score grid, as described by the French Technical Institute (ITP) (2002).The results of the individual lung scorings were amended by a questionnaire that included information on farm size, location, management techniques as well as routinely performed vaccinations.

The data were analyzed by a two factorial ANOVA, applying the proc glm procedure in SAS, version 8e.

Results

Completed questionnaires were returned from 691 farms which included results from 1220 pig batches. This response represented 72% of all questionnaires sent out. The Mycoplasma vaccines used in the respective farms included six different M. hyo vaccines: two belonged to the one-shot products and four products were two-dose products.

Lung scores of the vaccinated animals were significantly better than the unvaccinated controls providing evidence for the necessity of implementing vaccination protocols against *Mycoplasma hyopneumoniae* ($p \leq 0.05$).

As evident from table 1 both vaccine types (one-shot and two products) provided similar efficacy with regard to lung lesion scores.

Table 1: Comparison between one-shot and two
shot products

	One Shot	Two Shot
farms	192	236
batches	326	444
LS mean score	3.72 n.s	3.36 n.s

n.s. = not significant

Table 2 provides the results of the individual comparisons of the depot-one shot M.hyo vaccine and the other applied vaccines.

Table 2: individual comparisons between the depot one-shot product and the other vaccines

Individual comparison	Significance (p)
Depot one-shot vs. vaccine A (two-shot)	Not significant
Depot one-shot vs. vaccine B (two-shot)	Not significant
Depot one-shot vs. vaccine C (one-shot)	Not significant
Depot one-shot vs. vaccine D (two-shot)	Not significant
Depot one-shot vs. vaccine E (two-shot)	$p \leq 0.05$

In summary, based on a meta-analysis involving more than 70.000 lungs in Brittany it could be impressively shown that vaccination against EP significantly reduces the associated losses and the trend to using one-shot vaccines could be clearly laid down and explained. The trend for superiority to apply a depot one-shot vaccine over other products seems to be justified based on individual comparisons.

Induce farrowing in sows by using Cloprostenol (Planate®): studies on behavioral changes, timing of farrowing, duration of parturition, total born litter size and stillbirth rate

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Introduction

The use of Prostaglandins ($PGF_{2\alpha}$) for the controlled induction of farrowing has been successfully and widely used worldwide in pig farms, including Thailand. The advantages are to improve batch farrowing and to induce farrowing in the daytime which is easy to supervise farrowing in sows, prompt assistance may be given to sows which may have difficulty. This is safer delivery of the piglets and thereby reduces piglets mortality. This paper was performed to evaluate the efficacy of different doses and route of administration of Cloprostenol (Planate®)

Materials and methods

Twenty multiparous sows (Yorkshire x Landrace) were housed individual pens in a large enclosed fan-ventilated house under natural daylight and temperature varied between 25-32 ºC. They were selected for induction of parturition on days 113 to 114 of gestation. The average gestation period of sows on this farm was 115 days. Sows were fed with commercial feed and water was *ad libitum*. Parturition was induced by one of the following treatments:

- 175 µg $PGF_{2\alpha}$ (Cloprostenol,Planate®) administered IM, group A (n = 10);
- 85 µg $PGF_{2\alpha}$ (Cloprostenol,Planate®), half the manufacturer's recommended dose, administered IM at perivulva area, group B (n = 10)

Sows were observed for the sign of parturition, starting at 24 h after injection of Cloprostenol (from 7 am to 9 pm). The following parameters were evaluated: time from treatment to the onset of farrowing, expulsion intervals, duration of parturition, total number of piglets born (stillborn, mummy and born alive), umbilical cord morphology, meconium staining and bodyweight of each piglet.

In addition, sows were observed for mothering behaviour immediately after Cloprostenol injection for 1 h. The behavioural observations including posture (kneel, lie belly, lie side, sit, stand), nesting behaviour (grasping behaviour, paw floor, paw wall, root floor, root wall, step) and others behaviours (inactive, defecate, urinate, drink, chew fixure, object scratch, rear leg scratch, shake head) were also recorded.

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Results and Discussion

Times from treatment to the onset of farrowing are shown in Table 1. Zootechnical data relative to sows and piglets are shown in Tables 2, 3 and 4.

Table 1. Time from treatment to the onset of farrowing in which was divided into three groups.

Hours to farrowing	Groups (10 sows in each group)	
	A full-dose	B half-dose
<24 hr	5	4
24-30 hr	5	6
>30 hr	0	0

Table 2. Onset of farrowing, farrowing duration, expulsion interval, litter size, total born, presentation and piglet weight.

Variables (Mean ± S.D.)	Groups (10 sows in each group)	
	A full-dose	B half-dose
Hours to farrowing	26hr 3 min (n=6)	26 hr 2 min (n=9)
Farrowing duration	5hr 39 min (n=6)	4hr 29 min (n=9)
Expulsion Interval	22.88±9.97	24.20±8.90
Litter size	10.5±3.44	12.7±1.64
Ttotal piglets born	105	127
* liveborn	95 (90.48%)	118 (92.91%)
* stillborn	9 (8.57%)	7 (5.51%)
* mummy	1 (0.95%)	2 (1.58%)
Anterior presentation	65	82
Posterior presentation	22	30
Piglets weight	1.58±0.27	1.36±0.54

Table 3. Morphology of umbilical cord from 205 piglets

Umbilical cord morphology	Groups	
	A full-dose (n=93)	B half-dose (n=112)
Normal	66 (70.97%)	85 (75.89%)
Edema	0	0
Congestion	17 (18.28%)	24(21.43%)
Hemorrhage	10 (10.75%)	3 (2.68%)

Table 4 Degree of meconium-stained on liveborn and stillborn piglets (altogether 205 piglets)

Birth	Grading of stain	Groups	
		A full-dose (n=93)	B half-dose (n=112)
Liveborn	negative	51	70
Liveborn	mild	30	31
Liveborn	moderate	1	5
liveborn	severe	2	1
Stillborn	negative	4	3
Stillborn	mild	4	2
Stillborn	moderate	1	0
Stillborn	severe	0	0

The present data confirmed the observation in that a half dose of Cloprostenol (Planate®) administered at perivulva region was as effective for inducing farrowing as the full recommended dose injected at neck area (IM). For the other criteria relative to the sows or to the piglets no significant difference was observed between groups. The mothering behaviour (e.g. nesting, restless) was not observed in both groups. However, the behaviours such as lie on belly and drinking, defecations, kneel and root wall can be observed. The reason for the efficacy of the half dose of Cloprostenol administered at perivulva area for inducing parturition was not clearly elucidated.

In conclusion, the present data showed that induction of parturition using half dose of Cloprostenol can be as effective as using full dose, which in turn, reduce a cost of induce farrowing.

Acknowledgement

Planate® for this study was generously provided by Schering-Plough Animal Health (Thailand)

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Immunology and Pharmacology

Use of Suipravac® Killed PRRS vaccine to Control Respiratory disease in nursery pigs

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Introduction and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSv) can infect pig of all ages. PRRSv infection make pigs more susceptible to bacterial diseases (1, 2). Management strategies have been attempted to control PRRS and had inconsistent success (3, 4). The objective of this study was to evaluate the impact of gilt and sow vaccination with Suipravac® (Laboratories Hippra, S.A.) on nursery and grower performances in two different management and production systems.

Materials and Methods

Farm A is a 2000 sow farrow-to-finish farm. It had been PRRS negative for 4 years. The farm decided to import 30 PRRS-free boars and left them at a (believed) PRRS-free farm for a month before transported to the farm. PRRS broke few days after the new boars had arrived. Pregnant sows were feverish, cyanotic and 5 sows died within 2 days. Sow mortality stopped after Ceftiofur injection, but sickness and late/mid-term abortions continued. Preweaning mortality was almost 100%. Nursery and grow-finish pigs became sick one week after the first sing in breeding pigs. The boars were also sick.

Second blood samples of the boars were taken when they arrived on the farm. Serum samples from breeding pig were also collected at day 2 of the outbreak, and from the same sows 2 weeks later. Partial sow and nursery depopulation, all-in/all-out pig flow and twice blanket PRRS vaccination in breeding pigs 2 weeks apart with Suipravac® were implemented soon after PRRS virus infection has confirmed. Then 2 weeks pre-farrowing vaccination program was followed. Farrowing and nursery houses were changed to mechanical ventilated buildings. Nine weeks old pigs were moved to the opened finishing barn.

Farm B is a 1100 sow continuous flow farrow-to-finish farm. The farm was chronically infected with PRRSv (positive virology and serology) characterized by agalactia, light weight weaning pigs and severe respiratory diseases in nursery. Nursery mortality was 20 to 30%. The thumping sign began in 5 to 6 weeks old pigs. Nursery pigs were in an open building and then moved to finishing barns when they were 8 weeks of age. PRRS vaccination program was the same as Farm A. Seroprofiles were determined before and after PRRS vaccination.

Results and Discussion

Farm A. PCR test has confirmed the new boars were the source of PRRS virus. Isolation of replacement stock is one of critical biosecurity measures for PRRS. Pair serum of the sows and nursery seroconversion indicate PRRSv infection. Necropsy of the dead sows revealed pleuropneumonia disease. PRRSv infection in naïve sows appears to be severe, and *Actinobacillus pleuropneumoniae* could take this opportunity to kill the sows.

Farm B. Before PRRS vaccination, pigs of all ages were already PRRS seropositive. Necropsy of nursery pigs found polyserositis and pneumonia resembling mixed viral and bacterial infections. Pigs did not respond to antimicrobial treatment even though antibiotics were added in nursery diet at all time (Tiamulin, CTC, Colistin). Some pigs responded to amoxicillin/Enrofloxacin injection and then few days later became sick again.

One month after PRRS vaccination, reproductive problem return to normal in both herds. Within 6 months, farm A has nursery mortality less than 2%, but farm B is still having Streptococcosis and Glasser's diseases although nursery mortality reduces to 6%. Maternal antibody lasts only 3 to 4 weeks of age in farm A (Fig.1). This could be due to lack of wild type virus circulation in breeding pig. PRRSv infection occurs at around 9-10 weeks when the pigs were moved to finishing house (Fig.1). Older finishing pigs in an open building could spread PRRS virus to younger pigs. The pigs on farm A were depressed and having conjunctivitis, then recovered within 2 days. On farm B, Glasser's disease is difficult to eliminate because of a continuous pig flow system. Sow vaccination with Suipravac® combine with

partial depopulation of unhealthy looking pigs and running all-in/all-out pig flow in a close building can effectively control respiratory disease in nursery pigs.

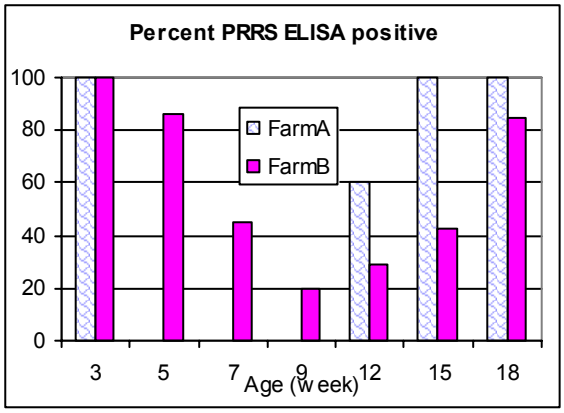

Fig.1 PRRS serum profiles of pigs after 6 months of Suipravac® vaccination. There is no PRRS virus infection during nursery. Pigs have active seroconversion at 12 weeks of age.

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EVALUATION OF MASS VACCINATION ON PRRS RESPIRATORY SYMPTOM IN SOWS AND PIGLETS

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Introduction and Objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) is generally characterized by reproductive disorders of sows, and respiratory disease of piglets. Also PRRS plays an important role in multi-factorial respiratory problems in the field which makes clinical signs often very variable. It has been reported that an acute clinical outbreak of PRRS in sows can dramatically exacerbate endemic diseases (White 1992a). The presence of PRRS may exacerbate both morbidity and mortality in *Actinobacillus pleuropneumoniae* disease. And It was demonstrated that PRRSV infection predisposes pigs for respiratory signs when they are subsequently exposed to bacterial lipopolysaccharides(LPS) (Labarque G,at al 2002 Vet Microbiol cond.accepted).

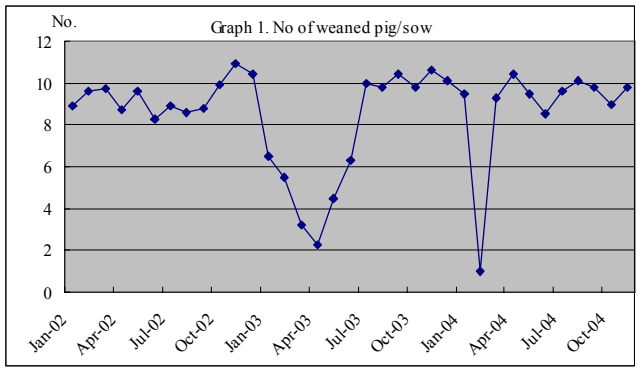
This paper discusses the successful control of respiratory disease associated with APP and PRRS in sows and piglets with the mass vaccination of Ingelvac® PRRS MLV.

Materials and Method

History : The 230 sows herd, is located in Kyoenggi province, Korea. The two sites of the farm are about 10km apart from each other. Routine vaccinations included Parvovaccination of the sows and 1-shot Mycoplasmavaccination of the piglets at the age of 14 days. In January 2003 respiratory problems were noticed in the sow herd. Sows had hyperthermia, anorexia and coughing. Although the farm was treated by antibiotics such as florfenicole and amoxaciline according to veterinary prescription the clinical signs did not improve. 17 sows (7%) died succumbed to respiratory disease until February. At the end of January diagnostic investigations were initiated by necropsy of 2 affected sows and blood sampling on the farm. APP and PRRSV were diagnosed using ELISA test, IFA test and isolation of PRRSV as well as gross and microscopic lesion. In February respiratory disease in suckling and nursery piglets was increasing and the mortality went up. In March the mortality of piglets was about 70%. Due to clinical signs and diagnostic results it was decided to mass-vaccinate all sows and piglets up to the age of 50 days in April.

Results and Discussion

The number of weaned piglets/litter is shown in Graph 1.



After the start of respiratory problem in the sow herd, the number of weaned piglets per sow decreased heavily due to the death of farrowed sows, reduction of nursing ability in sick sows and respiratory problems in piglets. The number of abortion was not remarkably increasing at that time. After mass vaccination of Ingelvac® PRRS MLV, the number of weaned piglets/litter quickly returned to normal and actually even exceeded the number before the outbreak. While the number was still down to 6.3 in June the disappearance of respiratory signs was paralleled by an increase to 10 in August. Production was back to normal except in February 2004, when the farm had massive PED problems in the farrowing house.

After the first whole herd mass vaccination, the vaccination with Ingelvac® MLV was done (according to the current Korean label) by piglet plus sow vaccination at the age of 7 to 10 days after parturition.

In this case report an acute PRRS + APP outbreak in the sow herd lead to massive respiratory disease and increased losses in sows and piglets. Mass vaccination with Ingelvac® PRRS MLV was effective method to control PRRS-related respiratory disease in sows and piglets and may also have reduced the susceptibility of the herd for APP.

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Effects of Mycoplasma hyopneumoniae Vaccines on Interferon-gamma and Performance in Pigs

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Abstract:
To prove the immune protection conferred by active immunization using Mycoplasma hyopneumoniae vaccines as opposed to acquired immunity from natural exposure to field Mycoplasma hyopneumoniae pathogen, taking into account its impact on pathological changes in the lungs and subsequent pig performance, we selected a farm for study in South China in which the disease was highly prevalent.

Three hundred (300) pigs were evenly separated into 3 groups. Group A was injected a dose of vaccine A on the 21st day, intramuscularly. Group B was injected a dose of vaccine B on the 7th and 21st day intramuscularly as well. Cohort C was left unvaccinated. Blood samples were sequentially collected beginning at 4 weeks, then at 10, 16 and 32 weeks old. The level of IFN-γ in the serum and the pigs' performance were recorded during the different phases of the growth cycle. Pigs which manifested clinical pneumonia were treated accordingly. Upon post-mortem examination, the affected organs, including the mediastinal lymph nodes, were checked for any visible pathological changes. All the lungs were coded and scored.

The level of IFN-γ in the vaccinated groups was significantly higher (P≤0.01) versus the unvaccinated group. Likewise, Group A exhibited higher levels of IFN-γ compared to group B (0.01≤P≤0.05). Post-mortem lung scoring showed significantly better scores in Group A versus Group B (P≤0.01), while that of Group B was better compared to Group C (P≤0.01). Moreover, the vaccinated groups had higher average daily weight gain (ADWG), better feed conversion rate (FCR), mortality rate, and lower treatment cost compared to the unvaccinated group (P≤0.01). Group A had higher ADWG, lower FCR, and lower treatment cost (0.01≤P≤0.05).
Key words: vaccination, IFN-γ, performance, pigs

Swine Enzootic Pneumonia (SEP) due to *Mycoplasma hyopneumoniae* infection has a very high incidence in China--seriously affecting majority of the pig farms chronically—usually with low mortality rate. Horizontal transmission from infected gilts and sows is not uncommon. Reportedly, the growth rate and feed conversion efficiency are reduced by 15.9% and 13.8% respectively, translating into an economic loss of about 2.8 $ per pig. The process by which the disease develops is quite complex, which includes pathogen entry, bacteremia and spread, pathogenesis, cytotoxicity, competitive substances, exclusion and immediate host immune response [(1)]. Vaccination induces cell- mediated immunity and humoral immunity synchronously. Studies show that antibodies present locally in mucous membrane of different animals, including cattle and mice, are able to mount a certain degree of protection against Mycoplasma hyopneumoniae. Cell-Mediated Immunity (CMI) plays an important role in this[(2,3)]. Mycoplasma hyopneumoniae antigens in vaccines stimulate the body to produce antibodies via the activated lymphocytes. The cells producing the IFN-γ induced cellular immunity. Kuhn MJ, et al, reported that positive rate of IFN-γ secreting cells increased after giving both the primary and secondary vaccination[(4)]. IFN-γ is secreted by the activated T-cell which is

the key element in a cell-mediated immunity (CMI). On the 6th day, the vaccinated pigs which were subsequently challenged with SEP showed remarkably higher levels of IFN-γ secreting cells than the others exposed through other means. On the 28th day post-infection, the unvaccinated pigs which were later challenged showed the highest level of IFN-γ secreting lymphocytes. This shows that the activated systemic T-cells play a very important role in mounting an effective protection against Mycoplasma hyopneumoniae in pigs after both active vaccination and natural field exposure [5]. The absence of IFN-γ affected the pig's innate immune system's ability to effectively clear the body of Mycoplasma, with a corresponding increase in disease severity [6]. The degree of pneumonic lesions in affected lung surface in vaccinated-challenged pigs was significantly reduced at day 70--vaccination stimulated the secretion of IFN-γ secreting lymphocytes in the blood against M. hyopneumoniae [7]. The extent of pathologic changes/damage among vaccinated pigs was lesser compared to non-vaccinated challenged pigs [8].

The objective of this study is to compare the efficacy of two commercially available Mycoplasma hyopneumoniae vaccines and their role in inducing a protective immunity to pigs against Mycoplasma hyopneumoniae challenge, as measured by the degree of gross pathological changes in the lungs, with its corresponding economic impact on farm productivity.

1. Materials and method

1.1 Choosing the farm and grouping the pigs.

1.1.1 Choice of farm

The farm has a history of exposure to M. hyopneumoniae infection, and exhibits clinical signs and lesions indicative of enzootic pneumonia. It is located in an area south of China. This farm has 640 sows and follows a continuous farrow-to-finish production system. The pigs are housed in either conventionally and/or specially designed farrowing,nursery, and growing-finishing barns. The boars are confined separately within the gestating barns with the sows, while the replacement herd is placed within developing houses situated apart from the rest of the buildings.

The farm still follows a natural breeding program using genetics originating from local suppliers.

1.1.2 Classification and vaccination:

Group A: 100 piglets of the same weekborn were vaccinated with Ingelvac® M.hyo at 3 weeks of age.

Group B: 100 piglets of the same weekborn as Group A were vaccinated with Respisure® at 1 and 3 weeks of age.

Group C: 100 piglets of the same weekborn as Group A were left unvaccinated.

1.1.3 Parameters observed and monitored

The following parameters were monitored: Morbidity rate, mortality rate, individual birth weights, individual weights at end of nursery, individual weights at market age, FCR, cull rates, treatment cost, days to market, and lung lesion score at slaughter. Statistical evaluation and interpretation was performed.

1.2 Trial

1.2.1 Cross-sectional sampling of groups A, B, and C

Production stage	Sample Size /
Start of weaning: 0 week old	5
At weaning: 4 weeks old	5
Grower-Finisher	
10 weeks old	5
16 weeks old	5
23 weeks old	5
Total	25

1.2.2 Serological test
Using IDXLL ELISA kits sera test IFN-r

1.2.3 Observations and record monitoring
Incidence of M.hyo, and clinical signs among each group, vis-à-vis other diseases; feed conversion ratio per group; ADG per group; treatment cost per group.

1.2.4 Lesions
Sacrifice chosen animals and perform necropsy per age group, record corresponding degree of M.hyo. lung lesion. Prepare Paraffin sections of lung tissue samples, stain with Hematoxylin – Eosin (H-E) dyes, do VIP immunohistochemistry test, observe the distribution of VIP in the lung tissues. Prepare paraffin sections of lymph nodes, stain with H.E., do VIP immunohistochemistry test, and observe the distribution of VIP and lymphocyte in the lymph nodes.

2. Results

2.1 Serological response (antibody production) in pigs at different weeks of age after vaccination with Ingelvac M. hyo and Respisure;

2.2 Degree of gross pathologic changes in the lungs post-vaccination and challenge.

Group	A	B	C
# of lungs samples	90	87	80
Average lung score	2 .85	3.56	4.65

Results showed a better average lung score in the vaccinated group compared to the unvaccinated group.

2.3 Impact on the lung tissue integrity
There were minimal pneumonic lesions seen microscopically in the lungs of vaccinated groups (Fig.1), whereas, serious microscopic lesions were seen in those of unvaccinated pigs (Fig.2). The amount of lymphocytic infiltration increased within and around blood vessels, bronchi, bronchioles and in mucous membranes lining the respiratory tract. There were abnormal proliferations of "**eyewinkers**" and monocytes in the alveolar sacs and respiratory passages, with pronounced thickening of the alveolar septum (Fig 3).

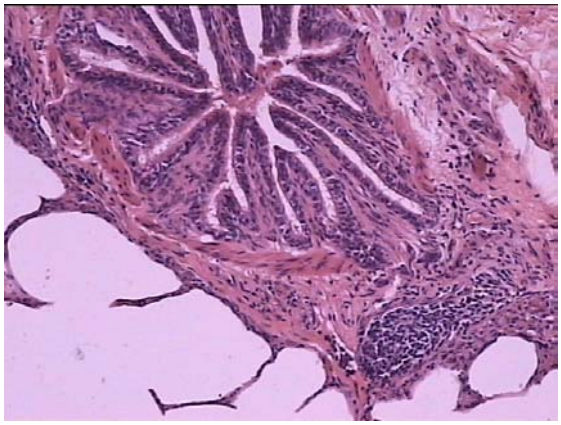

Fig 1. Normal bronchioles, lymph nodule

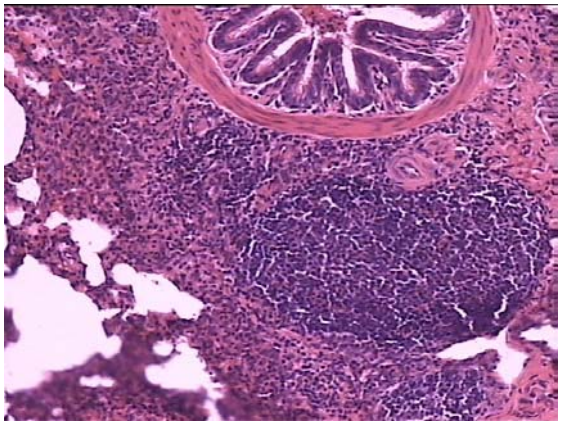

Fig 2. Increased Lymphocytic proliferation

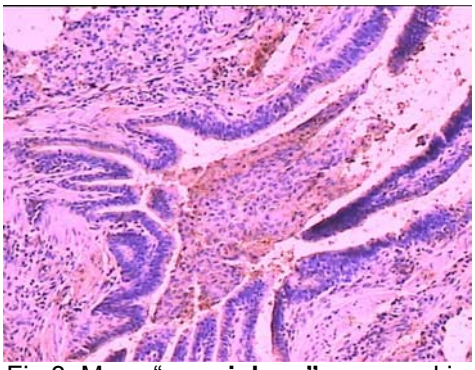

Fig 3. Many "**eyewinkers"** appeared in sacs and bronchioles

2.4 Impact on the mediastinal lymph nodes

In general, the mediastinal lymph nodes of the pigs belonging to the vaccinated group appeared normal (Fig. 4), whereas, so much proliferation was remarkably seen in the unvaccinated group, with increased lymphocytic infiltration (Fig. 5).

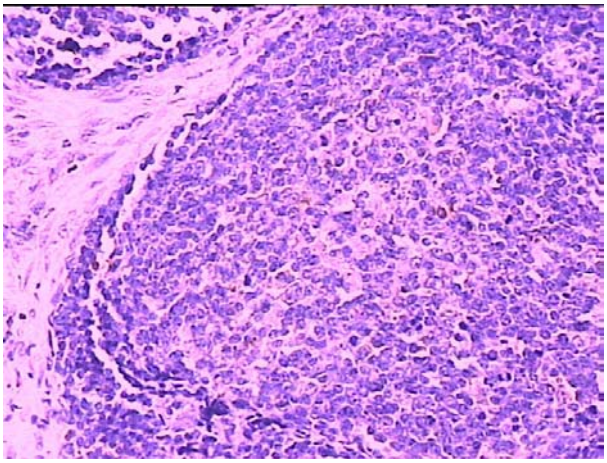

Fig. 4. Mediastinal lymph node appear normal.

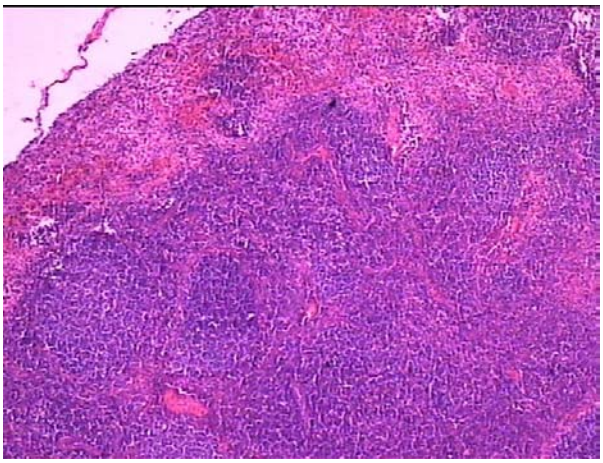

Fig. 5. Mediastinal lymph node swelled up

2.5 Cells containing Vasoactive Intestinal Peptides (VIP) increased in lungs as shown (Fig. 6) and in the mediastinal lymph nodes post-vaccination (Fig. 7).

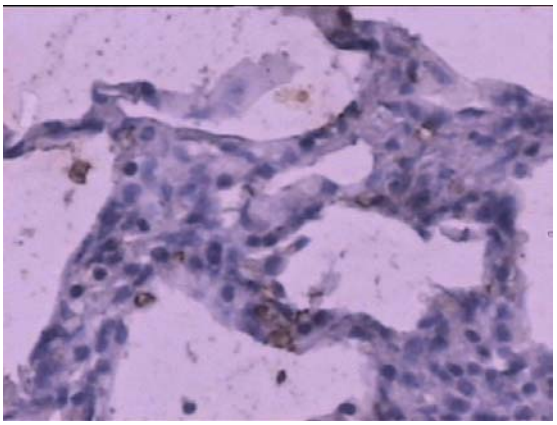

Fig. 6. Cells with decreased VIP—lung tissue

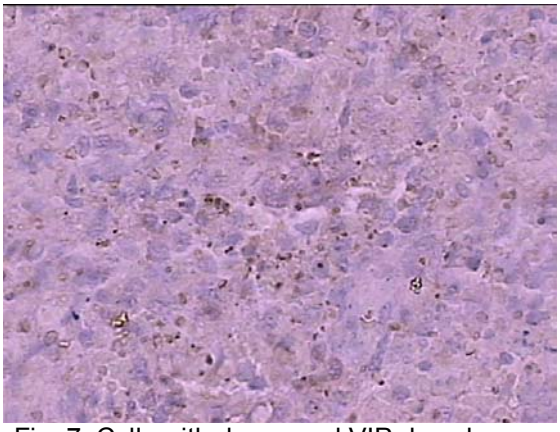

Fig. 7. Cells with decreased VIP--lymph nodes

2.6 Impact on farm productivity after vaccination

Group	A	B	C
Weaning weight, kg/pig	9.2	8.8	8.19
Nursery livability, (%)	99.8	99.5	98.4
Nursery FCR	1.6	1.65	1.7
Nursery treatment cost, Yuan/pig	0.88	0.90	0.96
End weight, kg/pig	22.3	21.5	19.2
Finisher livability, (%)	99.7	99.6	99.3
Finisher FCR	2.8	2.95	3.06
Finisher treatment cost, Yuan/pig	0.21	0.26	0.32
Days to market	161.5	162.1	168.7
Individual weight at market age, Kg	105.6	101.4	98.6
ADG , Kg/day	0.867	0.823	0.806

3 Discussion

3.1 Results showed a significantly increased level of IFN-γ in vaccinated groups versus the unvaccinated group (P≤0.05). Likewise, the level in group A was higher than in group B. This study shows that vaccination is able to trigger cellular immune response.

3.2 In general, pneumonia lung score in vaccinated groups was significantly lower (P≤0.05) compared to non-vaccinates, whereupon, the lung scores of group A were remarkably better than those of group B. This also indicates that the absence of IFN-γ in the immune system aggravates the degree of damaging effect of Mycoplasma hyopneumoniae lung infection.

3.3 The lung tissue sections of unvaccinated pigs showed hyperplastic lymphocyte infiltration around the bronchi, and the cells lining the alveolar septum showed remarkable increase with irregular array. Obvious "**eyewinkers**" appeared in the alveolar sacs. In contrast, the bronchi of the vaccinated pigs appeared normal, further corroborating the benefits of vaccination.

3.4 Mediastinal lymph nodes collect lymph mainly from the lungs, via the bronchi. The mediastinal lymph nodes in the unvaccinated group showed marked swelling due to natural infection, triggering lymphocyte proliferation.

3.5 VIP causes dilation of the vessels. In this study, a few VIP positive cells were seen in the vessels and interstitial substance surrounding the bronchi, inside the mediastinal lymph nodes. Further clinical study needs to be done in order to validate this.

3.6 The performance of the vaccinated group was significantly better than that of the unvaccinated group (P≤0.01). This result is in congruence with that of Isabelle A. Moreasl's[9].

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ILEITIS PREVENTION BY ORAL VACCINATION – EUROPEAN EXPERIENCES

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Introduction

Ileitis due to *Lawsonia intracellularis* infections is one of the most common enteric diseases of post weaning pigs in the world. The economic efficacy of Enterisol Ileitis, the first vaccine against *L. intracellularis*, is investigated in two commercial farms in Germany.

Material and Methods

The vaccine was given orally via the water in troughs during a period of 4 hours (Vaccination age farm A: seven weeks; farm B: three weeks.) All treatments were blinded to the investigators. There were no feed antibiotics applied in farm B. In farm A, feed antibiotics were only used at weaning and as interval supply at the first 18 nursery days.

For the statistical analysis a General Linear Model with the initial body weight as covariate effect was applied.

Results

Both farms tested positive for *L. intracellularis* in weaning, breeding and fattening units by PCR and IFAT but showed no clinical signs apart from increased growth variation around the18th week of life in farm B. This sub-clinical presentation of Ileitis is most typical for the majority of European farms infected with *L. intracellularis*.

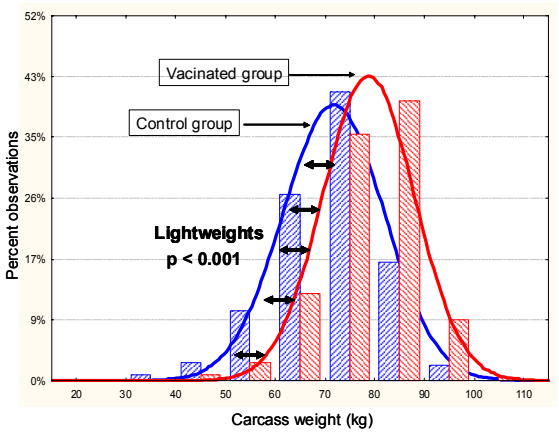
In both sub-clinical infected study farms the vaccinated groups performed substantially better than the control groups (Table 1).

Tab. 1: LS-Means of growth traits, slaughter traits and in the control and vaccinated groups

Trait/group		Farm A		Farm B	
		Vacc.	Contr.	Vacc.	Contr.
Pig number	(n)	555	278	546	271
ADWG	(g)	709^{a}	674^{b}	703^{a}	682^{b}
Feed intake*^{1}	(kg)	1.93^{a}	1.85^{b}	-	-
Feed conv. *^{1}	(kg/kg)	2.71^{a}	2.74^{a}	-	-
Mortality	(%)	4.30^{a}	4.68^{a}	5.3^{a}	3.68^{a}

*Different letters indicate significant differences within the respective farm ($p < 0.05$). *^{1} Feed intake was measured in farm A only

Vaccinated pigs showed significantly higher daily weight gains (21g to 35 g) and increased feed intake. The results agree with laboratory challenge experiments with virulent field isolates showing higher daily weight gain of pigs vaccinated with Enterisol® Ileitis compared to challenge-control pigs [1, 2]. In addition to the better growth, the variation in the slaughter weight was lower in vaccinated pigs than in controls. The lower percentage of lightweights (< 60 kg) led to significantly reduced weight discounts per kg laughter weight of vaccinated pigs in farm A (Graph 1).



Graph 1: Distribution of the carcass weight in vaccinates and controls in farm A

Based on the increased biological performance the economic impact of vaccination was analyzed (Tab. 2).

For the calculation of the slaughter sales and weight discounts the Euro-Reference grading grid with a base price of 1.4 € has been applied. A feed price of 175 €/t and

piglet prices depending on the weight were assumed. Mortality was considered in calculating all costs per pig sold.

Tab. 2: Sales, costs and gross margins of pigs vaccinated with Enterisol® Ileitis compared to non-vaccinated controls[*1]

Economic trait/group		Vaccinated group	Control group
Slaughter sales	(€/pig)	107.1[a]	98.6[b]
Costs			
Piglet costs	(€/pig)	50.3[a]	47.8[b]
Feed costs	(€/pig)	35.1[a]	33.7[b]
Mortality costs	(€/pig)	3.7[a]	3.6[a]
Gross margin	(€/pig)	17.98[a]	13.51[b]

[*1]Because of missing feed intake data in farm B, the economic analysis was done in farm A only.

It can clearly be seen from table 2 that vaccinated pigs yielded a considerably higher gross margin of + 4.47 € compared to non vaccinated pigs. This is mainly based on higher slaughter sales due to increased end weights and lower slaughter weight variation.

It is concluded that vaccination with Enterisol® Ileitis is an economically efficient tool to control sub-clinical Ileitis and to allow a better exploitation of the genetic growth potential of pigs.

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Marbofloxacin and *Actinobacillus pleuropneumaniae*

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Introduction and objectives

Actinobacillus pleuropneumoniae (APP) generates significant production losses in pig farms (1-2), worldwide and few successful treatments exist to eradicate this pathogen.
The APP susceptibility to marbofloxacin is good in Europe (CMI_{90} = 0.026 to 0.028 µg/ml between 1999 and 2004) and stable. Moreover, marbofloxacin has an excellent distribution in lungs and tonsils. Consequently, a protocol of treatment of APP infections was set up with Marbocyl® 10% through field trials.

Material and Methods

Three french farrow-to-finish farms (between 150 and 200 sows) with different sanitary status were implicated. They were all seropositive controlled for App.

Table 1: Sanitary status of the 3 farms

Farm 1	Farm 2	Farm 3
High sanitary status	Medium sanitary status	Poor sanitary status
APP	APP	APP + respiratory and digestive infections
No clinical signs of APP	Clinical signs of APP	Clinical signs of respiratory and digestive infections
-	Serovar 2	Serovar 9
High healthy status, separate buildings, no risk of external contamination	Attempts of vaccination and treatment of APP (Pulmotil, Amoxicilline)	Fruitless attempts of vaccination and treatment (Tilmicosine, Lyncomycine)

The treatment protocol consisted of two intramuscular injections of Marbocyl® 10% at a dose of 4 mg/kg, within four days, on all the animals, whatever sex and age, and also on all the new gilts at their arrival in farms.

The efficacy of the treatment was assessed by an observation of the clinical signs, the zootechnical parameters and especially the serologies against APP. Indeed, a series of blood samples was performed at various times (between approximately 2 and 6 months) after the Marbocyl injections.

Results and Discussion

In the farm 1, no emergency of clinical signs was observed and the serology was negative in almost all the animals.

In the farm 2, clinical signs and lung lesions disappeared. All the serologies became negative.

In the farm 3, clinical signs disappeared and an improvement of average daily gain, food conversion and lung lesions was observed. The serology was negative in all the animals.

As a conclusion, the use of Marbocyl® 10% in the treatment of APP infections improves the sanitary status of the farms and the zootechnical parameters. Besides, negative serologies can be observed on almost all the animals. Nervertheless, some sows remained healthy carriers. Thus, the words of “eradication” and “curative treatment” are not appropriate for this pathology.

As a consequence, our results show that Marbocyl® 10% is effective in the control of APP infections. In order to be more efficient, this protocol should be accompanied with an elimination of the healthy carriers, depopulated periods and control plans in batches.

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EFFICACY OF DICLAZURIL FOR OOCYST CONTROL IN SOWS AND PIGLETS

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Introduction and Objectives

Coccidiosis caused by *Isospora suis* and *Eimeria spp*. is one of the most important protozoal disease of swine. Piglets suffered from the major clinical signs of being yellowish to grayish diarrhea with loose, pasty or fluid feces, may have depressed weight gains and result in the poor health status in nursery and , may be, in starter pigs (1). During the six months prevalent survey, the oocyst was found in both sows and piglets feces. According to the farm practice in Thailand, piglets may receive Toltrazuril as an oral suspension at four days old. There are some anticoccidials mixed in sow feed to control the shedding of oocyst (2), however, some anticoccidials have the incompatibility to some antimicrobials which the users have to be aware. This study is to evaluate the effect of Diclazuril, the non-ionophore anticoccidial, to control oocyst shedding in lactating sows and sucking piglets in Thailand.

Materials and Methods

The study began by the prevalent survey of oocyst shedding in sows and piglets in the chosen farm to indicate the point of the most shedding in sow by collecting and counting oocyst during weaning, gestation, farrowing and lactation. After the survey, field trial was conducted at the same farm.

158 sows were divided into two groups, the control group with the conventional lactating feed of farm without anticoccidial and the treatment group with 1.5 ppm Diclazuril in feed from seven days before farrowing to the weaning day, at 17-18 days after farrowing. Piglets from the control group were given Toltrazuril 25 mg/piglet at four days old and none in another group.

Collecting feces from every sows at farrowing day, seven days after farrowing and weaning day and choosing one piglet from every sows to collect feces at seven days old, and weaning day. Oocyst count was reported individually for the statistical analysis by Chi-square test at $p<0.05$.

The following performance parameter had been evaluated: piglets coccidiosis , piglets birth and weaning weight, piglets wean per sow, and sow health condition.

Results and Discussion

The oocyst shedding from sows in the treatment group was significantly less than the control group and the oocyst shedding from piglets of the treatment group was also less than the control group but the different value was not statistically significant (Table 1). Sow at

farrowing (Table 2) and piglets at weaning (Table 3) were the most different oocyst shedding between control and treatment group. No piglet of both group suffered from coccidiosis. There was also no difference of the performance parameter from both groups.

The result in this study indicates that Diclazuril 1.5 ppm in lactating feed can reduce the oocyst shedding in sow and piglets from the treated sows show the equal result to

Group	Sows Shedding	Piglets Shedding
Control	22%[a]	3.7%
Treatment	2.6%[b]	1.3%

oocyst shedding comparing with piglets receiving Toltrazuril.

Table 1. Percentage of animals with oocyst shedding in sows and piglets feces.

Group	Farrowing Sow Shedding	Shedding at Lactating 7 days	Weaning Sow Shedding
Control	15.9%[a]	3.6%	3.6%
Treatment	0[b]	1.3%	1.3%

[a, b]: $p<0.05$

Table 2. Percentage of sows with oocyst shedding in

Group	7 days old Shedding	Weaning Shedding
Control	0	3.6%
Treatment	0	1.3%

different stages.

[a, b]: $p<0.05$

Table 3. Percentage of piglets with oocyst shedding in different ages.

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SEROLOGICAL RESPONSE AFTER HERD VACCINATION OF SOWS AND GILTS WITH PORCILIS AR-T DF

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Introduction and Objectives

For management reasons, a number of farmers prefer herd vaccination (i.e., all animals in the herd are vaccinated on the same day, irrespective of the stage of reproduction) over vaccination of sows at a fixed stage of gestation. This vaccination is repeated at regular intervals, e.g., every 6 months. Porcilis® AR-T DF was initially recommended for use in sows and replacement gilts during the second half of pregnancy.

The objective of this study was to determine the optimum herd vaccination scheme for Porcilis® AR-T DF, as determined by serological data.

Materials and Methods

The trial was carried out on a commercial pig-breeding farm in The Netherlands. The animals in the herd had not been previously vaccinated against Atrophic Rhinitis. Breeding sows and replacement gilts with a minimum age of 8 months were assigned at random to one of the following groups:

A: two basic vaccinations at a 4-week interval (T=0), a 3rd vaccination 3 months later (T=3), and a 4th vaccination 6 months later again (T=9). (n=69 animals)

B: two basic vaccinations at a 4-week interval (T=0), a 3rd vaccination 6 months later (T=6), and a 4th vaccination 6 months later again (T=12). (n=39 animals)

C: Unvaccinated control group. (n=10 animals)

Due to the yearly average animal replacement rate of 35%, the number of animals in the three groups decreased gradually. At the end of the trial 21, 22 and 7 animals were available for serology in groups A, B and C, respectively.

An inactivated vaccine (Porcilis® AR-T DF, Intervet) was used. One dose of 2 ml contained $\geq$ 5.9 $\log_2$ TN (toxin neutralization) titre of protein dO (non-toxic deletion derivative of *Pasteurella multocida* dermonecrotic toxin), $\geq$ 4.2 $\log_2$ agglutination titre of inactivated *Bordetella bronchiseptica* cells and 150 mg dl-alpha-tocopherol acetate (Diluvac® Forte, Intervet) as adjuvant.

Blood samples were collected at 0.5, 3, 4.5, 6, 7.5, 9, 10.5, 12 and 15 months after T0. The Blobel micro-agglutination test was performed to measure *B. bronchiseptica* antibody titres. For determination of the *P. multocida* toxin antibody concentration, a toxin neutralization test was carried out. For *B. bronchiseptica*, a titre of $\geq$ 4 was considered protective. For *P. multocida*, a titre of $\geq$ 1 was considered protective. The mean titres and the proportion of animals with protective titres were determined at each time of sampling.

Results and Discussion

Vaccination at T0, T3 and T9 (group A) resulted in high *B. bronchiseptica* antibodies and *P. multocida* toxin titres from 2 weeks after T0 onwards. For both components, the proportion of protected animals in this group was high at each time point (>95%).

Figure 1: *B. bronchiseptica* antibody titres (log_2) in groups A, B and C during the entire study period

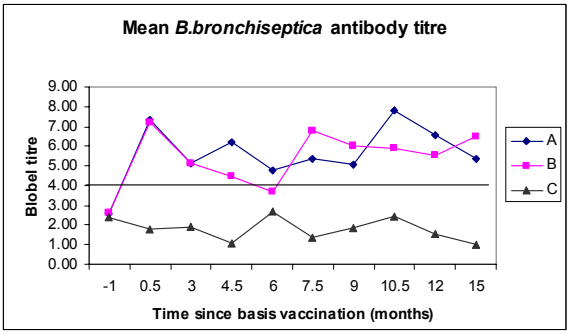
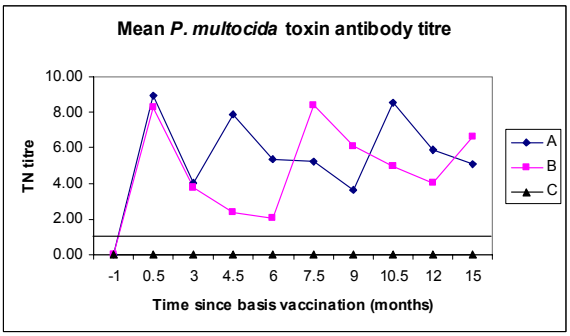


Figure 2: *P. multocida* toxin titres in groups A, B and C



At T6 (group B), the mean *B. bronchiseptica* antibody titre dropped below the protective level. The proportion of sows with protective *B. bronchiseptica* titres was 87% at 4.5 months and 56% at 6 months after T0.

For *P. multocida,* no relevant differences in protection were observed between the two schedules. From 2 weeks after T0 onwards, the percentage of protected animals was high (>94%) in both vaccinated groups.

Conclusion

Basic vaccination with Porcilis AR-T DF at a 4-week interval followed by a single vaccination 3 months later, resulted in protective antibody titres for *P. multocida* and *B. bronchiseptica* for at least another 6 months for >95 % of the vaccinated animals.

Therefore, after the basic and T3 vaccinations, a herd vaccination at regular 6-month intervals will provide on-going protection to the progeny of vaccinated sows.

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Posters

Virology

AN UPDATE ON PRRS PREVALENCE IN THE PHILIPPINES III. EFFECT OF PIG DENSITY ON SEROPREVALENCE

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Introduction and Objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) prevalence has been explored as early as 1995-1996[1], showing a 64.71% seropositive status from 170 herds tested. A previous study[2] reported a 100% seropositive status on 46 farms involving 2,441 samples. Seventy six percent (76%) of the samples were seropositive. This involved farms tested from a period of January 2000 to September 2001. This paper aims to determine the seroprevalence for PRRS of farms in the Philippines.

Materials and Methods

A total of 12,318 serum samples from 219 farms (167 in Luzon & 52 in Visayas-Mindanao) in the Philippines were tested for PRRS ELISA. The samples included in the study were taken from March 2000 to June 2004. Sampling for majority of the herd is based on the number of animals in the farm having a 95% confidence level and an assumed prevalence of 10-20%. The proportion of samples tested that were seropositive and seronegative was determined. A seropositive farm indicates the presence of even a single animal resulting to a positive ELISA result.

Results and Discussion

Table 1. Proportion of total samples and farms seropositive for PRRS virus in the Philippines.

	Positive	Negative	Total
Samples Tested	59%	41%	12,318
Farms Tested	90%	10%	219

Fifty nine (59%) of the serum samples tested were seropositive. This means that a total of 7,267 animals from which the sample was taken had been exposed to the PRRS virus. On a per farm basis, a total of 197 are seropositive for PRRS.

Table 2. Seroprevalence of the two regions compared to population density

	% Seropositive	
	Luzon	Vis-Min
Farms Tested	98.00%	65.00%
% of total population*	81.89%	18.11%

*PVDA data on commercial sow population

The higher percentage of seropositives in Luzon is correlated to the population density compared to that of Visayas & Mindanao combined (Table 2). As Luzon accounts for a little over than 80% of the swine population in the country, the proximity between farms would most probably cause the higher seroprevalence of PRRS relative to the other region.

In contrast, Visayas and Mindanao account for only less than 20% of the total commercial sow population. The distance from farm to farm would be farther.

With the high prevalence of PRRS-initiated PRDC in the Philippines, vaccination with a modified live vaccine is effective in controlling the complex infection[3]. In addition, for both Luzon and Vis-Min, an effective biosecurity measure should also be implemented to control PRRS and PRDC.

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3. Quilitis, MFE. et al, 2004. IPVS p. 44.

PSEUDORABIES PREVALENCE IN THE PHILIPPINES III. EFFECT OF PIG DENSITY

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Introduction and Objectives

The first, and most important part of the control of Pseudorabies is awareness. The use of the Enzyme- Linked Immunosorbent Assay (ELISA), particularly the gene deleted (gE type), have shown the capability of the test in identifying the infected animals [1,2]. Although the disease is being, or has been eradicated in some countries, Seroprevalence studies from countries in Eastern Europe[3], Latin America[4] and Asia[5] have shown that the disease is still present. This paper attempts to discuss the awareness in terms of seroprevalence of Aujeszky's Disease in the Philippines.

Materials and Methods

The study involved 219 farms from the period of March 2000 to Sept 2004 with a total serum sample of 2,204 tested. Breeders (gilts and sows) as well as 12 week old fatteners to market pigs were tested in the study. A differential (gE which later became gI) ELISA test (Idexx Laboratories) was used for this purpose. The proportion of samples tested that were seropositive and seronegative was determined. A seropositive farm indicates the presence of even a single animal resulting to a positive ELISA result.

Results and Discussion

Table 1. Proportion of total samples and farms seropositive for Pseudorabies virus in the Philippines

	Positive	Negative	Total
Samples Tested	23%	77%	8,642
Farms Tested	49.77%	50.23%	219

Thirty two percent (23%) of the samples tested and almost 50% of the farms tested registered positive for the test. Out of 219 farms tested, 109 were considered positive for exposure to the field virus.

Table 2. Seroprevalence of the two regions compared to population density

	% Seropositive	
	Luzon	Vis-Min
Farms Tested	58.08%	23.08%
% of total population*	81.89%	18.11%

*PVDA data on commercial sow population

The prevalence of Pseudorabies in Luzon is higher than in Vis-Min. Almost 82% of the pigs in the Philippines are concentrated in the former region (table 2).

In both Luzon and Vis-Min region, reducing seropositive prevalence and preventing the entry of the virus through latenly infected pigs respectively is crucial. Coupled with an efficient vaccination program[6], eradication can be achieved.

As Pseudorabies still confronts farms in terms of reproductive and respiratory form, control would be key.

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SEROLOGICAL PROFILES OF THREE PHILIPPINE COMMERCIAL PIG HERDS FOR SWINE INFLUENZA H1N1 VIRUS

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Introduction and Objectives

Recent serological surveys for swine influenza (SI) H1N1 virus in fattening pigs from commercial farrow-to-finish pig herds in the Philippines reveal that herd infection is widespread (1, 2). The present study describes the serological profile of 3 commercial farms for SI H1N1.

Materials and Methods

The study was conducted in 3 commercial farrow-to-finish pig herds designated as farms A, B and C, respectively. These farms had been previously identified as seropositive for H1N1 (1, 2). Farm A had 1,500 sows, while farms B and C had 400 and 300 sows, respectively.

Cross sectional sampling was done in farms A and B. In each farm, blood was collected from each of ten pigs of 3, 6, 9, 12, 15, 18 and 21 weeks of age. A total of 70 pigs were sampled in each farm. In Farm C, longitudinal sampling was done . Three sows and 3 to 5 pigs of their respective litters were followed. The piglets were tagged and serial blood samples were collected starting at 3 weeks of age and every 3 weeks thereafter until 15 weeks.

All serum samples were examined for SI H1N1 antibodies using a commercial ELISA kit (Idexx Laboratories, Westbrook, ME) following the manufacturer's instructions. Samples with S/P ratios equal to or greater than 0.4 were considered positive.

Results and Discussion

The results of the serological examinations in Farm A are shown in Fig. 1. Twenty-one out of the 70 pigs tested (26%) were seropositive for SI H1N1 virus. The number of seropositive pigs was low in pigs of 3 to 9 weeks of age (1-2). Generally higher numbers of seropositive pigs were observed in pigs from 12 weeks of age and older suggesting that active infection occurred between 9 and 12 weeks in Farm A. In Farm B (Fig. 2), 26 pigs were seropositive (33%). Progressively lower numbers of positive pigs were observed from 3 to 12 weeks. In 15 week-old pigs and older, the number of positive pigs were higher (4-9) compared to 12 week-old pigs, suggesting that active infection occurred between 12 and 15 weeks in this farm. The results of the serological examinations in farm C are shown in Fig. 3. All 3 sows were seropositive with S/P ratios of 1, 0.753 and 0.572 for litters 1, 2 and 3, respectively. Maternal antibodies were detectable until 3 weeks in litter 1 and 2. Seroconversion was detected between 9 and 12 weeks in litters 2 and 3. Seroconversion was not detected in litter 1.

Fig. 1. Cross-sectional serological profile for swine influenza H1N1 in Farm A.

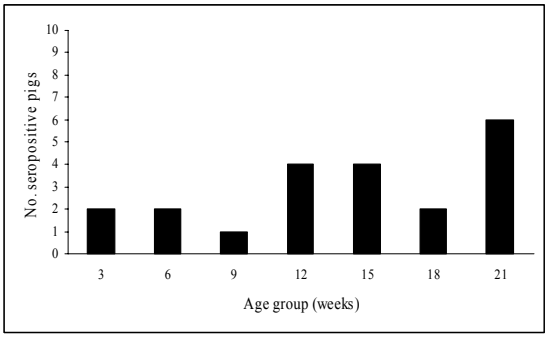


Fig. 2. Cross-sectional serological profile for swine influenza H1N1 in Farm B.

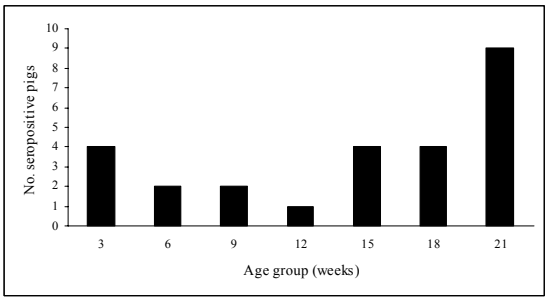
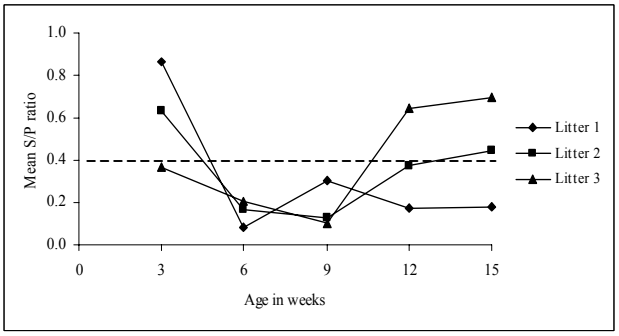


Fig.3. Swine influenza H1N1 virus antibody titers (S/P ratios) in 3 litters in Farm C.



The present results suggest that infection with H1N1 virus in the farms examined in this study occurs between 9-15 weeks of age, partly coinciding with the transfer of pigs from nursery pens to growing-fattening houses.

This study was supported in part by the Animal Health Division of Pfizer Philippines, Incorporated.

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ISOLATION OF INFLUENZA H3N2 VIRUS IN PIGS FROM PHILIPPINE COMMERCIAL SWINE HERDS

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Introduction and Objectives

Infections with influenza viruses in pigs are common in swine-producing regions throughout the world (1). The influenza H1N1, H3N2 and H1N2 virus subtypes are of major importance to pig health worldwide. In the Philippines, recent serological surveys for swine influenza (SI) H1N1 virus in fattening pigs from commercial farrow-to-finish pig herds reveal that herd infection with the virus is widespread (2, 3). The present study describes the first isolation of influenza H3N2 virus from a commercial herd in the Philippines.

Materials and Methods

Nine commercial farrow-to-finish pig farms (A-I) experiencing outbreaks of respiratory disease in growing-fattening pigs were examined. Nasal swabs from febrile pigs of 9 to 15 weeks of age were taken. A total of 57 pigs were swabbed (3-15 pigs per farm). Nasal swabs were suspended in transport medium and transported on ice to the laboratory. Fluids from swabs were either pooled or individually inoculated into 9-11 days old embryonating chicken eggs. After 3 days of incubation at 35 ^{o}C, chorioallantoic fluids (CAF) were harvested and tested for hemagglutinating activity using 0.5% chicken RBC. Positive samples in the HA test were checked for the presence of influenza nucleoprotein A antigen using a commercial immunomembrane filter assay (Directigen Flu A^{TM}, Becton Dickenson, MS, USA). Influenza A positive samples were then serotyped by hemagglutination-inhibition test using swine hyperimmune H1N1 and H3N2 antisera, respectively.

Results and Discussion

CAF from eggs inoculated with nasal swabs from 2 pigs coming from farm D showed hemaglutinating activity after 3 days of incubation. These samples were positive for influenza nucleoprotein A antigen in the Directigen Flu A^{TM} test. Hemagglutinating activity of the 2 samples was completely inhibited by hyperimmune H3N2 antisera (up to a dilution of 1:10,240). Inhibition of hemagglutination was not observed using the H1N1 antisera. These results suggest that the influenza A virus isolates are of the H3N2 subtype.

This study is the first report on the successful isolation of influenza virus in pigs in the Philippines. The present findings support previous serological studies showing that herd-infection with influenza viruses is common in commercial pig herds in the Philippines (2, 3, 4).

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The development and Preliminary application of immuno-gold filtration assay for porcine reproductive and respiratory syndrome

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Abstract: A dot immuno-gold filtration assay for porcine reproductive and respiratory syndrome virus (PRRS) was developed. The purified PRRS genetic engineering expressed antigen was labeled by colloidal gold. The results of specificity cross experiment indicated that detecting the PRRSV by GICA was of high specificity. And its sensitivity was improved through comparison with other methods. A batch of colloidal gold test papers was manufactured and 150 clinical samples were detected, 30 of which were positive by both ELISA and immuno-fluorescence. The development of this method provides a rapid and convenient means to detect the PRRSV antibody.

Key words: PRRSV; GICA; ELISA

THE ESTABLISHMENT OF A MULTIPLEX PCR OF PORCINE CIRCOVIRUS

Cui Shangjin, Quan Yanping
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Abstract: The nuclear sequence homology and diversity of 20 strains of PCV1 and PCV2 are compared to design two pairs of type-specific primers. The primers of P1 and P2 amplify the PCV virus-specific segment with the size of 938bp. The primers of P3 and P4 amplifies the PCV2 type-specific segment with the size of 490bp.A multiplex PCR is developed based on these two pairs of primers to type and differentiate PCV1 and PCV2.The optimization of the PCR reaction make the method more efficiently. The technique has been applied into the clinical diagnosis and epidemiological research.

Key words: Porcine circovirus PCV Multiplex PCR

THE DEVELOPMENT OF AN INDIRECT IMMUNOFLUORESCENT TECHNIQUE OF PORCINE CIRCOVIRUS

Shangjin CUI, Yanping Quan

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Abstract: An indirect immunofluorescent technique for the antibody detection (IFA) of porcine circovirus (PCV) was established using PK15 cell on 96 cell plates. The optimization of the concentration and time was done. The technique has high sensitivity and specificity. The method is valuable to the vaccine research providing the technique of detecting the antibody produced by the vaccine.

Key words: Porcine circovirus; IFA

The Molecular Cloned, Sequencing and Analysis of Genome of Different Porcine circovirus 2 strains

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Abstract: 3 strains of PCV2 were isolated from the affected pigs suffered with PMWS. The full-length genomes of three isolates are amplified with two pairs of primers and cloned. The whole genomes are sequenced and compared. The sequences of three isolates were submitted to GenBank. Genome comparisons show significant differences between PCV1 and PCV2. Phylogenetic analysis of 27 strains reveals overall nucleotide sequence homology of PCV2 is highly, and diversity of different geographic distribution not significant. As showed by the phylogenetic relationships, PCV2 has two large apparent branches: European series and American series. S2、P11 belong to European series, L belongs to American series.

Key words: Porcine circovirus; Molecular Cloned; Sequencing & Analysis

NIPAH VIRUS DISEASE – A REVISIT

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Introduction

The Nipah virus outbreak of encephalitis in human and respiratory and neurological syndrome in pigs in Malaysia and Singapore in 1999 has caused the death of about 109 people and the loss of more than a million pigs in Malaysia in order to contain the disease.

This paper attempts to make a revisit of the disease and to discuss what has happened after 6 years of post outbreak.

Materials and Methods

The information contained in this paper were obtained through review of available literatures on Nipah virus disease and an analysis of what had been done to control and eradicate the disease in human and animals in Malaysia

An overview of the epidemiology, clinical signs, pathology, strategies of control and eradication of the diseases were discussed.

Results and Discussions

Between late September 1998 and May 1999, a new pig disease with zoonotic implications, characterized by a pronounced respiratory and neurological syndrome and sometimes with sudden death of sows and boars, was discovered to spread among pig farms in Malaysia. Investigations had confirmed that Nipah virus was responsible for both the human and pig diseases.

Nipah virus caused a severe, rapidly progressive encephalitis in human that carried a high mortality rate (>40%). In pigs, mortality rate was low (< 5-15%) but morbidity rate could reach 100%. The disease spread rapidly in a farm but a large proportion of pigs could be infected without showing clinical signs (3).

The main presenting clinical signs were fever, headache, dizziness, vomiting, segmental myoclonus, reduced consciousness and coma in human (1). In pig, the presenting clinical signs varied according to the age of pigs. Pigs < 6 month old were presented with an acute febrile illness with respiratory signs ranging from rapid, labored respiration to a harsh non-productive cough or open-mouth breathing which may include neurological

signs like trembles, muscular twitching, spasm and myoclonus, hind leg weakness with varying degree of spastic or placcid paresis. In boars and sows, acute febrile illness with labored respiration, increase salivation and nasal discharges accompanied by neurological signs like agitation, head pressing or knocking, clamping of mouth, nystamus, tetanus-like spasm and seizures (3).

The main pathology in pig is the formation of syncytial cells in the lung tissues. In human, the lesions were widespread microinfarctions in brain due to underlying vasculitis and thrombosis of small blood vessels.

Serological evidence indicated that domestic animals, notably dogs, cats, horses and goats were infected with the virus from infected pigs. However, these animals were not involved in the spread of the virus to human.

Serological studies and isolation of virus from fruit bats implicated that fruit bats are the probable wildlife reservoir of the virus. The emergence of the virus was probably due to anthropogenic events which caused the migration and encroachment of fruit bats into index farms located among fruit orchards in Ipoh and the "jump" of virus from fruit bats to pigs and to human (1).

The immediate culling of pigs from infected and high risk farms as well as the continuous testing and surveillance programs had successfully rid the country of Nipah disease (2). Six years after the outbreak, there is no evidence of Nipah virus disease in domestic animals and in human indicating that the strategies applied in the control and eradication of the disease were effective.

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Genetic Diversity of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Isolated in Japan by Restriction Fragment Length Polymorphism (RFLP) analysis.

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Key words: *ORF 5, porcine reproductive and respiratory syndrome, PRRSV, RFLP*

Introduction and Objectives

Porcine reproductive and respiratory syndrome (PRRS) is a major emerging disease causing serious economic loss to the swine industries in the world (1). PRRS virus (PRRSV) was first discovered from the Netherlands in 1991 in the world (2). In Japan, PRRSV was first isolated in 1993 (3). Many genetic studies of the ORF5 of PRRSV has been carried out, because the E protein encoded by ORF5 is a predominant viral envelope protein. In addition, the ORF 5 is known as the most diversified gene of the PRRSV RNA (4). Wesley *et al*. analyzed the ORF 5 of PRRSV field isolates from the North America using restriction fragment length polymorphism (RFLP) (5). This RFLP method has been used extensively as a tool for the molecular epidemiological study of the North American type strains. But the epidemiological analysis of the RFLP for the Japanese PRRSV field isolates covering all over the country has never been reported. To confirm the origin of the PRRSVs in Japan, we analyzed the RFLP patterns of Japanese 95 field strains isolated in 1991 to 1998, regionally and retrospectively.

Materials and Methods

The PRRS viruses were isolated from the sera or tissue samples of field pigs during 1991 and 1998 in Japan (6). To distinguish the isolates between the European and the North American type, RT-PCR was performed to amplify the area ORF6 and 7 of PRRSV RNA, as described previously (7). Then, nested PCR was performed for distinction between the two types, using primer sets Pr22/Pr24 (designed for the North American type) and Pr23/Pr25 (designed for the European type). Another primer set, P420/P620 was used for amplification of the area including ORF5 to perform restriction fragment length polymorphism (RFLP) analysis. This RT-PCR protocol was followed as described by Andreyev *et al*., (8). The 716 base pairs PCR products was digested by restriction enzymes *Mlu*I, *Hinc*II and *Sac*II. The PRRSV isolates were classified in the three-digit code numbers according to the cutting patterns by these three enzymes described as Wesley *et al*., (5).

Results and Discussion

Ninety five PRRSV strains isolated from 1991 to 1998 in Japan were selected randomly to compare RFLP patterns of the ORF 6, 7 and the ORF5. These strains were isolated from 13 prefectures. All of the 95 strains were classified as the North American type by the reaction pattern of nested PCR of ORF 6, 7. Any European type isolates were not discovered in this study. In this study, we analyzed genetic diversity of PRRSV ORF5 genes of field isolates all over Japan. Eleven RFLP patterns were showed from the 95 Japanese isolates during 8 years from 1991 to 1998. These 11 RFLP patterns have been reported previously from U.S.A. (9). RFLP patterns from the four farms was compared between two to three times sampling. In the three farms of the four, two or more patterns has detected. And these patterns were different at the each sampling. In these farms we confirmed that 1 or 2 strains were existed at the same time, and changed to other strain before the next sampling. But in the other farm, only one RFLP pattern had been detected and unchanged during 12 months. Only this farm showing a homologous pattern excluded any live swine and semen importation during this period. Live swine and semen were seemed to be an important source of introducing PRRSV having different codes into the farms.

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IMMUNIZATION EFFECT OF ORF2 DNA VACCINE AGAINST PCV2 IN BALB/C MICE

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Keywords: **porcine circovirus (PCV), ORF2, DNA vaccine, mice**

Introduction Porcine circovirus (PCV) belongs to the family Circoviridae. PCV is a small (diameter 17nm), non-enveloped virus containing a single-stranded circular DNA genome of 1.76Kb. There are two major open reading frames (ORFs) encoded by the 1.76Kb genome: ORF1 and ORF2. ORF1 gene encodes Rep protein, it is essential for circoviral replication. ORF2 gene encodes the structural protein of PCV viral capsid(1). There are four B-cell epitopes in ORF2 gene. Two genotypes of PCV have been identified: PCV1 and PCV2. PCV1 was first detected as a containment in PK-15 cell line, it is wild spread in swine and is non-pathogenic. PCV2 is associated with post-weaning multisystemic wasting syndrome (PMWS)(2), it is an important economic disease affecteing pig industry. To prevent PMWS, a PCV2 vaccine is needed. In this experiment, we construct two PCV2 DNA vaccines, immunoreaction to Balb/c show they can stimulate anti-ORF2 antibodies in mice.

Materials and Methods

Construction of expression plasmid and DNA vaccines ORF2 and BVP22 (Bovine herpesvirus 1 VP22) gene are acquired by PCR. PCR products were cloned into pMD18-T vector (TaKaRa) and sequenced. Then the ORF2 gene and BVP22 gene were subcloned into expression vectors: pCDNA3.1(+) and pEGFP-N1 by appropriate enzyme digestion, which resulted: pCORF2, pCBVP22ORF2 (BVP22 and ORF2 gene are in the same ORF, express as a fusion protein), pNORF2, pNBVP22. Sketch map of these plasmids are showed in Fig.1 and Fig. 2.

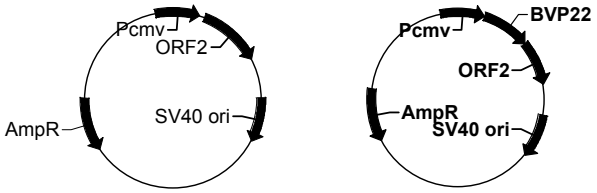

Fig.1 Sketch map of pCORF2 and pCBVP22ORF2

Expression of ORF2 and BVP22 gene in *Vitro* Hela cells grown to 60% confluency in 24 wells dishes were transfected with 2μg DNA of pNORF2, pNBVP22 or pEGFP-C1 with the help of 2μl liposome (Invitrogen). 18h post transfected, cells were tested by a fluorescent microscope.

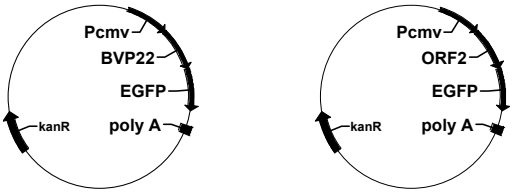

Fig.2 Sketch map of pNORF2 and pNBVP22

Animal Experiment 15 Balb/C mice (6 weeks) are random divided into 3 groups, and each group were muscular injected by pCDNA3.1(+), pCORF2, pCBVP22ORF2, 100μg plasmid per mouse. A second injection applied to enhance the immunoreaction 2 weeks after. Serums are collected 14, 42 days after the first immunization. PCV2 ORF2 specific antibodies were tested by ELISA (3).

Result and Discussion

Construction of expression vector and DNA vaccine vector **pCORF2, pCBVP22ORF2 are identified by HindIII and EcoR V digestion. pNORF2 is identified by Hind III and Stu I digestion. pNBVP22 is identified by Bgl II and BamH I digestion. (Fig. 3 and Fig. 4)**

ORF2 and BVP22 expression in *vitro* **18h post transfection, cells were observed by a fluorescent microscope. Both ORF2 and BVP22 transfected cells can show green fluorescent in Hela cell line, but the localization of the two proteins are obviously different from EGFP. ORF2 only localized in the nucleus, whereas BVP22 was mainly localized in the nucleus, but can transduct into cytoplasm. (Fig. 5)**

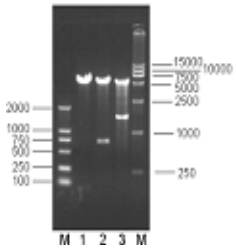

Fig.3 HindⅢ and EcoRⅤ enzyme digestion identification of the DNA vaccine vectors

1. pCDNA3.1(+); 2. pCORF2;

3. pCORF2BVP22;

4. pCBVP22ORF2

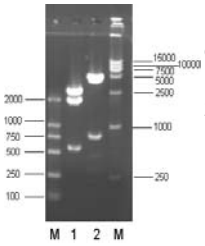

Fig.4 Enzyme digestion identification of pNORF2 and pNBVP22
1. pNORF2/HindⅢ+StuⅠ;
2. pNBVP22/BglⅡ+Bam HⅠ
M. .DL Marker 2,000 or 15,000

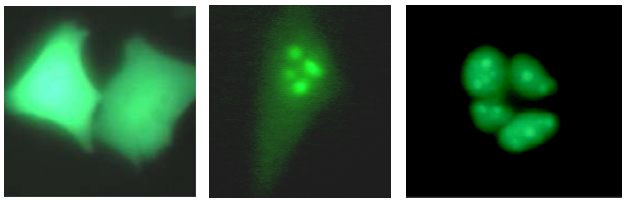

Fig.5 ORF2 and BVP22 gene expression in *vitro*

ELISA test antibodies against ORF2 **Serums from mice 14, 42 days after initial immunization were tested antibodies by an ELISA method. At 14 days anti-ORF2 antibodies show a low level, but 42 days after a higher level were tested. This result show that BVP22-ORF2 fusion expression can enhance the ORF2 immunoreaction. (Fig.6)**
In this experiment we has constructed two ORF2 DNA vaccine that can stimulate immunoreaction in mice. ORF2 is the unique structural protein of PCV2. Baculovirus expressed recombinant ORF2 protein can self-assembled to capsid-like particles in vitro (1). We can forecast that anti-ORF2 antibodies could be protectable against PCV2.
From Fig.5 we can see that PCV2 ORF2 is strictly localizated in nucleus, so it can stimulate high level humoral antibodies. BVP22 gene has the function of protein transduction and has been proved that it could enhance immunoreaction (4).

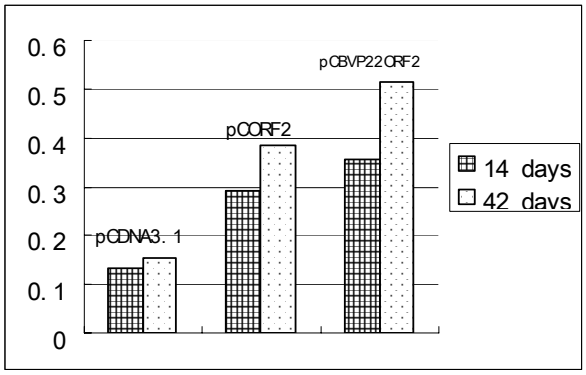

Fig6. ELISA antibodies difference between the three groups

So we can presume that BVP22 could carry the ORF2 protein to the cytoplasm or even out of cell. In this situation ORF2 has more chance to contact with humoral, immunoreaction enhanced.

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INHIBITION OF REP EXPRESSION OF PORCINE CIRCOVIRUS TYPE 1 IN PK-15 CELLS BY SIRNA

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Key words: inhibition; Rep expression; porcine circovirus; siRNA

INTRODUCTION

Porcine circovirus (PCV) recently emerged as an important infectious pathogen for pigs in the world. It has been associated with many diseases, such as PMWS (1), PDNS (2), PRDC (3)). PCV is very popular in pigs and porcine-derived commercial product. It also raises concern for potential human infection through food and xenotransplantation. So it is imperious to find an efficient way to prevent pigs from PCV infection.

In this study, three siRNA(A,B,C) targeting Rep mRNA of PCV1 were synthesized and used to inhibit Rep expression in PK-15 cells line. It will provide useful information for further research on inhibition of PCV replication by siRNA.

Materials and Methods

PCV1 strain

Isolated from IBRS-2 cells line. siRNA design and expression vector construction

Three siRNA(A,B,C) targeting Rep mRNA of PCV1 were synthesized and three siRNA expression plasmids (p*S*CMV-PCV1ORF1A,p*S*CMV-CV1ORF1B, p*S*CMV-PCV1ORF1C) were constructed by ligating siRNA into the p*Silencer* 4.1-CMV neo vector (Ambion).

Rep-EGFP fused expression

Rep-EGFP fused expression plasmid (pCDPCV1ORF1-EGFP) was constructed by inserting Rep gene in the N terminal of EGFP. Then PK-15 cells line free of PCV1 was transfected with pCDPCV1ORF1-EGFP. 24h after transfection, fluorescence in cells was detected by fluorescence microscope.

Inhibition of EGFP and Rep expression by siRNA.

PCV free PK-15 cells were co-transfected with pcDREP-EGFP and siRNA expression plasmids(table 1). Fluorescence of EGFP was detected by fluorescence microscope and flow cytometry, mRNA level of Rep, EGFP and GAPDH were also evaluated by semi- quantitative RT-PCR.

Table 1: PK-15 cells were co-transfected with pCDPCV1ORF1-EGFP and siRNA expreesion plasmids.

Results

Rep-EGFP fusion protein was expressed in PK-15cells and exhibited a diffuse staining pattern throughout the transfected cells. Compared with the cells in the No 1, fluorescence intensity was deduced 60%, 80% and 90% in No 2, No 3, and No 4, respectively. It indicted that EGFP and Rep expression were prominent inhibited by siRNA, and siRNA(B) and siRNA(C) were more efficient than siRNA(A).

Semi-RT-PCR result suggested that mRNA level of EGFP and Rep in the No 2,No3, No4 were decreased when compared with No1. However, there is no essential difference in mRNA level of GAPDH among the No1-No5 (fig 1). It also indicated that Rep expression was inhibited by siRNA.

Discussion

PCV infection is an urgent disease in the world. It has had potentially serious economic impact on the swine industry. But there is no efficient method to deal with PCV infection until now.

RNAi is a powerful new method for intracellular Immunization against several viral infections. Inhibition of virus replication by means of induced RNAi has been reported for several viruses like HIV-1(4), hepatitis B virus(5)), influenza A virus(6) and SARS coronavirus(7).It has been demonstrated that *Rep* and *Rep' are* essential for PCV replication and Stop mutations in *Rep* or *Rep'* can cause greater than 99% reduction of protein synthesis and complete shut down of viral DNA replication (8).In this study, Rep expression was efficiently inhibited by three siRNA. It suggested siRNA could be used as a new way to inhibit replication of PCV in further study.

No	Plasmids
1	pCDPCV1ORF1-EGFP+p*Silencer* 4.1-negative
2	pCDPCV1ORF1-EGFP+p*S*CMV-PCV1ORF1A
3	pCDPCV1ORF1-EGFP+p*S*CMV-PCV1ORF1B
4	pCDPCV1ORF1-EGFP+p*S*CMV-PCV1ORF1C
5	Cells control

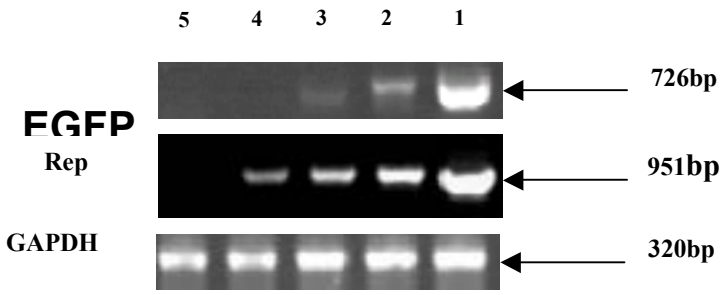

Fig 1 detection of mRNA of EGFP,Rep and GAPDH by semi- quantitative RT-PCR

1：pCDPCV1ORF1-EGFP＋pSilencer 4.1-negative
2：pCDPCV1ORF1-EGFP＋pSCMV-PCV1ORF1A
3：pCDPCV1ORF1-EGFP＋pSCMV-PCV1ORF1B
4：pCDPCV1ORF1-EGFP＋pSCMV-PCV1ORF1C
5：cell control

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ENHANCING IMMUNOLOGICAL RESPONSE TO PORCINE REPRODUCTIVE AND RESPIRATORY SYDROME VIURS DNA VACCINE BY CO-EXPRESSING GP5 AND M PROTEINS

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Key words: GP5-M heterodimer, co-expression, PRRSV, DNA vaccines

Introduction:

Porcine reproductive and respiratory syndrome virus (PRRSV) is responsible for reproductive failure in pregnant sows and inappetence, fever and respiratory disease mostly in young piglets (3-8 weeks of age). The GP5 protein (which is encoded by ORF5 gene) and M protein (which is encoded by ORF6 gene) of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), forming a disulfide-linked heterodimer in the virus particle, are the known primary protecting antigen (1). It is possible that the GP5-M heterodimer formation may enhance the specific immune response. To address the possibility, we investigated the GP5-M heterodimer formation with the eukaryotic expressing plasmids that contained either one or two CMV promoters. Furtherless, The immunogenicity of the recombinant plasmids in BALB/c mice was determined.

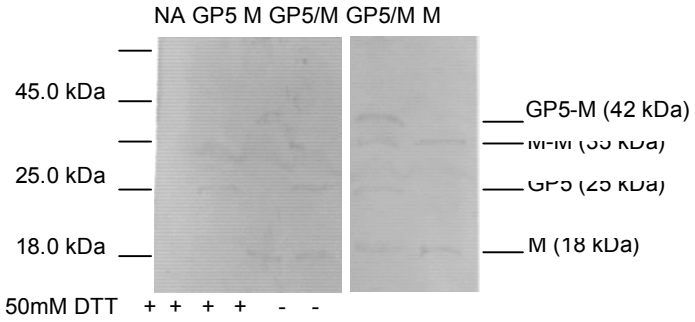
Materials and Methods:

We constructed the eukaryotic expressing recombinant plasmids pCI-ORF5 and pCI-ORF6 expressing GP5 or M protein individually, and pCI-ORF5/ORF6 co-expressing GP5 and M proteins with the two independent CMV promoters based on the eukaryotic expressing plasmid pCI-neo.The expressing authenticity GP5 and M proteins and disulfide-linked heterodimer formation were confirmed by Western immunoblotting. The immunogenicity of individual (pCI-ORF5, pCI-ORF6, and pCI-ORF5/ORF6) or co-administering (pCI-ORF5+pCI-ORF6) DNA vaccines in BALB/c mice was determined at the age of 6 weeks. All vaccinated mice received a booster vaccination at 2 weeks after primary vaccination.

Results:

The expression authenticity and homodimer or heterodimer of the expressed GP5 and M proteins were demonstrated in BHK-21 cells lines at 24 h post-transfection by Western immunoblotting (Fig.1).

Figure 1: Western immunoblotting of expressing recombinant proteins and PRRSV with PRRSV-convalescent pig serum.



The results showed that the anti-GP5 ELISA antibodies (Fig.2), neutralization antibodies (Table 1) and celluar immunological responses (Fig.3) to DNA vaccine (pCI-ORF5/ORF6) co-expressing GP5 and M proteins were significantly higher than the individual expressing DNA vaccines (pCI-ORF5 and pCI-ORF6). In contrast, co-administering (pCI-ORF5+pCI-ORF6) DNA vaccines exhibited inferior enhancement compared to the individual expressing DNA vaccines.

Figure 2: Anti-GP5 specific antibodies from the vaccinated mice were analyzed by ELISA of the expressing product of the recombinant GP5 protein of pGEX-53 in *E coli*.

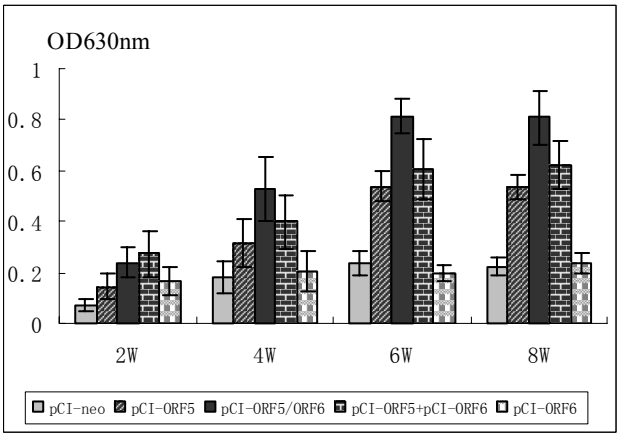
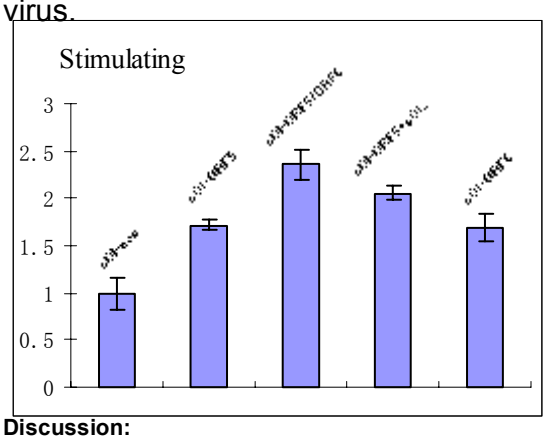


Table 1: Titers of SN antibodies in mice vaccinated with the different vaccination project.

		<1:8	1:8	1:16	1:32	mean
pCI-neo	6W	-	-	-	-	-
	8W	-	-	-	-	-
pCI-ORF5	6W	5[a]	1	0	0	NR[b]
	8W	2	4	0	0	NR
pCI-ORF5/ORF6	6W	0	4	2	0	10.67
	8W	0	2	3	1	16
pCI-ORF5+ pCI-ORF6	6W	1	3	2	0	NR
	8W	0	4	2	0	10.67
pCI-ORF6	6W	6	0	0	0	NR
	8W	4	2	0	0	NR

a: The number of mice; NR[b]: no result.

Figure 3: Lymphocyte proliferative responses of the vaccinated mice after in vitro stimulation with inactivated virus.



Discussion:

There are sufficient published studies on characterization of the molecular biology, the immune responses to the GP5 and M proteins of PRRSV (2, 3). Moreover, DNA immunization of PRRS virus was determined to elicit protective humoral and cellular immunological response and to protect the immunized pigs from developing the intensive PRRSV-induced lesions (4). recent research revealed that the heterodimeric GP5-M complexes are essential for arterivirus assembly, but that they do not determine viral tropism (5).

In this experiment, detectable SN antibodies induced by the pCI-ORF5/ORF6 plasmid were significantly earlier and higher than that induced by the pCI-ORF5 or pCI-ORF6 plasmid. Co-administration (pCI-ORF5+pCI-ORF6) also showed the higher capability of neutralizing antibodies compared to the pCI-ORF5 or pCI-ORF6 plasmid expressing alternative protein of the GP5 and M proteins, but inferior to the pCI-ORF5/ORF6 for the probability of the heterodimer formation of the GP5 and M proteins. Similarly, the cell-mediated immunity of the DNA construct had reciprocal tendency. Our data showed that the expressing GP5 protein of PRRSV resulted in remarkable immunogenic characterization after co-expressing the M protein in pCI-ORF5/ORF6. It demonstrated that the M protein can boost authentic post-translational modification and conformational maturation of the GP5 protein, and enhanced humoral and cellular immunity.

Then the GP5-M heterodimer formation effectively enhanced the immunological response to PRRSV DNA vaccines, as provide a new approach for the further design of the PRRS vaccines.

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ENHANCED IMMUNOGENICITY OF THE MODIFIED GP5 OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Key words: DNA vaccine, modified GP5, PADRE epitope, Porcine reproductive and respiratory syndrome virus (PRRSV)

Introduction

PRRS is one of the most economically devastating diseases affecting swine industry worldwide. At present, a limited number of PRRSV vaccines, which are either modified live or killed vaccines, are available on the market. However, both types of vaccine have inherent drawbacks (1). Therefore, a new generation of vaccines with higher safety and protective efficacy is required to control this disease.

GP5, which is associated with the development of neutralizing antibodies and protection, has been a leading target for vaccine design (2,3). However, relatively weak and slow antibody responses, especially neutralizing antibodies, have been observed in DNA vaccines encoding GP5 (2,3). More recently, both a non-neutralizing and a neutralizing epitope located at the N-terminus of the GP5 of the North American PRRSV strain have been identified by Ostrowski et al. (4). The non-neutralizing epitope is highly immunodominant and exhibits some features of a decoy epitope. It is possible that the decoy epitope of PRRSV GP5 is responsible for the reduced and delayed neutralizing antibody response. To address this possibility, we modified the GP5 of the PRRSV strain YA by inserting a Pan DR helper T-cell epitope (PADRE) (5)between the neutralizing and decoy epitopes in order to display the neutralizing epitope and determine whether the modified GP5 can induce earlier and more effective immune responses or not.

Materials and methods

The eukaryotic expression plasmid pCI-52, in which the full-length cDNA of the native ORF5 gene of the PRRSV strain YA was cloned into the expression vector pCI-neo (Promega) under the control of the human cytomegalovirus (hCMV) promoter, has been described previously(6). To generate the expression plasmid pCI-52M encoding the modified GP5, a PADRE was inserted between residues 32 and 33 of the native GP5 using PCR methods. Briefly, the nucleotide sequence corresponding to amino-acid residues 1 to 32 of GP5 was obtained using PCR with the following primers: 5′-GAACTCGAGAGTATGTTGGGGAAAT GCTTGA CC-3′(forward) and 5′-TTTCTGCAGAGCGGCAGCCTTCAGG GTCCAGGCAGCCACGAACTTGGCGT TGGCGTTGACGAGC-3′ (reverse). The sequence encoding the PADRE was synthesized in the reverse primer directly downstream of residue 32. The nucleotide sequence corresponding to amino-acid residues 33 to 200 of GP5 was amplified using the following primers: 5′-TTTCTGCAGAGCAACAGCAGCTC TC-3′ (forward) and 5′-

TTTTCTAGAGACGACCCCATTGTT CCGC-3′ (reverse). The two resultant PCR products were subcloned into the *Xho*I and *Xba*I cloning sites of pCI-neo, resulting in the expression plasmid pCI-52M. The ability of each plasmid to express the encoded antigen was confirmed *in vitro* by transient transfection of Hela cells followed by immunofluorescence analysis.

Groups of 5–6 week old female BALB/c mice (five mice per group) were injected twice at 2-week intervals into the quadriceps muscle with 100μ g of plasmid DNA: pCI-52, pCI-52M, or empty vector (pCI-neo). Serum samples were collected from the retro-orbital plexus 2, 4 and 6 weeks after DNA immunization to detect GP5-specific ELISA antibody and neutralization antibody against PRRSV. The mice were sacrificed and the splenocytes were harvested for a lymphocyte-proliferation assay 4 weeks after the secondary immunization.

Results and Discussion

We used a modified method that has been demonstrated to detect early neutralizing antibody, as described by Yoon et al. (7), but no neutralizing antibodies (<1:4) were detected in the sera from pCI-52-immunized mice 2 weeks after the primary immunization. However, as shown in Fig. 1, four of the five mice immunized with pCI-52M developed detectable neutralizing antibodies (>1:4) 2 weeks after the primary immunization, and the serum titers of two mice were up to 1:8, indicating that the DNA vaccine encoding the modified GP5 induced earlier neutralizing antibodies.

By 2 weeks after boosting, all of the mice immunized with pCI-52 or pCI-52M had developed detectable neutralizing antibody. However, the mean titer of the group immunized with pCI-52M was significantly higher than that of the group immunized with pCI-52 (8.8 versus 4.8, $P<0.01$). At 4 weeks after boosting, a greater increase of the neutralizing antibody levels in the sera of pCI-52M-immunized mice was observed; four of the five mice developed >1:16 neutralizing antibodies and one had levels as high as 1:32. The mean titer also increased up to 17.6, which was significantly higher than that in the group immunized with pCI-52 (mean titer 6.4, $P<0.01$), indicating that the DNA vaccine construct encoding the modified GP5 induced higher levels of neutralizing antibody.

To further compare the ability of pCI-52 and pCI-52M to induce GP5-specific antibody responses, the serum samples were analyzed using a standard single dilution (1:20) ELISA with recombinant GP5 as the antigen. As shown in Fig.2, all of the mice immunized with pCI-52 or pCI-52M developed detectable GP5-specific ELISA antibodies 2 weeks after the primary immunization, and the levels increase consecutively with boosting and over time. However, the mean GP5-specific ELISA antibody level in the group immunized with pCI-52M was higher than that of the group immunized with pCI-52 at all time points ($P<0.05$). We also observed that, relative to the enhanced neutralizing antibodies, the enhanced ELISA antibody was slight.

To investigate whether this vaccine could also enhance cell-mediated immune responses, the lymphocyte proliferative responses were analyzed 4 weeks after the secondary immunization. As shown in Fig. 3, the proliferative response was significantly higher ($P<0.05$) in mice immunized with pCI-52M than in those immunized with pCI-52. These results indicated that pCI-52M also induced an enhanced Th1-type immune response.

These data presented here clearly showed that the modified GP5 had better immunogenicity than the native GP5. The enhanced immunogenicity of the modified GP5 might be valuable for the design of a new generation of vaccines against PRRSV.

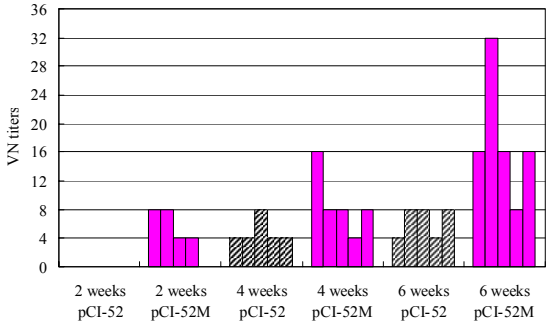


Fig. 1. Neutralizing antibodies in mice immunized with pCI-52 or pCI-5M.

Fig. 2. GP5-specific IgG responses of mice immunized with different DNA constructs.

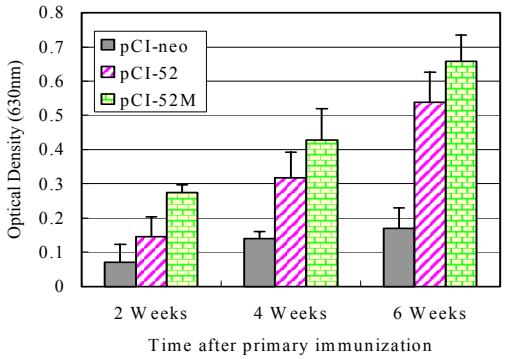
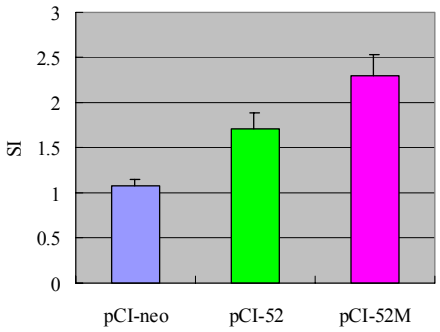


Fig.3. Lymphocyte-proliferative responses after *in vitro* stimulation with purified PRRSV proteins



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BACULOVIRUS-INSECT EXPRESSION AND IMMUNOLOGICAL STUDIES OF PORCIN CIRCOVIRUS TYPE 2 (PCV2) CAPSID PROTEIN

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Keywords: Baculovirous; porcine circovirus type 2; PMWS; Orf2; subunit vaccine

Introduction and Objectives

Porcine circovirus type 2 (PCV2), a single-stranded DNA virus associated with post-weaning multisystemic wasting syndrome (PMWS) of swine. In most cases of PMWS, clinical signs are characterized by pallor, fever and progressive wasting, together with respiratory and digestive disorder [1]. Gross lesions consist of generalized lymphadenopathy, hepatitis, nephritis, and pneumonia [1][2]. Since no PCV2 vaccine was available so far, considerable effort is under way to develop a specific PCV2 vaccine candidate. In the present study, the Orf2-encoded protein of PCV2 was expressed and detected in insect system.The main objective was to evaluate the properties and immunogenic of baculovirus-expressed Orf2 protein, pave the way for a good efficacy of the subunit vaccine of PCV2 , that would induce both antibodies and cell-mediated immunity.

Materials and methods

Recombinant baculovirus carring the ORF2 gene (Ac.Orf2) was constructed as described previously [3]. Expressed Orf2 was confirmed by indirect immunoflurescent assay and western blotting. Virus-like particles formation was determined by the electron microscopy with negative staining using 2% PTA.

The protein vaccine was pepared immediately before use, as previously reported [4]. A Total of 20 conventional 21-old-day piglets, seronagative to PCV2, were assigned to 4 groups. Group 1, 2, 3 were immunized subcutaneously with crude lysate from Ac.Orf2 at dose of 0.1ml, 0.5ml, 1.0ml emulsified with complete Freunds adjuvant (Sigma). After first priming, the piglets were boosted on week 4 with the same volume of the initial dose in incomplete Freunds adjuvant (Sigma). Group 4 was mock-immunized with 1.0ml of crude lysate from wild-type AcNPV under the same conditions and serve as control. Serum samples were collected at 0, 2, 4, 8 weeks. The immune response was monitored by an enzyme-linked immunosorbent assay (ELISA) for ORF2-specific antibody and lymphocyte proliferative responses.

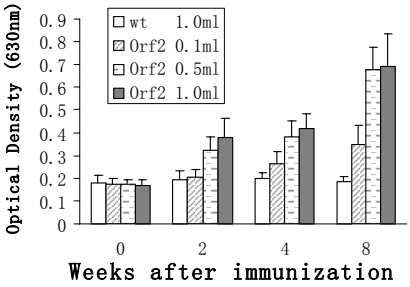
Results

Expression of the Orf2 protein was confirmed by IFA test using polyclonal antiserum to PCV2. It can be seen that recombinant baculovirus infected cells gave a clear positive signal characterized by the presence of strong fluorescence in the cytoplasm of the cells, not in the nucleus. Uninfected or wild-type infected cells yielded a negative signal. Western blotting analysis showed that a 28-kDa band corresponding to the PCV2 Orf2 protein was present only in the infected cell pellets, but not in the pellets of wt-AcNPV infected and non-infected sf9 cells.

Electron micrographs of recombinant ORF2 products showed that number of aggregates. The aggregates consisted virus-like particles, with a morphology

very similar to the original virus PCV2. The diameter of the particles was estimated to be in the order of 20nm. Most of the observed particles had dark strained centre that made them appear to be empty capsides

Levels of ORF2-spcific antibody in serum were determined by an ELISA at varied time points, titers were expressed as the genometric mean and stantard error for five piglets. No detectable ORF2-specific antibody response was induced by 0.1ml of crude lysate before the boost (Fig.4). In contrast, 0.5ml of crude lysate induced a detected primary response and 1.0ml of crude lysate induced an even higher response. After the boost, piglets treated with 0.1ml of crude lysate showed no sign of a memory response. Piglets primed and boosted with either 0.5ml or 1.0ml of crude lysate showed a high memory response that did not differ significantly between the groups.



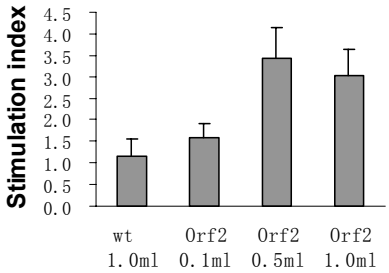


Fig.4. Serum levels for ORF2-specific antibody were determined for individual piglets by response an ELISA.

Fig.5. Lymphocyte proliferative response.

Lymphocyte proliferative were analyzed at 2 weeks after boost immunization, as show in Fig.5, a low backgroud of T-cell proliferation was observed. 0.1ml of crude lysate induced a level of lymphocyte proliferative resoponse, there is no significantly difference, compared to the control group. Both 0.5ml and 1.0ml of crude lysate induced significantly higher lymphocyte proliferative response than control group, the mean SI of 0.5ml of crude lysate from Ac.Orf2 was nearly up to three fold of control group with the mean SI of 3.40 and 1.17, respectively.

Baculovious expressed Orf2 protein in this study is concordance with previously reported[25], having the ability to spontanenously assemble into particles that closely resemble intact PCV2 virions. We demonstrated that relatively high ELISA titers can be elicited by either 0.5ml or 1.0ml of crude lysate from Ac.Orf2 to both prime and boost immune response that did not differ significantly between the groups. In addition, the higher cell-mediated immune response was developed in piglets immunized with 0.5ml. Because of the concentration of the crude lysate from Ac.Orf2 tested in these experiment was varied 0.1ml to 1.0ml, it is possible that 0.5ml does not represent the optimal crude lysate concentration. It has been demonstrated that virus-like particles are easy to be picked up by dendritic cells for subsequent processing and class1-restricted presentation to $CD8^{+}T$ cells [5]. We speculate that the enhanced cell-mediated immune response elicited by crude lysate from Ac.Orf2 may be due to the formation of virus-like particles. Using the same dose of Orf2 protein to first prime and then boosted the immune response would be greatly facilitated. These exciting results indicated that the Orf2 protein based subunit vaccine have promise to be developed as safe and efficient vaccine against PCV2.

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ENHANCEMENT OF THE EFFICACY OF A DNA VACCINE ENCODING THE FMDV CAPSID PRECURSOR POLYPEPTIDE (P1) BY LINKING BOVINE HERPESVIRUS 1 VP22 TO ANTIGEN

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Introduction

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating disease of cattle, swine and other cloven-hoofed ruminants. Epidemics of FMD are constant threats to domestic livestock throughout the word [1]. The causative agent of FMD is Foot-and-mouth disease virus (FMDV). The capsid precursor polypeptide P1 of FMDV is a structural protein encoding by P1 gene about 2.2kb, which site recognized by most neutralizing antibodies [2], and the second cleavage products of P1 polyprotein is VP1-VP4 [3]. The animals immunized with yeast expression of P1 or with recombinant adenovirus expressing P1 showed good ELISA and SNT titer and were protected from FMDV challenging [4].

Control of the disease is achieved by vaccination with a chemically inactivated whole virus vaccine emulsified with adjuvant, providing only short-term protection [5]. As outbreaks of foot-and-mouth disease virus (FMDV) have been associated with inadequately inactivated virus or by escape of virus from sites of vaccine production, considerable but unsuccessful effort has been directed towards the development of DNA vaccines based on the sequence of P1 gene [6]. This approach is being widely investigated to protect against a large number of infectious disease including FMD, is able to elicit both cell-mediated and humoral immune responses [7].

One major limitation of DNA vaccines is their inability to amplify and spread in vivo as some replicating viral vaccine vectors are able to do. Therefore, a strategy that facilitates the spread of antigen may significantly enhance the potency of naked DNA vaccines. Studies have shown that alphaherpesvirus virus VP22 protein, one of the tegument proteins, possesses the property of intercellular transport: VP22 can ferry various genetically fused effector proteins to bystander celler without loss of effector function [8]. Recently, studies have shown that bovine herpesvirus 1 VP22 (BVP22) has been able to enhance the potency of DNA vaccines and revealed that the genetic adjuvant properties of BVP22 is much more effective than other VP22. The BVP22-antigen DNA has shown the capability of a remarkable new type of bystander effect, and a DNA construct encoding BVP22-linked antigen could elicit enhanced immune responses than compared with a DNA construct encoding antigen alone in mice [9].

So, in this study, we constructed DNA vaccines encoding FMDV P1 protein and BVP22 fused P1 protein and evaluated the efficacy of P1 DNA vaccine and the potency of BVP22-P1 DNA vaccine.

Methods

Cells and Virus:

BHK-21 and IBRS-2 cells were obtained from ATCC, maintained in Dulbecco's

modified Eagle's medium (DMEM,Invitrogen) supplemented with 10%(v/v) fetal calf serum(FCS) and 100μg/ml streptomycin and penicillin each at 37ºC in 5% CO_2. FMDV O/ES/2001 strain was propagated and tittered in BHK-21 cells, the supernatants of infected cells were clarified and stored at -70ºC .

Construction of recombinant plasmid pcDP1 and pcDBP1

The resulting plasmid pcDP1 and pcDBP1, which carry the P1 sequence, were under the transcription control of the human cytomegalovrus (hCMV) immediate-early promoter/enhancer provided by pcDNA3.1 (+) (Invitrogen). During all plasmid constructing , the Escherichia coli stain $DH_{5α}$was used. The constructed plasmids were subsequently purified using an EndoFree Plasmid Mega kit (Qiagen).

Mouse immunization

Plasmid DNAs, pcDP1 、pcDBP1 and pcDNA3.1(+) were delivered into BALB/c mice by intramuscular injection. Each plasmid was dissolved in PBS at a concentration of 1μg/μl. Each BALB/c mice was injected with total 100μl plasmid solution at the inner side of the two hinder legs averagely. The booster was done with the same quantities of DNAs 2 weeks later. Sera were collected at 4,6 weeks after primary immunization to detect P1-specific ELISA antibodies and neutralizing antibodies against FMDV.

Assay for antibody titer by ELISA

P1- specific antibody responses were determined by ELISA test using the recombinant P1 protein produced in E.coli as antigen, the recombinant P1 protein was purified by GST purification kit (Ame Bio 27-4574-01) .96-well microtiter plates were coated with recombinant P1 protein (4.15μg/ml in 50mM sodium carbonate coating buffer ,pH 9.6;100μl/well) and incubated at 4ºC overnight. The plates were washed three times with wash buffer PBST(0.05% Tween-20 in PBS) and blocked for 1 h at 37ºC with blocking buffer (1% BSA in PBST). After three washes, serum samples were diluted to 1:40 in blocking buffer,added to each well (100μl/well) in duplicate and incubated for 1 h at 37ºC. Plates were washed three times with PBST, and treated with 100μl HRP-conjugated goat anti-mouse IgG antibody (SBA; diluted to 1:5000 in blocking buffer) each well for 1 h at 37ºC .After three washes, 50μl of substrate solution TMB was added to each well for 20 min at room temperature in dark and the reaction was stopped by addition to each well of 50μl 1% SDS. The absorbance at 630 nm was measured by an ELISA reader (Labsystems MK3).

Assay for neutralizing antibodies

Before testing ,Serum samples from test animals were inactivated at 56ºC for 30 min. 50μl of each sample was added to a 96- well tissue culture plate with a two-fold serially dilution, and mixed with 200 $TCID_{50}$ of FMDV in a 50μl /well, then incubated for 1 h at 37ºC in 5% CO_2. After incubation, 100μl of 10^6 cells/ml BHK-21 cell suspension was added to each well and plates were incubated for 4 days at 37ºC in 5% CO_2. Thereafter, cells were monitored for FMDV-specific cytopathic effect (CPE) and neutralization titers were calculated as the log_2 of the reciprocal of the highest dilution resulting in complete neutralization.

Lymphocyte proliferative response

Mouse splenocytes were prepared as follows. Spleens were removed from the immunized mice and were homogenized in PBS (pH7.4). The erythrocytes cell suspension were lysed with 0.75% Tris-NH_4CL (pH7.4). After being washed three times with PBS, the splenocytes were resuspended at $4×10^6$ cells/ml with the supplemented RPMI 1640 containing 10% FBS, 14mM HEPES, 50mM 2-mercaptoethanol, 100μg/ml streptomycin and 100 IU/ml penicillin. Splenocytes were plated in

96-well flate-bottom plates at 100µl / well. Subsequently 100µl / well of medium with or without 200 $TCID_{50}$ FMDV were added and mixed. Each splenocytes sample was plated in triplicate. After 72 h of incubation , 20µl of MTS (3-(4,5-dimethylthiazol-2-ly)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; Promega) was added to each well and plates were incubated for a further 4 h. At the end of the incubation, the plates were read at 492 nm. The stimulation index (SI) was calculated as the ratio of average OD value of wells containing antigen-stimulated cells to average OD of wells containing only cells with medium.

Statistical analyses

All data of VN tests, Lymphocyte proliferative response assays and ELISA was analyzed by the *t*-test. P-values of < 0.05 were considered statistically significant.

Results

Construction and expression of the P1 gene in expression plasmid.

A P1 cDNA of FMDV type O/ES/2001 strain was subcloned into pCNA3.1 (+) and pcDBVP22 with the VP22 gene of bovine herpesvirus 1 (BHV-1), creating four cDNA expression plasmid, namely, pcDP1 and pcDBP1.

Comparison of humorol immune responses in mice immunized with pcDP1 and pcDBP1

To determine the ability of different DNA vaccine constructs to induce P1-specific antibody responses, groups of five mice were immunized i.m. with 100 µg of each of different DNA constructs, as described in Section 2. Serum samples were collected at various time points and P1-specific lgG was analyzed by indirect ELISA. As shown in Fig 1, at 4 weeks after primary immunization, the group immunized with pcDP1 or pcDBP1 could induce P1-specific ELISA antibodies and the significant difference ($P < 0.05$) was observed between groups immunized with pcDP1 and pcDBP1. After a booster immunization, a stronger increase in the sera of pcDP1 or pcDBP1-immunized mice was observed and the antibody level anti-P1 lgG in the sera of pcDBP1 is higher than that of pcDP1.

Sera samples were further evaluated the ability to neutralize FMDV in vitro in serum neutralization assay. As shown in Fig2, after a single immunization, detectable neutralizing antibodies were developed in the sera of mice immunized with pcDP1 or pcDBP1. However, after a booster, the higher neutralizing antibody was observed in the sera of mice immunized with plasmid pcDBP1, similar to the ELISA antibodies responses.

Cell-mediated immune responses

Not humoral immune responses induced by pcDBP1 were higher than those of pcDP1, but it provided more efficient protection after virus challenge. To test the cellular immune responses, mice were immunized with different DNA constructs as described earlier. Lymphocyte proliferative responses were analyzed at 4 weeks after secondary immunization. As shown in Fig. 3, similar to the humoral immune responses, the P1-specific proliferative response was significantly higher in mice immunized with pcDBP1 than in mice immunized with pcDP1 ($P<0.05$). Low level of lymphocyte proliferative responses was observed in control group. These results indicated that pcDBP1 induced an enhanced Th1-type immune response compared with pcDP1.

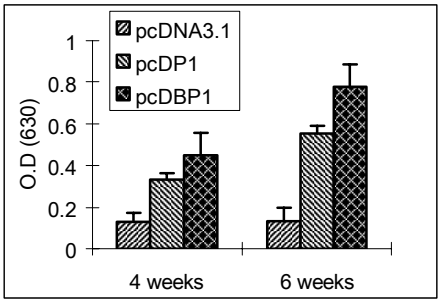

Fig.1 P-specific IgG responses of mice immunized with different DNA constructs. Each group of mice (n=5) was immunized i.m. with 100 □g of DNA at 0 and 2 weeks. Sera were collected at various time points and the specific anti-P1 antibody response was analyzed by indirect ELISA.

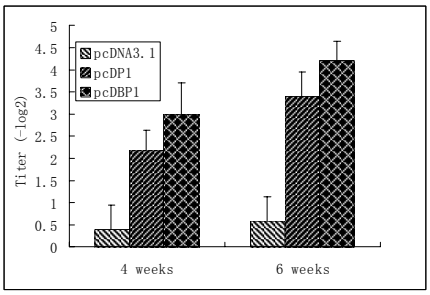

Fig.2 Development of neutralizing antibodies in sera of mice after DNA immunization. Four weeks after the second immunization, titer of FMDV-specific antibodies in mice immunized with pcDBP1is much higher than that in mice immunized with pcDP1.

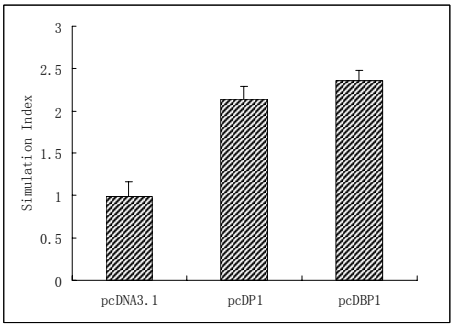

Fig.3 Lymphocyte proliferative responses induced by DNA vaccines. Either pcDP1 or pcDBP1 had induced lymphocytes proliferation responses (SI>2), but pcDBP1 induced a higher a Lymphocyte proliferative responses (P<0.05).

Discussion

The previous has reported that DNA vaccines encoding FMDV VP1 protein, which included major antigen site, didn't induce either humoral responses or cell-mediated responses [10]. In our experiment, we found that DNA vaccine pcDP1encoding FMDV P1 protein induced not only humoral responses but also cell-mediated responses. At the same time, we obtained the result that DNA vaccine pcDP1 resulted in clearance of FMDV from the sera of immunized mice challenged by FMDV, while the DNA vaccine of VP1 couldn't [19]. This is why we choose P1 for this study.

In this study, we compared DNA vaccine pcDP1 encoding P1 protein alone and DNA vaccine pcDBP1 encoding BVP22-P1 fusion protein for the induction of humoral and cell-mediated immune responses. We found that humoral or cell-mediated immune responses of mice immunized with pcDBP1 was significantly increased than that of mice immunized with pcDP1. The potential explanation for this enhancement of P1-specific may be that intramuscular administration of pcDBP1 DNA can introduce DNA directly into professional APCs of the muscle, allowing APCs to directly present P1 through the MHC-I class I pathway

In summary, our results revealed that DNA plasmids encoding P1 are effective to induce significant and specific immune responses in tested mice. Our findings illustrate the promise of enhancing vaccine potency by linkage of BVP22 to antigen, allowing for enhanced immune responses leading to clearance of virus in sera of immunized mice. This approach provides a strategy for enhancing the efficacy of immunization.

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GENETIC CHARACTERIZATION OF TYPE 2 PORCINE CIRCOVIRUS(PCV2) ISOLATES FROM DIFFERENT GEOGRAPHIC REGIONS OF CHINA

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Summary. PMWS has been diagnosed in China since 1999. Increasing evidence indicates that type 2 porcine circovirus(PCV2) is the etiological agent of postweaning multisystemic syndrome(PMWS). To determine the heterogenecity of PCV2 isolates, the complete genomes of nine PCV2 isolates from animals affected with PMWS from different geographic representative regions of China were amplified by PCR and sequenced. It is demonstrated that the complete genome of 4 isolates is 1,768bp in length and 5 isolates 1,767bp in length. All the PCV2 isolates sequenced are closely related to each other with a 97.84% nucleotide sequence identities. Homology and Phylogenetic analysis of all PCV2 isolates of China origin yet published in the database showed a nucleotide identity ranging from 95.4%-99.8%. Results also indicated that genomic sequences of PCV2 isolates from China show a high homology with those of North America and Europe origin. The data from this report showed that although the genome of PCV2 is generally stable in a worldwide scale, PCV2 isolates from different origins have minor variance in their genomic sequences. Several minor branches of PCV2 isolates of China origin were identified. Within China Mainland, although isolates from East China and Southeast share a homology more than 99.0% and the isolates from East China are more closely related to each other, with identities ranging from 99.5%~100%, it doesn't seem there is correlation between the phylogenetic minor branches of PCV2 isolates and the geographic regions.
Key words: PMWS; PCV2;Genetic Characterization

Introduction. Postweaning Multisystemic Syndrome(PMWS) is an emerging disease first described in 1991[1,2]. The disease occurs in swine herds aged 5-12 weeks old[1,3]. It now has been recognized in pigs in Canada and most of United States[2,3,4~11], many European countries[12,13,14~18], and some countries in Asia[19,20]. It has been diagnosed in China since 1999. PMWS has been brought huge economic loss to pig industry. Virus isolation from the affected pigs revealed a new type of porcine circovirus[5,11], which has been designated Type 2 porcine circovirus(PCV2) to distinguish with PCV1.

Accumulated evidence indicates that PCV2 is responsible for PMWS[6,7,12, 21]. Recently, it has increasingly been described to be associated with various disease syndromes in pigs worldwide, including porcine dermatitis and nephropathy syndrome (PDNS), porcine abortion and reproductive failure, proliferative and necrotizing pneumonia (PNP), and congenital tremors (CN)[10,17,21~23]. PCV2 often infected with porcine reproductive and respiratory syndrome virus (PRRSV) or porcine parvovirus (PPV)[12,19,23,24], and/or coinfected with various bacteria to make the illness deteriorated.

Phylogenetic analyses based on complete genomic sequences of PCV1 and PCV2 confirmed that two distinct genotypes of PCV exist[22,25]. The nucleotide sequence between the entire genomes of PCV1 and PCV2 only shares about 70% identity[25,26,27,28]. The genome sequences of different PCV2 isolates share a high homology. But within the major genotype of PCV-2, a few minor branches were identified, and some of these minor branches appear to be associated with the geographic regions of the isolates. More data are needed to gain a better understanding of the genetic relationship and evolution of PCV2. The virulence of PCV2 isolates of different origin were under further investigation.

In this report, we first described the isolation, identification of PCV2 in China mainland and conducted the sequencing analysis of nine isolates of PCV2 from PMWS-affected pigs from Beijing, Tianjin, Shandong, Guangdong province and Hebei Province.

MATERIALS AND METHODS

Sample sources

Various tissue samples(lung, liver, spleen, lymph nodes, kidney, etc) from cases of suspected PCV2-associated PMWS that originated in Beijing, Tianjin, Shanghai, Hebei Province, Shandong, Guangdong and Shenzhen of China mainland were collected and stored at -80□ until use.

PCV2 identification

Equal amounts of these tissue samples were pooled together and DNA extracted for PCR detection for the presence of PCV2 nucleic acids. A pair of PCV2 specific primers were designed on the basis of published PCV1 (U49186) and PCV2 (AF027217). They are Pw1 and Pw2 (Table 1), amplifies a 472bp fragment. The PCR was performed in a 50μl volume, containing 10×PCR buffer 5μL, 25mM $MgCl_2$ 5μL, 10mM each dNTPs 1μL, 5U/50μL Taq DNA polymerase 0.5μL, 25μM each primer 1μL, total DNA sample 3μL, DDW 33.5μL. The circling parameters consisted of predenaturation at 94□ for 2 min; then 30 cycles of denaturation at 94□ for 45s, annealing at 58□for 45s, and extension at 72□ for 45s,then by a terminal extension at 72□ for 8 min. The PCR products were subsequently digested with a unique restriction enzyme, *EcoR*I, which is present in PCV2 isolates but not in PCV1 isolates. The PCR products and the digested PCR products were all analyzed with 1%(w/v) agarose gel electrophoresis.

Virus isolation

Tissue samples that were PCV2 positive detected by PCR were processed to attempt virus isolation. 10%(W/V) tissue suspension was prepared in Earle's minimal essential medium (MEM). These suspensions were homogenized and subsequently clarified by centrifugation at 4000×g for 30min (4□). The supernatant was used for virus isolation. Virus isolation was performed as described by Allan et al. [34]. PCV-free PK-15 cells, kindly provided by Bureau of Veterinary Medicine Supervision, China, were used to isolate PCV2 from tissue suspensions. The infected PK-15 cell passages were detected by PCR. At the 4th passage, D-Glucosamine-induced Cells were harvested at the 24th, 48th, and 72th hr post-induction for transmission electron microscopic (TEM) examination. On each 24hr, one flask was harvested by scraping all cells and culture medium into a 50ml conical test tube. The tube was then centrifuged at 1500 rpm for 10 min and

the cell pellet was fixed in 2% phosphate-buffered glutaraldehyde overnight. Thin sections were made by routine method, examined and photographed using TEM.

DNA extraction and PCR amplification of complete genome of PCV2

Nine PCV2 isolates from pigs with PMWS that originated from different representative geographic regions(two isolates from Beijing(designated BX, BJ-HB), two from Tianjin(dedignated HR,TJ), one from Hebei(BF), one from Shandong(SD), two from Guangdong(GD, GDTS), one from Shenzhen(SZ)) were selected and their DNA samples were prepared from 2nd infected PK-15 cell passages and the complete genome was amplified(Table 2).

Two sets of PCR primers were designed on the basis of the published PCV2 sequence. These primers amplify two overlapping fragments that represent the entire genome of PCV2. The first set of primers, P1 and Pw2(Table 1), amplifies a 1165bp fragment(D fragment). The second set of primerswere C1 and C2(Table 1), amplifies a 989bp fragment(C fragment).

PCR was carried out using a commercial PCR kit (Sangon) scaled down to a final reaction volumes of 50μl. The final concentration of reagents were: 2.5mM $MgCl_2$, 200mM each dNTPs, 0.5μM each primer, and 2.5U Taq polymerase . 3μL of the total DNA extraction sample was used as the template. We have adopted different reaction procedures for these two fragments. For the 1165bp fragment, the PCR consisted of predenaturation at 94□ for 3 min, then 30 cycles of denaturation at 94□ for 1 min, annealing at 58□ for 1min, and extension at 72□ for 2min, followed by terminal extension at 72□ for 10 min. For the 989bp fragment, the PCR consisted of predenaturation at 94□ for 2 min, and then 30 cycles of denaturation at 94□ for 50s, annealing at 58□ for 50s, and extension at 72□ for 1.5min, followed by terminal extension at 72□ for 8 min. The PCR products were analyzed by electrophoresis on 1%(w/v) agarose gels.

Sequence and phylogenetic analysis

The PCR products of the expected sizes were purified with the DNA purification Kit (Bio-Tech inc.) according to the guide of manufacturer. Purified DNA fragments were then cloned into pGEM-T-easy vector（Promaga inc.）, respectively. The target gene was sequenced automatically using SP6 and T7 universal primer conjunct sites located on the both side of pGEM-T Easy vector polyclone sites(Takara inc., Dalian and Sangon inc., Shanghai). The nucleotide sequences were compiled using DNAsis1.0 and then analyzed through DNAMAN package. The percentages of sequence identities among different PCV2 isolates from this study and other PCV-2 isolates of China origin accessed in GenBank database were determined and Phylogenetic analysis was conducted with DNAMAN program according to .Multiple Sequence Alignment. Observed Divergence option of Distance Methods were used to produce a phylogenetic tree.

Table 1 Oligonucleotide primers used in the study

Primer	Primer sequence	position	Fragment length
Pw1	5'ACGGATATTGTAGTCCTGGT3'	1094-1113nt	472bp
Pw2	5' CAAGGCTACCACAGTCAGAA 3'	1546-1565nt	
P1	5' TGACCTGTCTACTGCTGTGA 3'	401-420nt	1165bp
Pw2	5' CAAGGCTACCACAGTCAGAA 3'	1546-1565nt	
C1	5' AGGGCTGTGGCCTTTGTTAC 3'	1329-1348nt	989bp
C2	5' TCTTCCAATCACGCTTCTGC 3'	530-549nt	

Table 2. PCV-2 isolates used in the present study as well as those accessed in Genbank.

No.	Isolate	Geographic Category	GeneBank accession number	Reference or source
1	AY294310CHNJXL		AY294310	Genbank
2	AY288135CHNHLJ	Northeast China(3)	AY288135	Genbank
3	AY288134CHNHLJ		AY288134	Genbank
4	AY391729CHN		AY391729	GenBank
5	AY188355CHN	East China(3)	AY188355	Genbank
6	AY217743CHN		AY217743	Genbank
7	CHNBF		AF381175	This study
8	CHNBJ-HB		AF538325	This study
9	CHNBX	North China(6)	AF381177	This study
10	CHNTJ		AY181946	This study
11	CHNHR		AF381176	This study
12	CHNSD		AY181947	This study
13	AF035820CNHZ	Middle China(1)	AF035820	GenBank
14	CHNGD		AY177626	This study
15	CHNGDTS	Southeast China(3)	AY181945	This study
16	CHNSZ		AY181948	This study
17	AF027217CAN	Canada	AF027217	Genbank
18	AY094619USA	United States	AY094619	Genbank
19	AF055394FRA	France	AF055394	19,Genbank
20	AJ293869UK	UK	AJ293869	Genbank
21	AF201897(NETH)	Netherlands	AF201897	Genbank
22	AY146992(TW)	Taiwan	AY146992	Genbank
23	AF364094(TW)	Taiwan	AF364094	Genbank

RESULTS

PCR amplification of overlapping fragments of PCV2 complete genome

To determine the extent of the heterogeneity of different PCV2 isolates, nine PCV2 isolates from pigs with PMWS that originated from different representative geographic regions(two isolates from Beijing(designated BX, BJ-HB), two from Tianjin(dedignated HR,TJ), one from Hebei(BF), one from Shandong(SD), three from Guangdong(GD, GDTS, SZ)) were selected and their DNA samples were prepared from 2nd infected PK-15 cell passages. By two set of specific primers, two overlapping fragments that cover the entire genome of PCV2 were successfully amplified. The PCR products were analyzed by electrophoresis on 1%(w/v) agarose gels, it showed that the D fragment was 1165bp in length, the C fragment was 989bp.

Sequence and Genetic Characterization of PCV2 isolates from different geographic regions

Purified target DNA fragments were cloned into pGEM-T-easy vector（Promaga inc.）, respectively. The positive recombinant plasmid was selected and identified by using an unique restriction enzyme site (EcoRI) that is present in the amplified fragments. After digesting the PCR products with EcoRI, the resulting patterns revealed that D fragment produced two fragments of 1042bp and 113bp, the C fragment produced 876bp and 123bp. The target gene was automatically sequenced(Takara inc., Dalian and Sangon inc., Shanghai). The nucleotide sequences were compiled using DNAsis1.0 and then analyzed through DNAMAN package.

The sequence of nine complete genome were abtained. The results showed the complete genome of five isolates is 1,767bp in length, including one from Beijing (isolate BJ-HB, GenBank accession number AF538325), one from Tianjin(isolate TJ, GenBank accession number AY181946), one from Shandong(isolate SD, AY181947) and two from Guangdong(GD, AY177626; GDTS, AY181945). The other 4 isolates showed a 1768bp in the complete genome length, among which are one from Beijing(isolate BX, AF381177), one from Tianjin(isolate HR, AF381176), one from Hebei province(isolate BF, AF381175) and one from Guangdong(SZ, AY181948). All the isolates sequenced were closely related to each other, displaying nucleotide sequence identities of 95.4%～99.7%. Two isolates both from Tianjin(isolate TJ, AY181946; isolate HR, AF381176) showed the most sequence divergence with an identity of 95.31%. We also selected the complete genome of PCV-2 isolates of China origin accessed in GENBANK, which includes three isolates from Eastern China(accession number AY391729,AY188355,AY217744), one from Middle China(AY035820) and three from Northeast China(AY288134,AY294310,AY288135) to conduct sequence divergence analysis. So our isolates cover Northeast China(3 isolates), North China(6 isolates), East China(3 isolates), Middle China(1 isolates) and Southeast China(3 isolates). All these isolates show high sequence identities. The genomic sequences of PCV2 isolates from different geographic regions in China vary to some extent, several minor branches were identified. Results also indicated that genomic sequences of PCV2 isolates from China show a high homology with those of North America and Europe origin. Within China Mainland, it doesn't seem there is correlation between the phylogenetic minor branches of PCV2 isolates and the geographic regions except that isolates from East China and Southeast share a homology more than 99.0%

and the isolates from East China are more closely related to each other, with identities ranging from 99.5%~100% and the isolates from East China are more closely related to each other, with identities ranging from 99.5%~100%.

ORF2 of PCV is believed to code for the putative capsid protein. Pairwise sequence comparisons revealed that the ORF2 genes of all PCV2 isolates shared 89.7%~100% nucleotide sequence identity.

DISCUSSION

PMWS is an emerging disease in pigs. Since it is first described in 1991, PMWS has become an economically important global disease of swine. The etiology of PMWS is rather complicated, but accumulated evidence suggested that PCV2 is the causative agent[6,7,12,21]. Various methods have proved the presence of PCV2 in tissues of PMWS-infected pigs, among which the lung, lymphoid and splenic tissues harbor a great amount[3,23,30]. Breeding sows and even boars kept in less crowded conditions were found to be infected with PCV2 with similar frequency, which indicate that PCV2 can be vertically transmitted. The presence of published complete genome of PCV make PCR method a quick and important means to detect and identify the virus. We designed PCV2 specific primers in the lowest homology area of PCV1 and PCV2 according to the published PCVs sequence and developed PCV2 specific PCR method. The specific PCR product was identified by *EcoR*I restriction enzyme which is unique in PCV2.

In this report, pigs with wasting syndrome were autopsied, histopathological lesions comparable to those previously documented for post-weaning multisystemic wasting syndrome were observed. Lungs, lymph nodes and spleens which are the major affected organs were abtained as materials to isolate and identify PCV2.

According to geographic distribution, in this study, nine isolates were selected to conduct genome sequencing and phylogenetic analysis to fully characterize these new circovirus isolates.

In this study, we also selected the complete genome of PCV-2 isolates of China origin accessed in GENBANK, which includes three isolates from Eastern China(accession number AY391729,AY188355,AY217744), one from Middle China(AY035820) and three from Northeast China(AY288134,AY294310,AY288135) to conduct sequence divergence analysis. So our isolates cover Northeast China(three isolates), North China(six isolates), East China(three isolates), Middle China(one isolates) and Southeast China(three isolates)(see Table 2). This strategy is better for getting a full understanding of PCV2 isolates of China origin. All these isolates show high sequence identities. The genomic sequences of PCV2 isolates from different geographic regions in China vary to some extent, several geographic minor branches were identified. Results also indicated that genomic sequences of PCV2 isolates from China show a high homology with those of North America and Europe origin. Within China Mainland, it doesn't seem there is correlation between the phylogenetic minor branches of PCV2 isolates and the geographic regions except that isolates from East China and Southeast share a homology more than 99.0% and the isolates from East China are more closely related to each other, with identities ranging from 99.5%~100%. Pairwise sequence comparisons revealed that the ORF2 genes of all PCV2 isolates shared 89.7%~100% nucleotide sequence identity.

By far, the homology data and the phylogenetic analysis showed that PCV2 isolates from worldwide are closely related to each other and they share a high genome sequence homology. But within the genotype of PCV2, a few minor branches were identified, some of these minor branches appear to be correlated with the geographic regions. These results indicated that in a worldwide scale, PCV2 was generally stable. PCV2 isolates from different geographic origin vary in their genomic sequences to some extent.

ORF2 of PCV is believed to code for the putative 233 amino acid capsid protein[28,31,32~34]. Sequence of ORF2 in some PCV2 isolates display lower Amino acid identity suggest that some antigenic difference lie in PCV2 isolates. The role of genetic mutations of ORF2 gene on the antigenicity of virus and the virulence of PCV2 isolates of different origin were under further investigation.

Some scholars speculated the possibility of the difference of virus morphogenesis in the natural target cell in vivo versus virus in cell lines in vitro[22,35]. The cell cycle(affected with glucosamine treatment) and passage level of target cells and virus in vitro may also affect virus morphogenesis. In this study, DNA samples were prepared from 2nd infected PK-15 cell passages and the complete genome was amplified, in order to maximally avoid the possibility of in vitro mutations, we choose less passage level virus, but we can't definitely rule out it.

Various researches confirmed the mutations in PCV2 genome. Available evidence indicates that a small number of point mutations and a resultant substitutions of 1 or 2 amino acids in another small DNA virus, feline parvovirus, allow a shift in host range from cats to dogs. These mutations result in a highly pathogenic virus to dogs. Whether the minor change of genome of PCV2 will get the same result is still unknown. But recently, it has increasingly been described to be associated with various disease syndromes in pigs worldwide, including porcine dermatitis and nephropathy syndrome (PDNS), porcine abortion and reproductive failure, proliferative and necrotizing pneumonia (PNP), and congenital tremors (CN)[10,17,21,23]. This may be reflective of the suspicion. So the research on genetic characterization and functional genomics of PCV2 are of significant importance.

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Abstract

Four weeks old SPF piglets were inoculated with porcine reproductive and respiratory syndrome virus (PRRSV) BJ-4 strain. The porcine alveolar macrophages (PAM) was collected at 1, 3, 7, 14, 21, 28, 42, 56 and 84 day post infection, respectively. Simultaneously, the PAMs from SPF piglets were infected with PRRSV BJ-4 in in vitro. The MHC I molecules (SLA-1) and the MHC II molecules (SLA-DR) on PAM were detected by flow cytometry. The IL-1 level secreted by infected PAM following LPS stimulation for 24 hours was detected by ELISA kit. It was shown that the percentage of SLA-I+ and SLA-DR+ cells of PAM were decreased in two weeks in the early stage of PRRSV infection in vivo, and in vitro experiment showed the same pattern. The amount of IL-1 secreted by PAM infected with PRRSV in vivo were persistently elevated in two weeks of early stage, then decreased slowly and steadily. In *in vitro* infection experiment, the level of IL-1 β showed a pattern of linear increasing till 96 hours after infection. It was concluded that PRRSV infection could decrease the presentation function of PAM to exogenous and endogenous antigen, thus affect the generation of immune response in piglets. PRRSV infection seemed to up-regulate the IL-1 secretion of PAM during the early infection. These may help to understand the humoral immune and cell-mediated immune response against PRRSV infection.

Keywords: Porcine reproductive and respiratory syndrome virus; MHC molecules; IL-1; Porcine alveolar macrophages

1. Introduction

Since its emergence in the late 1980s, porcine reproductive and respiratory syndrome virus (PRRSV) has been recognized worldwide as one of the most economically important etiological agents of swine (Goyal S.M.,1993; Meulenberg. J.J.M et al., 1998; Rossow. K.D.,1998). PRRSV is an enveloped, positive-stranded RNA virus that belongs to the family of Arteriviridae (Cavanaugh. D.,1997). The mechanisms by which PRRSV undertakes to invade the host immune system is still unclear, but studies suggest that PRRSV negatively modulate the host immune system. Following infection, PRRSV persists in infected pigs for at least 150days and infectious virus can be shed (Albina. E., 1994; Benfield. D.A., 1996,1997; Allende. R., 2000;), which suggests the immune responses are not able to completely eliminate the virus from the infected host.

PRRSV infection appears to induce an ineffective or at least delayed immune response. Immune responses to PRRSV have been studied extensively and virus specific humoral and cellular responses, including lymphocyte proliferation, antigen presentation, antibody production, cytotoxic activity and cytokine production have been demonstrated in PRRSV-infected pigs (Suradhat.S., et al., 2003). Macrophages play a central role in resistance to and infection, this may due to function-deficiency recovery from many viral infections. Results showed PRRSV replication appears to be limited mainly to phagocytic cell populations (Molitor.T.W et al., 1996). There appears to be a poor innate immune response of PAM at the time of PRRSV infection. Cytokines play an important role in the modulation of immunological processes. Production of pro-inflammatory cytokines by virus-infected macrophages is a prelude to activation of effector cells of innate resistance. But pro-inflammatory cytokines were mostly detectable or minimally

increased following exposure to the virus(Van Reeth. K and Nauwynck, J. 2000). IL-1 is the first pro-inflammatory cytokine to be produced following pulmonary virus infection, which importantly functions in cellular and humoral immune responses.

The objective of this study was to determine the presentation function and IL-1 secretion of PAM in vivo and vitro. These may help to further understand the humoral immune and cell-mediated immune response against PRRSV infection.

2. Materials and Methods

2.1 Animals and experimental design

Thirteen four weeks old SPF piglets were purchased from Beijing SPF Pig Center. The piglets were randomly assigned in two groups. Nine piglets were inoculated with strain BJ-4 of PRRSV by intranasal instillation of 2 ml inoculums with a $TCID_{50}$ of $10^{-5.6}$/0.5ml and were bled and euthanized at 1, 3, 7, 14, 21, 28, 42, 56 and 84dpi, respectively. The other four piglets served as non-infection control group, were bled and euthanized at 0, 28, 56, 84d of experiment, respectively. Two SPF piglets were prepared for PAM isolation for the purpose of in vitro PRRSV infection.

2.2 *Samples preparation*

After the infected piglets were bled, the lungs were lavaged with Hank's solution. The recovered bronchoalveolar lavage fluids were filtered and separated into cells and supernatants by centrifugation. The cells were then resuspended with RPMI-1640 supplemented with 20% FBS and incubated for 2 hours at 37℃, 5% CO_2, then 0.02% EDTA was added to digest and collect PAM.

2.3 Analysis of IL-1 expression on PAM in in vitro PRRSV-infection experiment

Porcine IL-1βELISA kit (BIOSOURCE, USA) was used to detect dynamics of IL-1βproduced by PAM lavaged from infected piglets, following LPS stimulation for 24 hours in vitro, according to manufacturer's instructions. As control, the PAMs from SPF piglets were infected with PRRSV BJ-4 in vitro, the supernatants of different incubation period were harvested and level of IL-1β was detected as above.

2.4 Analysis of SLA-1$^+$ and SLA-DR$^+$

Mabs(VMRD, Inc. and PHARMINGEN, Inc.) specific for SLA-1 and SLA-DR were added to PAM suspension, incubated at 4℃ for 30min, then FITC-goat-anti mouse ($F(ab')_2$, SOUTHERN BIOTECHNOLOGY Inc.) was added to label positive cells, incubated at 4℃ for 45min in shadow place. The suspension was subjected to centrifugation to separate cells, then the cells were resuspended for flow cytometric analysis (EPICS^{TM}Elite, Coulter, Inc.).

3. Results

3.1 Analysis of IL-1 expression on PAM in in vitro PRRSV-infection experiment

The standard concentration-OD value curve was established based on standard product supplied with IL-1 β ELISA kit, according to manufacturer's instructions. And the dynamics of IL-1 β secreted by PAM of SPF piglets experimentally infected with PRRSV BJ-4 was determined(Fig. 1). It was shown that the amount of IL-1 β secreted by

PRRSV-infected PAM was persistently elevated and peaked at 14d post infection, then decreased gradually and steadily. The amount of IL-1 β secreted by PAM of control group was measured at different days post infection, however, dramatic changes were not shown. In in vitro infection experiment, the level of IL-1 β showed a pattern of linear increasing till 96 hours after infection(Fig. 2).

3.2 Analysis of SLA-1$^+$ and SLA-DR$^+$ cell percentage of PAM from PRRSV-infected SPF piglets in in vivo and in vitro

After SPF piglets was infected with PRRSV, the PAM was lavaged at different days post infection, the SLA-1$^+$ and SLA-DR$^+$ cell percentage were measured with flow cytometry (Fig.3). Meanwhile, the PAM from SPF pigs was inoculated with PRRSV in vitro. PAM was harvested at different time intervals, and the SLA-1$^+$ and SLA-DR$^+$ cell percentage were measured as described above(Fig.4). It was shown that the percentage of SLA-1+ and SLA-DR+ cells of PAM were decreased in two weeks in the early stage of PRRSV infection, then increased to the highest at three to four weeks post infection, and later decreased slightly and then remained stable till the end of experiment. Usually, the SLA-I+ and SLA-DR+ cells of PAM from normal piglets remain constant. The results in vitro tests showed the same pattern. The percentage of SLA-1+ cells decreased slightly in 24 hours post infection, then recovered. But the percentage of SLA-DR+ cells decreased dramatically in 24 hours post infection, then persistently increased and reached its peak at 48 hours post infection. In contrast, the SLA-1+ cell percentage of PAM from control group remained generally constant.

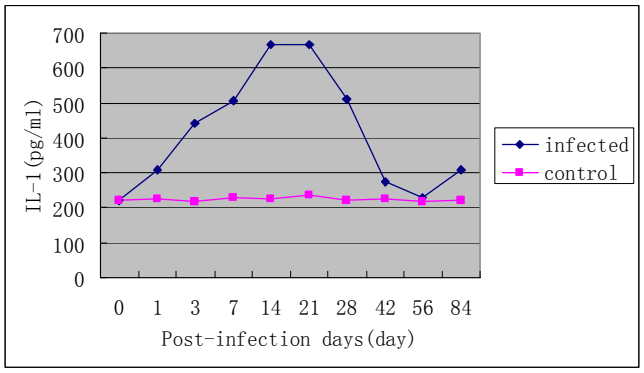


Fig.1 The dynamics of IL-1 β secreted by PAM of SPF Piglets experimentally infected with PRRSV BJ-4 in vivo.

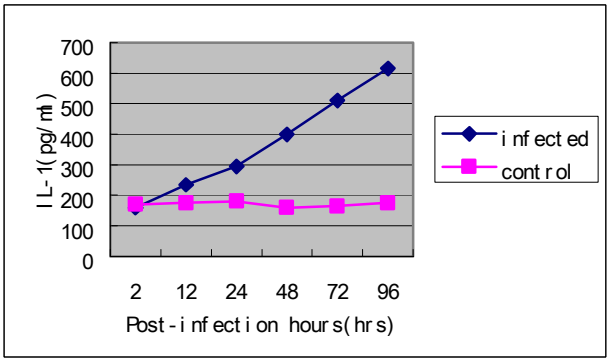
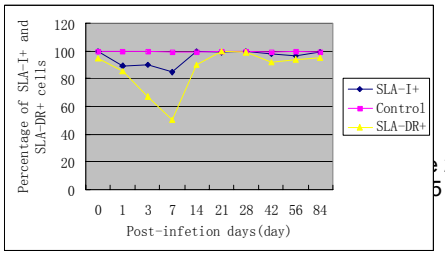


Fig.2 The dynamics of IL-1 β secreted by PAM of SPF Piglets experimentally infected with PRRSV BJ-4 in vitro.



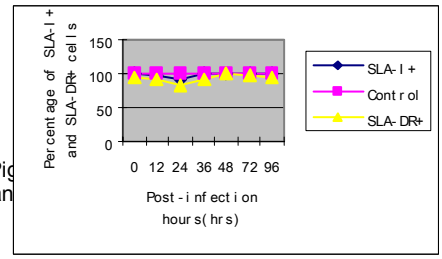


Fig.3 The dynamics of SLA-I^+ and SLA-DR^+ cells percentage of PAM from SPF piglets experimentally infected with PRRSV BJ-4 in vivo.

Fig.4 The dynamics of SLA-I^+ and SLA-DR^+ cells percentage of PAM experimentally infected with PRRSV BJ-4 in vitro.

4.Discussion

PRRS virus infection can disturb the immune response by down-regulating immunocyte and cytokine and other factors. In contrast, the immune system boost up the immune response by up-regulating these factors to control and eliminate the PRRS virus. We previously reported the experiment on the antibody response to class swine fever virus vaccination during PRRS virus BJ-4 strain infection (Li. H et al.,2003), the results demonstrated that PRRS virus infection can suppress the antibody response to class swine fever virus vaccination. In addition, we analyzed the proportional change of lymphocyte subpopulation during PRRS infection(unpublished data), we found that on the early stage of infection, the proportion of CD3+,CD4+,CD8+ and SLA-DR+ T cells in peripheral blood mononuclear cells decrease. What's more, the data about change of the level of IFN –γ, IL-10 and IL-12 in peripheral blood mononuclear cells of pigs infected with porcine reproductive and respiratory syndrome virus has reported (Johnsen. C. K. et al 2002; Sanipa. S et al 2003; Feng. W. –H et al., 2003;). But data about PRRSV influence on function of pulmonary alveolar macrophage (PAM) which is the target cell during PRRS virus infection is unkown. In this study, we made an attempt to elucidate the influence to PAMs and their function by the change of secretion of IL-1 and dynamics of SLA-I and SLA-DR type molecules in the surface of PAMs during PRRS virus infection in vivo and in vitro.

Pulmonary alveolar macrophages (PAMs) play an important action in pulmonary immunity. They eliminate the pathogens by ingesting and digesting them in non-specific immunity. They act as modulator by adjusting antigen-presenting and secreting cytokine during specific immunity. The target cell of PRRS virus infection is PAM. In order to escape from the action of PAM they decrease the number of PAM and intervene their functions such as pathgocytosis and antigen presentation during infection.

IL-1 is a critical element in cytokine network immunity, mainly secreted by activated mononuclear macrophage and mediates immune response and inflammatory reactions of body to get rid of the pathogens. In this study, our results showed that the level of IL-1βsecreted by PAM elevates slowly during the early stage of PRRS virus infection, then slowly recovers to normal level, contrast with the control. In a previous study, during ATCC VR-2332 strain infection, IL-1β showed high level expression in serum, but the change of the control was unclear (Zhou et al 1992). Other study demonstrated that the mRNA of IL-1αand IL-1βin peripheral blood mononuclear cells obviously ascends during 48 hours of PRRS virus infection using semi-quantitative competitive RT-PCR (Ronngroje. T et al.). The up-regulate of the IL-1 and other cytokine can help activate the cellular

immunity and inflammatory reactions to clear up the pathogens. So we thought the ascent of IL-1β contributes to help induce the inflammatory reactions and immune responses to control and eliminate PRRS virus of lung during the early stage of infection. But later, the level of IL-1β decreased, maybe it was due to the suppression of other cytokines.

Effective immune response depends on successful antigen presentation. In order to complete antigen presentation and induce immune response, there must have adequate MHC molecules on surface of macrophages. MHC-Ⅰ(SLA-Ⅰ)and MHC-Ⅱ(SLA-Ⅱ) are both expressed on the surface of PAM of pig, what's more, SLA-DR molecule dominates in SLA-Ⅱmolecules. SLA-Ⅰand SLA-DR molecules with high-level expression on the surface of macrophage not only indicate that strengthen of capability of antigen presentation, but also marker of activation of the cells. In this study, we analyzed the dynamics of expression of SLA-Ⅰand SLA-DR molecules on the surface of PAM using flow cytometry. The results illuminated that SLA-Ⅰand SLA-DR molecules declined during the early stage of infection, then the expression of SLA-DR molecule elevated for a period of time, it accorded with zhang's experimental results(unpublished data), but different with Martin B's data (Martin B et al., 1999), that may ascribed to the virus strain used and dose of virus inoculated. The decline of expression of SLA-Ⅰand SLA-DR molecules may suppress effective immune response and resistance to the pathogens of respiratory tract and help proliferation and re-infection of the virus; the re-elevation of SLA-DR molecules may enhance the immune response of body and eliminate the pathogens, that may be characteristics of pigs recovering to health. Not only in vivo, but also in vitro, the influence to the level of expression of SLA-Ⅱis much more than to SLA-Ⅰ, we thought one reason is that SLA-Ⅰis stably expressed on the surface of macrophage and little influenced by foreign factors such as virus; the other reason is that the virus play a key role in counteract host humoral immunity during the early stage of infection.

In conclusion, We validated that the level of IL-1 secretion during the early stage of infection, that may help immune system to eliminate PRRS virus; the percentage of SLA-I+ and SLA-DR+ cells of PAM decreased in two weeks during the early stage of PRRSV infection, these may help the virus escape from immunity response and come into being persistant infection.

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Bacteriology

SALMONELLOSIS PREVALENCE IN THE PHILIPPINES

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Introduction and Objectives

Salmonella sp. infection is one of the most overlooked diseases worldwide. Its historical discovery was only in association with Classical Swine Fever (Hog Cholera). The identification and eradication of the viral etiology of the latter relegated Salmonellosis to an opportunistic pathogen in swine. It was only in the 1980's in North America that the pathogenic potential of *S. cholerasuis* was fully realized[2]. The purpose of this study is to determine the prevalence of Salmonellosis in 55 cases from commercial swine farms in the Philippines.

Materials and Methods

The study involved 55 cases from Philippine commercial farms from the period of January 2002 to November 2004 where 330 tissue samples (e.g. spleen, lungs, kidneys, stomach and lymph nodes) were tested using *Salmonella* PCR. Most of these farms experienced high mortalities in the nursery and early growing phase. A positive case indicates the presence of even a single sample positive result.

Results and Discussion

Twenty eight percent (28%) of the samples tested and 56% of the cases tested registered positive for the test. Out of 55 cases, 31 were positive to the test.

Table 1. Prevalence of Salmonellosis

	n	% Seropositive
Sample	330	28%
Cases	55	56%

This indicates that in at least 5 out of 10 cases, Salmonellosis was confirmed in Philippine farms. While most of these farms are implementing control measures through antimicrobials directed towards *Salmonella* infection, results at best, are variable.

This has a tremendous impact in possible zoonotic and epidemiologic implications. Reduction of the impact of infection is possible and should be implemented in farms confirmed for the said disease[3,4].

In the light of the ban using antimicrobials as growth promotants and the consciousness for "clean meat", reduction of *Salmonella* contamination and infection becomes an even more urgent and important objective in the industry.

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MYCOPLASMA HYORHINIS IN THAILAND AND ITS SUSCEPTIBILITY

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Introduction

Mycoplasma hyorhinis is one of the porcine mycoplasmal causes of the systemic diseases in pigs named Mycoplasmal Polyserositis-Arthritis. Lameness in the nursery and respiratory problems or Glasser's Disease- like symptoms found on the farm can be caused by this pathogen so *M. hyorhinis* has to be taken into consideration when these symptoms occur.

Mycoplasma hyorhinis was isolated from nasal cavities of pigs and named by Switzer in 1955. It is commonly present in the nasal cavity of swine and in pneumonic pig lung. Some authors have the opinion that it is only a secondary invader without primary pathogenic effect on the porcine lung but others consider that it can induce pneumonia in the pig. It occasionally causes polyserositis and arthritis in young pigs and it is also associated with otitis media in pigs. The high incidence of infection in piglets a few weeks old demonstrates that the spread of infection takes place in the farrowing unit. *M. hyorhinis* can be commonly found in pigs of all ages. Strains of *M. hyorhinis* can be isolated from brain, liver, conjunctivae, joint, tonsils and respiratory tract, e.g. pneumonic lung tissue, and nasal cavities. It has also been isolated from pigs with Porcine Reproductive and Respiratory Syndrome (PRRS) (Friis, 1974; Ross ,1999; Radostits et al., 2000; Wu et al., 2000).

Aetiology and Epidemiology

Mycoplasma hyorhinis infections are transmitted to young pigs from infected sows in farrowing pens or from older pigs in nursery and grower units. It colonizes the mucosa of sucking piglets and, under stress conditions, may spread with subsequent polyserositis and arthritis. Disease commonly occurs between 3-10 weeks old but may occasionally occur in older pigs. Outbreaks can occur within and between litters. Mortality is lower than 10% but chronic disease has a negative effect on growth performance including slow growth and runts. Many infected pigs evidence no clinical signs. However, it may become more

important as a cause of piglet pneumonia (Gyles and Thoen, 1993; Ross, 1999; Radostits et al., 2000).

During the year 2002-2003, National Institute of Animal Health (NIAH), Thailand, found *M. hyorhinis* from 43 samples which were from lung 39 samples, heart one sample, brain one sample and joint of leg 2 samples. Most of the samples were pigs at 5-12 weeks old (Thongkamkoon, 2004).

Pathogenesis

M. hyorhinis commonly inhabits the upper respiratory tract and, under stress conditions, may cross the epithelial barrier and become systemic. Infections with *M. hyorhinis* may localize in the joints and the serous-membrane-lined body cavities to stimulate acute inflammation. It also precedes acute eustachitis and inflammation of other sites of the ear in the first week of life. The pathogen can be isolated from acute stage lesions of polyserositis or arthritis and during the subacute stage, 2 weeks to 3 months after the clinical signs, it can still be isolated but decreased in numbers.

Johannsen et al. performed an experiment to challenge 10 day old piglets with *M. hyorhinis* under swim stress and no swim stress and they found that the infection rate for *M. hyorhinis* in pneumonic lungs is higher than in non-pneumonic lungs (66.7% vs 25.0%) and highest in experimentally infected stressed piglets (56.2%) (Johannsen et al., 1991; Gyles and Thoen, 1993; Ross, 1999).

Clinical Signs

M. hyorhinis , as the causal pathogen of Mycoplasmal Polyserositis-Arthritis, leads to arthritis and inflammation of the lining of the thoracic and abdominal cavities including the epicardium and the membrane around the testis.

Pigs from the late sucking to nursery period, at around 6 to 27 kg body weight or between 3-10 wks old, are the most susceptible group with respiratory signs and lameness.

Clinical signs start 3-10 days after exposure or stress. Slight fever, less than 40.6° C, is present for 4-5 days. Pigs show moderate anorexia, are unwilling to move, have low growth rate, roughened hair coat, huddled appearance, laboured or difficult breathing and abdominal breathing but usually no coughing. Piglets show severe runting after weaning. The main clinical signs consist of

lameness and swollen joints from polyarthritis and polyserositis. Some pigs remain affected for up to 6 months. The lameness and swollen joints in young pigs which can be also caused by *Streptococcus suis* and *Haemophilus parasuis* (Glasser's disease) may be confusing for veterinarians and farm managers for differential diagnosis (Ross, 1999; Taylor, 1999; Radostits et al., 2000).

M. hyorhinis is commonly found in the Eustachian tube of young pigs. This mycoplasma can be the primary cause of porcine otitis media (Ross, 1999).

Gross and Microscopic Lesions

Necropsy findings are serofibrinous pleuritis, pericarditis and peritonitis. In chronic cases, pleural-pericardial adhesions and adhesions between the organs in the affected cavity can be found. Swelling of the joints with or without blood in the synovial fluid and membrane depends on the degree of infection. In acute arthritis, fibrinous sero-haemorrhagic fluid and hyperaemia of the synovial membrane is found. Thickening of the synovial membrane (joint capsule), increasing amounts of fluid and erosion of cartilage are found in chronic cases. Synovial fluid from chronic cases does not contain organisms which can be cultured.

Microscopic lesions of the respiratory tract show loss of cilia, immature epithelial cells and hyperplasia of the pneumocyte II in affected lung. In acute cases, the joints contain fibrinous sero-haemorrhagic fluid with lymphocytes, neutrophils and exfoliated synovial cells. In the chronic phase, mononuclear infiltrations around the subsynovial capillaries are found (Johannsen et al., 1991; Gyles and Thoen, 1993; Ross, 1999; Taylor,1999; Radostits et al.,2000).

Diagnosis

Samples for confirmation of diagnosis are as follows:

Histology: synovial membrane, liver, lung and heart for Light Microscopic diagnosis.

Mycoplasmology: culture swabs from serosal surface and joint from acute or early phase.

Samples for diagnosis by cultivation have to be taken from pigs killed in the acute stage of disease because the autolytic changes in dead pigs may reduce the chance of isolating *M. hyorhinis*.

Differential diagnosis from *Streptococcus suis* and *Haemophilus parasuis* infection can be made by clinical signs, necropsy findings and laboratory examination. The necropsy findings vary as follows:

Streptococcus suis: Produces more purulent arthritis with swelling of joints. More severe peritonitis.

Haemophilus parasuis: The gross lesion is very similar but more severe.

Bacterial and mycoplasmal culture is necessary for differential diagnosis.

The criterion to differentiate from other Mycoplasmas is not only Mycoplasma identification or culture, but also the age of animal since *M. hyosynoviae* may occur in fattening or older pigs (Johannsen et al., 1991; Ross, 1999; Taylor, 1999; Radostits et al.,2000).

PCR can also be used for DNA detection of Mycoplasma from lung tissue at the sensitivity of 10^4 cfu/g (Thongkamkoon and Narongsak, 2002). This technique can be avoid the problems of *M.hyorhinis* contamination and overgrowth in *M. hyopneumonia* cultures. It is also the faster technique than Mycoplasmal culture, taking 1-2 days comparing with 7-30 days for culture and identification, since *Mycoplasma spp.* are the fastidious organism (Thongkamkoon, 2004).

Susceptibility Test of Mycoplasma hyorhinis to Antimirobial Agents

Thongkamkoon et.al. (2005) studied MIC of *M.hyorhinis* and to determine the minimum inhibitory concentrations (MICs) for ten antimicrobial agents against 26 *M. hyorhinis* fields isolates in Thailand.(See Table 1)

All isolates were highly susceptible to valnemulin, tiamulin, aivlosin, josamycin, lincomycin, doxycycline and tylosin, moderately susceptible to tilmicosin, oxytetracycline (OTC), and chlortetracycline (CTC). Valnemulin showed very highly activity against all *M.hyorhinis* isolates. MIC 90 was less than 0.006 ug/ml, ten times lesser than in Hungary (Stipkovits, et al, 2004)(See Table 2). The reason might because valnemulin is still not used in Thailand. Tiamulin was less active with MIC90 at 0.19 ug/ml which were 5 times lesser than in Hungary (Stipkovits, et al, 2004) but almost same as the test by Ter Laak et al 1991 and in Japan (Kobayashi et al, 1996). The MIC90 for OTC and CTC were high

compare to the data in Japan which were performed 9 years ago (Kobayashi et al, 1996) and in USA (Williams, 1978).

From Thongkamkoon's study and other previous study, it can be concluded that *M.hyorhinis* is susceptible to the same antimicrobial as *M.hyopneumonia* .

Table 1. MICS for *M.hyorhinis* determined by microdilution method (Thongkamkoon et.al , 2005)

Antimicrobial	MIC (ug/ml) from *M.hyorhinis* (n = 26)		
	Range	MIC_{50}	MIC_{90}
Aivlosin	0.024-6.25	0.097	0.19
Chlortetracy-cline	6.25-50	25	25
Doxycycline	0.097-3.12	0.78	1.56
Josamycin	0.19-6.25	0.39	0.78
Lincomycin	0.19->12.5	0.78	0.78
Oxytetracycline	1.56-25	6.25	12.5
Tiamulin	0.024-0.39	0.097	0.19
Tilmicosin	0.19-50	0.78	3.12
Tylosin	0.39-6.25	0.78	1.56
Valnemulin	≤0.006	≤0.006	≤0.006

Antimicrobial	MIC (ug/ml) from *M.hyorhinis*		
	Range	MIC_{50}	MIC_{90}
Doxycycline	0.06-8.0	0.5	8.0
Valnemulin	0.06-0.5	0.06	0.25
Tylosin	4.0-32.0	8.0	32.0
Vancomycin	1.0-8.0	2.0	8.0
Chlortetracycline	4.0-32.0	16.0	32.0
Tilmicosin	4.0-32.0	16.0	32.0
Tiamulin	0.06-2.0	0.125	1.0

Table 2. MIC range, MIC_{50} and MIC_{90} (mcg/ml) of tested antibiotics for *Mycoplasma hyorhinis* (Stipkovits, et al, 2004)

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THE PREVALENCE AND BIOCHEMICAL PROPERTIES OF *BRACHYSPIRA HYODYSENTERIAE* IN THAILAND

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Introduction

Swine Dysentery (SD) is known as a highly contagious disease causing bloody diarrhea in pig (Pearce, 1999; Barcellos *et al*., 2000). The dominant clinical symptoms included diarrhea at various age of pig, particularly at 15-70 kg of body weight. The diarrhea is characterized by black scours, bloody scours or mucohemorrhagic scours (Wills, 2000). The disease caused by *Brachyspira (Serpulina) hyodysenteriae* (a spirochete, anaerobic, gram negative, motile and hemolytic bacteria) (Fellström and Gunnarsson, 1995). The disease was reported first time in 1921 and nowadays the incidence was found all over the world. The prevalence of this disease in USA in 1993 was 11% and in western Austraia was 33% in 1992 (Harris *et al*., 1999). In United Kingdom, this disease is reported as the second most common enteric disease after enteric colibacillosis (Harris *et al*., 1999). This disease is transmitted mainly via oral infection. The animal recover from the disease for 70 days still shed the bacteria via feces. The disease is distributed within and between herd by contamination of feces with booth, clothes and with the carrier such as infected pig, mice, rat and bird.

Objectives

The objective of the present study is to investigate the prevalence of *Brachyspira spp* in bloody diarrhea pig and to study the biochemical properties of all the spirochete that were isolated from swine herd in Thailand.

Materials and Methods

The fecal sample and intestinal mucosa were obtained from the pigs with clinical symptoms of bloody diarrhea and from pig in the herd with a history of bloody diarrhea. A total number of 126 samples, 74 bloody diarrhea/mucus diarrhea and 52 normal feces, were submitted for bacterial culture and identification. The samples were primarily investigated for the organisms by fresh smear and gram staining. All samples were cultured with a specific media in anaerobic environment. The colonies were determined under light microscope and were tested for hemolysis and biochemical properties including indole and hippurate hydrolysis test.

Results

Fifteen isolations were obtained from 74 fecal samples of diarrhea pig. *Brachyspira hyodysenteriae* could not be isolated from the pigs with normal feces (n=52), although the herds have a history of bloody diarrhea. The prevalence, base on the isolation, was 20.3% (15/74) among the pig with diarrhea. *Brachyspira hyodysenteriae* was found in 9 out of 19 herds in this study representing 47.3% of prevalence among the herds. Most of the pig that found the spirochete was between 20-24 wk of age and one spirochete were isolated from a sow. It was revealed that the bacteria were a gram negative staining, long and spiral shape and had a strong beta hemolysis properties in 10% sheep blood agar. Most of the spirochetes were positive for indole test (14/15) and negative for hippurate hydrolysis test (15/15). One strain of indole negative spirochetes (1/15) was also isolated. Compared with the references stain, all of the spirochete were identified as *Brachyspira hyodysenteriae*.

Discussion

In the present study other species of *Brachyspira spp.* was not found among these 19 herds. This for some extent indicated that *Brachyspira hyodysenteriae* is the most common organisms among the *Brachyspira spp.* group in Thailand. However, *Brachyspira pilosicoli* have been reported in Thailand using Polymerase Chain Reaction (PCR) technique. Kramonthong *et al.* (1996) found that 23% of normal pig from herd was sero-positive for SD.

In the present study, none of the spirochete could be isolated from non-diarrhea pigs. The biochemical properties of most *Brachyspira hyodysenteriae* reacted positively for indole test. However, some strain of *Brachyspira hyodysenteriae* show a negative reaction for the indole test (Fellström *et al*., 1999). This study reported first time on the finding of *Brachyspira hyodysenteriae* indole-negative strain in Thailand. This strain have also been isolated in Belgium and Canada (Hommez *et al*. 1998; Belanger and Jacques, 1991). The reason that both strains of *Brachyspira hyodysenteriae* was found in Thailand might be due to the import of pig from many sources including Canada and Belgium. This finding suggested that the intensive control of swine dysentery among the import pig should be concerned.

Conclusions

The prevalence of *Brachyspira hyodysentery*, base on bacterial isolation, among the bloody diarrhea pig was 20.3%. *Brachyspira hyodysentery* could be isolated in most case from the finisher and some case from the sow. The biochemical properties revealed that *Brachyspira hyodysenteriae* isolated from pig herd in Thailand included both indole-positive and indole-negative strains.

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A case of *Aeromonas hydrophila* infection in Pigs causing Reproductive failure

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Introduction and Objectives

Aeromonads are gram-negative, catalase and oxidase positive facultative anaerobic bacilli (1). The bacteria are distributed worldwide in aquatic environment (2). *Aeromonas hydrophila* is a primary pathogen of variety fresh water fishes and amphibians (3-6) of all Asian countries (7, 8). *A. hydrophila* was first isolated from human and proposed as a potential cause of disease in 1937 (9). Its ubiquitous nature in fresh water lakes and streams, domestic tap water and sewage (2) provides opportunity for human and animals to come into contact with and to ingest organisms. A few cases of human infection have been documents (9-11). *A. hydrophila* has been found in market meats, e.g. beef, pork, lamb and raw chicken (10). However, infection in worm blooded animals has never been documented. This is the first report of *A.hydrophila* infection in pigs. The paper describes two infection cases resembling PRRS disease in two different herds in Thailand.

Materials and Methods

Farm A and B are continuous flow, farrow-to-finish pig operations. The farms locate on the opposite sites of the road. Both farms had been utilizing chlorine treated underground water. Farm B uses fish meal in the diet whereas farm A does not. Vaccination is similar on both farms. Sows' vaccines are Parvo-Lepto-Erysipelas, Aujezsky's disease, Classical swine fever, Foot and mouth disease, and inactivated PRRS. Pigs' vaccines are Mycoplasma, Classical swine fever, Aujezsky's disease, and Foot and mouth disease.

Farm A is a 2000 sow farm. On August 24, 2004, the farm decided to use water from urban canals. Due to the big demand of water, chlorinating in the reservoir tank was not complete. Pigs on the farm began to sick within 4 days after drinking the water. Affected sows (10%) were off-feed, vomiting, aborting or farrowing before term with weak born litters that died 2-3 days post-farrowing. The placenta has grayish or cloudy characteristics. Some finishing pigs were diarrheic. Sows and piglets were obviously anemic. The pigs did not respond to amoxicillin injection. A month later, the farm stopped using an outside water source.

Farm B is an 1100 sow farm. On November 21, 2005, the farm began to remove chlorine from drinking water and add effective micro-organism (EM) instead. Wasted water was subjected to sedimentation and filtration and used for barn cleaning. On March 4, 2005, six sows farrowed with cyanotic weak born piglets that died within 2 days. The sows exhibited inappetence and agalactia.

Pigs' blood and organs, water and feed were collected for laboratory diagnosis and analyses. Weak born piglets were sacrificed and necropsied.

Results and Discussion

Gross lesions were pettichial hemorrhages in kidney and intestinal mucosa. We could not isolate *Salmonella spp*. from the piglets. The pure culture of *A.hydrophila* was found in blood, brain, liver and lung of weak born piglets from both farms. Serum was PRRS PCR negative. Also, tonsil was Aujezsky's disease and Classical swine fever FA negative, and kidney was Leptospirosis FA

negative. Sows responded well to Enrofloxacin injection and 600 g/tonne CTC in feed medication for 10 days. Additional samples were tested for other causes of the disease. Farm A; sow sera-PRRS PCR negative and Toxoplasma and Brucellosis serology negative, feed-aflatoxin, vomitoxin and T2 negative. Farm B; Fish meal-no bacterial growth, drinking water-*E.coli* positive. This is suggesting *A.hydrophila* infection in pigs.

Reproductive failure in a sow such as abortion, early farrwoing with low viability piglets, and agalactia can be mistaken to PRRS virus infection. Interestingly, nursery pigs did not show obvious sings. This is probably due to a few antimicrobials were added into their diet. *A.hydrophila* infection in pigs can cause septicemic, anemic, and gastroenteritis. This was similar to human infection (10). However, experimental infection is under investigation to determine its pathogenesis and residue effect in pork meat.

Since *A.hydrophila* is a potential human pathogen and included on the U.S. EPA's candidate contaminant list (9), it is critical to identify the source where this bacterium get through pork production system. The source could be from water although we did not confirm it. On farm A, clinical sings occurred soon after the use of water. On farm B, recycling water could be the source of bacterium because there was no more case after the chlorine treatment.

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DEVELOPMENT OF DIAGNOSTIC METHODS AND VACCINES FOR PORCINE PLEUROPNEUMONIA

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Key words: *Actinobacillus pleuropneumoniae*, Apx toxin, ELISA, vaccine

Porcine pleuropneumonia is a highly contagious disease caused by *Actinobacillus pleuropneumoniae* (APP), a member of *Haemophilus-Actinobacillus-Pasteurella* group in the family *Pasteurellaceae*, which can be isolated from nasal cavities, tonsils, middle ear cavity and lungs of infected pigs. The disease is characterized by a haemorrhagic necrotic pneumonia and fibrinous pleuritis, and causes increasing economic losses of the swine industry worldwide (1). This Gram-negative bacterium can be divided into 2 biotypes and 15 serotypes (2). The prevalence of serotypes varies in different regions. The most prevalent serotypes in China are 7, 1 and 2 or 3. A number of potential virulence factors have been identified, such as capsular polysaccharide (CPS), lipolysaccharide (LPS), transferrin binding proteins (Tbp), urease and Apx exotoxins (1). The Apx toxins, members of the RTX toxin family, are the key virulence determinants of APP. To date, 4 types of Apx toxins, ApxI, ApxII, ApxIII and ApxIV, have been identified. Different serotype of APP secretes different combinations of the 4 toxins. Apx toxins are generally encoded by operons consisting of 4 contiguous genes *apx*C, A, B and D (3). The *apx*A gene encodes the structural toxin, whose activities must be activated by a post-translational activator encoded by the *apx*C gene. The *apx*B and *apx*D genes encode proteins required for secretion of the activated toxin. Variations in virulence and immunogenicity exist among serotypes, and very limited cross protection can be conferred between serotypes, thus the diagnosis and prevention of this disease is a great challenge. The present report summarizes some diagnostic methods and vaccines developed in our laboratory.

1. Diagnostic Methods

1.1 ApxII-ELISA

ApxII is one of the most important virulence factors and protective antigen. Except for serotypes 10 and 14, all other 13 serotypes of APP secrete ApxII (1). So, ApxII-based immunological methods can be used not only to detect APP infection, but also to evaluate efficacy of most vaccines. According to the DNA sequence of the ApxII operon (GenBank: M30602), a 3.5 kb *apx*IICA gene was cloned from the genome of APP strain HB (serotype 1) and expressed in *E. coli* using the pET-17b expression system (Novagen). The recombinant protein ApxIICA was purified using His-tagged affinity column chromatography and used as coating antigen to develop an indirect ELISA. Sera from rabbits and pigs immunized and/or experimentally-infected with APP strains of different serotypes, inactivated and bacterin-toxiods vaccines were tested with the ApxII-ELISA. The results show that antisera against APP serotypes 1-9, 11-13 and 15 immunized or infected animals are positive, whereas those against serotype 10 are serologically negative. This agrees completely with the results of the indirect hemagglutination assay (IHA).

The titers of serum antibodies against ApxII were in the same pattern as those of IHA antibodies in the pigs immunized with an inactivated APP bacterin (KeQian APP-a) or bacterin-toxiod vaccine (KeQian APP-b).

1.2 ApxIV-ELISA

Unlike other 3 Apx toxins, ApxIV is produced by all 15 serotypes only during infection of animals, but not under *in vitro* conditions (1). Pigs infected with APP show specific antibodies against ApxIV, whereas antibodies against the other 3 Apx toxins are also found in APP-free pigs, that might be induced by other *Actinobacillus* species such as *A. rossii* and *A. suis*. We developed an indirect ELISA using a recombinant protein based on the N-terminal 814 amino acids of ApxIVA. The analysis of sera from pigs that were experimentally or naturally infected with different serotypes of APP, and of sera from APP-free pigs, revealed that the ApxIV-ELISA had a specificity of 100% and a sensitivity of 95%. Pigs immunized with the inactivated APP bacterin or bacterin-toxiod vaccine were serologically negative in the ApxIV-ELISA. Our data demonstrated that ApxIV-ELISA can be used to specifically detect APP infection of all serotypes and to differentiate naturally-infected pigs from inactivated and subunit vaccine-vaccinated ones.

2. Vaccine development

2.1 Inactivated vaccines

Two killed vaccines were developed: KeQian APP-a and KeQian APP-b. The first one is a multi-valent oil-adjuvanted bacterin made from 3 local APP isolates of serotypes 1, 2 and 7, the prevalent serotypes in China. KeQian APP-b is an oil-adjuvanted bacterin-toxiods containing a killed APP strain of serotype 1 and recombinant ApxI, ApxII and ApxIII proteins. 30 to 35-day-old of APP-negative piglets were intramuscularly vaccinated with either KeQian APP-a or KeQian APP-b at a dose of 1.5ml and 30 days later received a second vaccination. The serum antibodies were tested using IHA, ApxI-, ApxII- and ApxIII-ELISA at 0, 14, 21, 30, 45, 60, 75 and 90 day(s) post primary vaccination (dpi). The titers of the antibodies elevated at 14 dpi, and begun to decrease at 21 dpi. The antibodies peaked at 15 days for IHA, and 30 days for ELISAs after second vaccination, and then decreased slowly. The IHA titers were significantly higher in the pigs vaccinated with KeQian APP-a than KeQian APP-b, whereas the ELISA titers against the 3 Apx toxins were significantly higher and lasted longer in the KeQian APP-b vaccinated group. This suggests that KeQian APP-b may be more effective than KeQian APP-a. Protection experiments are performing to confirm this hypothesis.

2.2 Gene-deleted vaccines

Killed vaccines can protect pigs from serious pleuropneumonia and economic losses, but can not inhibit infection and colonization of APP in the upper respiratory tract of vaccinated pigs. Attenuated live vaccines may meet this demand. ApxI, ApxII and ApxIII are the most important virulence factors for APP. Their A protein is the structural toxin and the most important protective antigen, but its toxicities must be activated by the C proteins. We suppose that *apx*C-deleted mutants might be candidates for live vaccines of APP. APP of serotype 7 produces only 1 type of toxic Apx toxins, i.e. ApxII. We have deleted its *apx*IIC gene using a sucrose counter-selectable marker system and obtained a mutant strain that had no antibiotic resistance marker and expressed a nontoxic ApxII (4). The mutant strain caused no adverse effects in mice at doses up to 2×10^9 cfu via the intraperitoneal route while the parental strain induced total mortality at a dose of 2×10^7 cfu. Mice vaccinated intraperitoneally with the mutant strain had 100% and 70% protection against homologous

(serotype 7) or heterologous (serotype 1 and 3) challenge with APP, respectively. To increase protective rate and serotype range of the mutant, two approaches are been pursued in our group: (a) insertion of an expression cassette of *apx*IA gene from an APP strain of serotype 1 in the deleted locus of the genome of the existed APP *apx*IIC-negative mutant strain and (b) construction of an *apx*IC/*apx*IIC double negative mutant strain of APP serotype 1. These 2 mutant strains shall express both nontoxic ApxI and ApxII and obtain a broader and higher protection. If these approaches are demonstrated to be effective, the mutant strains will be promising live vaccine candidates and may be also used as potential live vectors to express protective antigens of other pathogens for developing multi-valent recombinant vaccines against swine respiratory infectious diseases.

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Immunology

CONTROL OF PRRS IN THE PHILIPPINES: A TALE OF 2 FARMS

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Introduction and Objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) continues to be the leading cause of Porcine Respiratory Disease Complex (PRDC). Several reports discusses the use of mass vaccination in both breeders and fatteners to control PRRS [1,2,3&4].

The objective of this study is to show the efficacy of mass vaccination with Ingelvac[R] PRRS MLV in two Philippine swine farms.

Materials and Methods

From 2000 to 2003, two swine farms experienced varying degrees of both reproductive and respiratory problems in the breeder and fattener herd, respectively.

Farm A: 600 sows, continuous farrow-finish. Before vaccination, there were 2 phases: a pre-outbreak phase from January to October 1999. Sometime in November of the same year, the farm experienced an outbreak phase extending up to June of 2000. On July to December of 2000, the farm implemented vaccination with a modified live vaccine (MLV) against PRRS.

Farm B: 600 sows, continuous farrow-finish. High mortalities mainly in the nursery (average of 6.71%) and growing finishing (average of 6.63%). Total mortalities went as high as 34.87% in September of 2002.

Based on clinical signs, post-mortem lesions and serological evaluation (PRRS ELISA, IDEXX[R]) as seen in figures 1 to 2, PRRS was determined to be the primary pathogen involved in the PRDC.

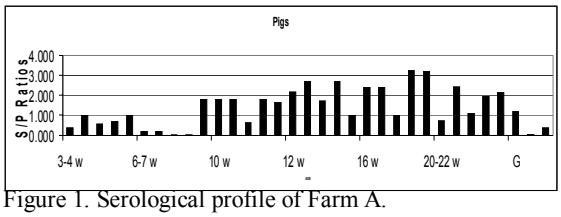

Figure 1. Serological profile of Farm A.

An initial mass vaccination of breeders and pigs up to end of nursery (10-11 weeks old) was done, followed by a maintenance program where sows are vaccinated 1 week post-farrow and pigs 1 week pre-weaning. The production data before and after vaccination were then compared.

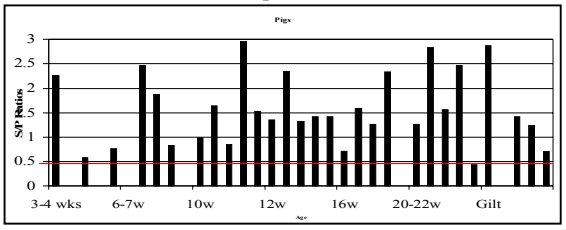

Figure 2. Serological profile of Farm B.

Results and Discussion

Farm A and B had a mortality reduction after the mass vaccination with Ingelvac[R] PRRS MLV, 7.98% and 8.28% respectively.

Table 1. Production data before and after PRRS Vaccination

Farm A			
Mortality Rates (%)	Before Vac	After Vac	P < 0.05
Pre-wean	10.12	7.75	0.11
Post-Wean	1.59	0.95	0.02
Pre-starter	6.04	3.32	0.1
Starter	5.42	4.46	0.41
Grower	3.00	2.08	0.03
Finisher	1.03	0.66	0.36
Total	27.20	19.2	0.002

Farm B			
Mortality Rates (%)	Before Vac	After Vac	P < 0.05
Pre-wean	5.83	3.79	0.14
Nursery	6.71	5.81	0.74
Grow-Finish	6.63	1.29	0.02
Total	19.17	10.89	0.06

Conclusion

Mass vaccination with Ingelvac[R] PRRS MLV, is effective in controlling PRRS and consequently PRDC in the two Philippine field cases.

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SALMONELLOSIS CONTROL THROUGH VACCINATION IN THE PHILIPPINES

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Introduction and Objectives

The successful use of Enterisol® SC 54 a live avirulent *Salmonella cholerasuis* vaccine had been demonstrated by several groups[1,2]. The purpose of this study is to demonstrate the control efficacy of Salmonellosis using Enterisol® SC 54, a modified live *Salmonella cholerasuis* in two Philippine herds.

Materials and Methods

This study involved two herds located in the densest area in the Philippines. Enterisol® SC 54 was given intranasally at 2 ml per dose at 1 to 14 days of age in piglets. The vaccination protocol was adjusted to avoid antibiotic therapy for scouring caes. No antibiotics were given 3 days before and after vaccination.

Herd 1: 2,500 sows, 6 months study period, total number of animals involved 27,360 pigs and parameter observed was the clinical incidence (clinical signs and post-mortem lesions) of septicemic salmonellosis. Differential diagnosis was done with classical swine fever and other diseases.

Herd 2: 3,000 sows, 10 months study period, total number of animals involved 32,832 pigs and parameter observed was the improvement in average daily gain (ADG).

The limitation of this study was the absence of negative control animals due to reluctance of the farm owners to leave unvaccinated animals in the herd; variation in production parameters for comparison, co-infections with other pathogens and inability to conduct statistical analysis due to the format of data. Instead a numerical comparison was made before and after Enterisol® SC 54 was used.

Results and Discussion

In herd 1, a clinical reduction of 11.60% incidence was seen (Table1). Almost a day reduction in pig days (0.80 day) was seen with a 26.00 g/day improvement in ADG was seen in herd 2.

Table 1. Clinical comparison of clinical salmonellosis incidence before and after the use of Enterisol® SC 54 in herd 1.

	Before	**After**
Incidence of Salmonellosis/ month	**12.10% (N=265)**	**0.50% (N=332)**

Table 2. Comparison of pig days and ADG before and after the use of Enterisol® SC 54 in herd 2.

	Before	**After**
Pig days	**184.39 (N=16,416)**	**183.59 (N=16,416)**
Daily Gain	**0.382**	**0.408**

Conclusion

Salmonellosis control was proven to be effective using a modified live vaccine, Enterisol® SC 54.

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INCIDENCE REDUCTION OF PSEUDORABIES THE PHILIPPINES

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Introduction and Objectives

Control and eradication of pseudorabies virus has been a challenge for Philippine swine herds. The successful use of modified live gE$^-$ vaccines given intranasally (IN) has been shown by several authors in reducing the incidence of pseudorabies in swine herds[1,2]. It was also demonstrated that a gE $^-$ Bartha strain vaccine is more efficient than a gE $^-$ & Tk $^-$ vaccine in reducing latent infections[3].

Latent infection is important because it is the mechanism by which pseudorabies field virus persists in populations of susceptible animals.

This study shows the efficacy of Ingelvac® Aujeszky MLV in reducing the prevalence of pseudorabies virus in 3 continuous farrow-finish herds in the Philippines.

Materials and Methods

This study involves 3 herds located in the densest pig raising area in the Philippines.

Herd 1: 250 sows, started to use Ingelvac® Aujeszky MLV in July, 2001 and period of comparison for 12 months.

Herd 2: 600 sows, started to use Ingelvac® Aujeszky MLV in February, 2002 and period of comparison for 8 months.

Herd 3: 600 sows, started to use Ingelvac® Aujeszky MLV in August, 2002 and period of comparison for 4 months.

Table 1. Pseudorabies vaccination program before and after the use of Ingelvac® Aujeszky MLV.

Stage	Herd 1		Herd 2		Herd 3	
	Before (d=days)	**After (days)**	**Before (days)**	**After (days)**	**Before (days)**	**After (days)**
SOWS	93 d gestation	mass 3x/year	72 d gestation	72 d gestation	93 d gestation	93 d gestation
BOARS	2x/year	2/year	2/year	2/year	2/year	2/year
PIGS: First Dose	day 70 (im)	**d 1 (IN)**	d 70 (im)	**d 1-3 (IN)**	d 70 (im)	**d 7 (IN)**
Second Dose	None	day 70 (im)	none	day 70 (im)	none	day 60 (im)
GILTS	2x pre-breeding	2x pre-breeding	2x pre-breeding	2x pre-breeding	2x pre-breeding	2x pre-breeding

Serological evaluation was done with IDEXX® Pseudorabies Gp1 Antibody ELISA Test. The percent reduction in incidence before and after intervention was computed individually.

Results and Discussion

After using Ingelvac® Aujeszky MLV, the proportion of animals tested seropositive for pseudorabies field virus declined in all 3 herds.

Table 2. Percent seropositive reduction in the 3 herds before and after the use of Ingelvac® Aujeszky MLV.

	Farm 1	**Farm 2**	**Farm 3**
Sows	50.0%	27.66%	11.67%
Gilts	100.0%	100.00%	0.00%
Finishers	60.6%	100.00%	86.79%

Conclusion

Ingelvac® Aujeszky MLV dramatically reduced the prevalence of pseudorabies field virus seropositve animals in 3 Philippine herds.

Eradication of pseudorabies field virus can be achieved even in herds located in the densest area in the Philippines with the intranasal use of Ingelvac® Aujeszky MLV. Vaccination for breeders 3-4 times per year would help reduce the shedding to the piglets. A semi-annual vaccination would then be a recommended program once the breeders become negative.

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Efficacy of Enterisol® Ileitis when concurrently administered with Enterisol® Sc-54 and Ingelvac® Ery-Alc via oral drench to pigs.

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Introduction and Objectives

Porcine Proliferative Enteropathy (PPE) caused by the intracellular organism *Lawsonia intracellularis* is a ubiquitous enteric disease of swine with an estimated swine herd prevalence of >95% worldwide. Combining the licensed *Lawsonia intracellularis* product (Enterisol® Ileitis) with the *Erysipelothrix rhusiopathiae* (Ingelvac® ERY-ALC) and *Salmonella choleraesuis* (Enterisol® SC-54) licensed products would give excellent protection to young pigs against enteric disease. The objectives of the study were to demonstrate the efficacy and lack of interference of the *Lawsonia intracellularis* vaccine product in young pigs when given in combination with ERY and SC-54 vaccines.

Materials and Methods

The study consisted of 3 treatment groups of weaned 3 week old (+/- 5 days) *L. intracellularis*-negative test pigs. All vaccinations were given on day 0 of the study.
Treatment group 1 (n=20) received a dose of Enterisol® Ileitis in combination with Ingelvac® ERY-ALC and Enterisol® SC-54 (FF) via oral drench.
Treatment group 2 (n=20) was designated as study "challenge controls" and received a placebo.
Treatment group 3 (n=10) was designated as study "Strict Controls" and did not receive a vaccine or challenge treatment.

On day 23 of the study, treatment group 1 and 2 (20 pigs each) received a target dose of greater than 1 x $10^{7.0}$ $TCID_{50}$ of heterologous virulent low passage pure culture challenge material by gavage.

Three weeks after challenge administration (day 44), all treatment groups will be euthanized and necropsied for gross and microscopic analysis for lesions from PPE.

Daily health observations were made from initiation of study to the day of challenge for each test animal. Clinical signs due to *L. intracellularis* (diarrhea, behavior, and body condition) were scored daily from day of challenge (day 23) to Statistical analysis consisting of Chi-Square/Fisher's Exact analysis was performed on gross lesions data for positive animals per group. ANOVA analysis was performed on the gross lesion scores by treatment group.

Table 1: Average gross lesion scores by treatment group and number of animals with a positive score within groups.

Group ID	Treatment	Ileum score	Caecum score	Colon score	Overall group score	Nr. of pos per group total
1	Vaccinates	0.3	0	0	0.3^{a}	$4/20^{a}$
2	Challenge Controls	1.2	0.4	0.3	1.9^{b}	$13/18^{b}$
3	Strict Controls	0	0	0.2	0.2	2/10

[a,b] Scores with different letters denote statistically significant differences ($p \leq 0.05$). Strict controls where not part of the statistical analysis.

There was a statistically significant difference between vaccinates versus the challenge controls for gross lesion scores overall and by treatment group ($p=<0.0001$).
There was also a significant difference between vaccinates versus challenge controls in number of positive animals ($p=<0.0001$).
There was a statistical ($p<0.05$) difference between vaccinates and the challenge control group for clinical signs at 10 and 11 days post challenge.

Conclusion

The combination of Ingelvac® Ery-ALC, Enterisol® SC-54, and Enterisol® Ileitis, ALC proved to be efficacious against a *Lawsonia intracellularis* challenge, and, therefore, the data in this study demonstrates that there is no interference of the *Erysipelothrix* and *Salmonella* vaccines to the *Lawsonia* vaccine, in providing protection against *Lawsonia intracellularis* infection.

Reducing the Incidence of Pseudorabies Using Ingelvac^R Aujeszky MLV in Three Chinese Herds

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INTRODUCTION AND OBJECTIVES

Control and eradication of pseudorabies has been a challenge for Chinese swine herds, particularly those farms situated in the pig-dense regions. The successful use of modified live gE$^-$ vaccines given intranasally (IN) in reducing the incidence of pseudorabies in swine herds has been shown by several authors[1,2]. It was also demonstrated that a gE$^-$ Bartha strain vaccine is more effective than a gE$^-$ & Tk$^-$ vaccine in reducing latent infections[3].

Latent infection or latency, plays a major role in the subsequent persistence of the virus within a susceptible swine herd, hence, is given considerable attention when it comes to effectively controlling pseudorabies field infection.

This study aims to serologically demonstrate the efficacy of Ingelvac^R Aujeszky MLV in reducing the incidence of pseudorabies infection in three continuous farrow-to-finish swine herds in China.

MATERIALS AND METHODS

The study involves three herds located amidst the densest pig raising area in China.

Herd 1: A 2,100 sow unit in Jiangxi Ding Nan, started using Ingelvac^R Aujeszky MLV in June, 2003; period of study, 18 months

Herd 2: A 1,200 sow farm in Guangdong Guangzhou; started using Ingelvac^R Aujeszky MLV in January, 2003; period of study, 12 months

Herd 3: A 600 sow unit in Guangdong Shenzhen; started using both Ingelvac^R Aujeszky MLV and Intervet Aujeszky MLV for piglets (IN) in January, 2004; period of study, 6 months

Table 1. Pseudorabies vaccination program before and after the use of Ingelvac^R Aujeszky MLV.

Stage	Herd 1		Herd 2	
	Before (days)	After (days)	Before (days)	After (days)
SOWS	4x/year	mass 4x/year	86d gestation and14d after forrowing	4x/year
BOARS	4x/year	4x/year	3x/year	4x/year
PIGS: First Dose	d1 (IN) 0.5dose	d 1-3 (IN)	d 35 (im)	D 1-3 (IN)
Second Dose	d21 (IN) 0.5dose	day 70 (im)	d 77 (im)	day 70 (im)
Third Dose	day 70 (im)	none	none	
GILTS	2x pre-breeding	2x pre-breeding	2x pre-breeding	2x pre-breeding

Serological evaluation was done using IDEXX^R Pseudorabies Gp1 Antibody ELISA Test. The percent seropositive before and after intervention was computed individually.

RESULTS AND DISCUSSION

After using Ingelvac^R Aujeszky MLV, the proportion of pseudorabies seropositive pigs declined dramatically in all three herds.

1.Serological profiling

Table 2. Herd 1: Proportion of seropositive animals before and after using Ingelvac^R Aujeszky MLV

	Before	After	Difference
Parity 1-3	12%	0%	**-12%**
Parity 4-6	50%	20%	**- 30%**
All sows	37% (N=120)	12%(N=120)	**-25%**
6 months	10%	0%	**-10%**
7.5 months	15%	0%	**-15%**
All gilts	12.5% (N=40)	0% (N=40)	**-12.5%**
Finishers 12 weeks	(N=60) 15%	(N=60) 10%	**-5%**
16 weeks	0%	0%	**0%**
20-22 weeks	10%	0%	**-10%**

Table 3. Herd 2: Proportion of seropositive animals before and after using Ingelvac^R Aujeszky MLV

	Before	After	Difference
Parity 1-3	33%	20%	**-13%**
Parity 4-6	100%	67%	**-33%**
All sows	67% (N=30)	44% (N=30)	**-23%**
6 months	40%	0%	**-40%**
7.5 months	60%	0%	**-60%**
All gilts	50% (N=20)	0% (N=20)	**-50%**
Finishers: 12 weeks	(N=30) 20%	(N=30) 10%	**-10%**
16 weeks	30%	0%	**-30%**
20-22 weeks	70%	0%	**-70%**

Table 4. Herd 3: Proportion of seropositive animals before and after using Ingelvac^R Aujeszky MLV and Intervet Aujeszky MLV

AGE	Before	After		Difference
		Intervet	BI	
8 wks	100% (N=10)	90%	90%	**0%**
12 wks	100% (N=10)	20%	50%	**+30%**
14 wks	40% (N=10)	40%	10%	**-30%**
16 wks	90%	90%	10%	**-80%**
18 wks	80%	70%	20%	**-50%**

Results in Table 4 prove that a gE-Bartha strain vaccine is more effective than a gE-&Tk- vaccine in reducing latent infections.

2. Production data monitoring:

Production parameters to be monitored before and after the vaccination with Ingelvac Aujeszky MLV.

Table 5: Herd 1Production data monitoring.

Production data:	Before	After
Reproductive and Pre-weaning:		
Farrowing rate %	83	86
Recycle rates%	11	10
Abortions %	4	1.5
Premature farrowings %	0.1	0.1
Pigs born alive/sow	9.01	9.3
Stillborn %	5.3	3
Mummies %	0.1	0.01
Pre-weaning Mortality %	7	5
Pigs weaned/sow	8.38	8.84
Pigs weaned/sow/year	18.44	19.89
Post-weaning		
Mortality, % 7.5 KgW—30 KgW	3-18	2
Treatment Cost/Pig, RMB	30	24
Days to Market	170-190	165-180
Culled Pigs %	15	7

Table 6:Herd 2 Production data monitoring.

Production data:	Before	After
Reproductive and Pre-weaning:		
Farrowing rate %	78	85
Recycle rates%	8	8
Abortions %	4.5	2
Premature farrowings %	0.7	0.3
Pigs born alive/sow	9.5	9.8
Stillborn %	7	3
Mummies %	1.2	1.4
Pre-weaning Mortality %	5	5
Pigs weaned/sow	9.03	9.31
Pigs weaned/sow/year	19.87	20.49
Post-weaning	?	?
Mortality, %	4	2
Treatment Cost/Pig, RMB	38	33
Days to Market	170	165
Culled Pigs %	11	10

CONCLUSION

This study clearly demonstrates the efficacy of Ingelvac^{R} Aujeszky MLV in effectively reducing the incidence of pseudorabies among susceptible pigs of varying ages, including cases wherein latent infection is considerably present. Proportions of animals turning completely seronegative after vaccination further corroborates earlier studies showing the power of Ingelvac^{R} Aujeszky MLV to eradicate pseudorabies infection from swine herds situated even in pig-dense localities of China.

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Prevailing overview of the pig Pseudorabies and application of the Pseudorabies vaccine in our country

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Abstract: Pseudorabies also call Aujeszky's disease is one acute, hot infectious disease of such many kinds of domestic animals and wild animals as the pig, ox, sheep, etc. infecting caused by Pr V that have another name called Suis herpesvirus I. The hazard to the pig of this disease is the greatest ,regard generate heat and neural symptom as the characteristic, cause the breeding pig not to sterile , the pregnant sow miscarries, Stillborn fetus and fetus of the mummy , the new born piglet and weaning piglet's death, fatten pigs increase weight slowly ,etc. It causes the enormous economic losses to pig industry. At present, this disease has already endangered one of the global pig industry's most serious epidemic diseases and has already taken place in the world more than 40 countries. In recent years, with of our country pig industry towards intensify, the scale direction is developed rapidly, raise the density bigger and bigger and breeding pig exchange more and more frequent, it has created the condition for this disease emergence, spread and spreading. So this disease is already prevalent and broken out in Guangdong , Henan , Hubei , Shandong , Beijing , Liaoning , Jilin , Heilongjiang ,etc. pig farm of 19 provinces and cities of our country, present the trend that is expanding and spreading constantly , the sound development of the great influence and animal husbandry of our country of restriction. Greatly influence and restrict the sound development of the animal husbandry of our country. There is no effective method to the treatment of Pr yet, use the vaccine immunity to prevent and cure is the most important means of the disease. Pr vaccine is divided into the attenuated vaccine of disappeared gene that including naturally and artificial disappearance and deactivation vaccine of the whole virus mainly. Weak virus vaccine has good immunity and cheap price, the American-European occupation rate of market is more than 90% ;deactivation vaccine security kind, but immunity is relatively bad, occupation rate fewer than 10%. Pr vaccine that our country uses far and wide at present is that Harbin veterinarian's research institute utilized gE/gI that the weak virus vaccine of two disappeared genes succeeded in developing introduced from foreign countries in 1979. This vaccine has the security such characteristics as strong , one long , cheap , easy to use of immunity with effectual immunity ,etc. use extensively in the domestic pig farm , has already obtained the formal products authentication code of the Ministry of Agriculture. This vaccine order is inoculated once, can produce the strong immunity in 6 days, the immunity one is as long as one year, produce sow inoculate 2ml / head in front of each breeding , it produce sucking pig can through milk win passive immunity for the first time. It can prevent from and control the breaking out and prevailing of Pr effectively, and the urgent immunization campaign of pig farm that can break out at this disease, control the spreading of epidemic situation, for the prevention and cure and putting out giving play to on important function positively of Pr.

Key words: pseudorabies ; pseudorabies virus ; prevalence ; pig ; vaccine

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NO. 1 factory of Heilongjiang province biological product

SEROLOGICAL RESPONSE AFTER INTRAMUSCULAR AND INTRADERMAL ADMINISTRATION OF THREE AUJESZKY VACCINES

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Introduction and Objectives

In Thailand, Aujeszky's disease (AD, pseudorabies) is one of the most important infectious diseases in pig production. After intradermal administration dendritic cells act as antigen presenting cells (APC) and present the antigen to the immune system in a more effective way than macrophages and B-cell lymphocytes can.
The objective of this study was to compare serum neutralizing (SN) antibody titres against Aujeszky virus after vaccination by either the intramuscular (IM) or intradermal (ID) route.

Materials and Methods

The trial was carried out on two commercial pig-breeding farms in Thailand.
Farm A: 45 male finishing pigs were assigned at random to 3 groups (Table 1) and vaccinated once at 12 weeks of age (T=0) (according to the vaccination program of this farm). Blood samples were collected at 4, 8 and 12 weeks after T0.

Table 1: Farm A, vaccination groups 1, 2 and 3

Group	Vaccine	Dose	Route	n
1	Porcilis® Begonia ID	0.2 ml	ID	15
2	Vaccine X	2 ml	IM	15
3	Porcilis® Begonia	2 ml	IM	15

Farm B: 20 finishing pigs were assigned at random to 2 groups (Table 2) and vaccinated once at 12 weeks of age (T=0) (according to the vaccination program of this farm).
Blood samples were collected 3 and 6 weeks after T0.

Table 2: Farm B, vaccination groups 1, 2, and 3

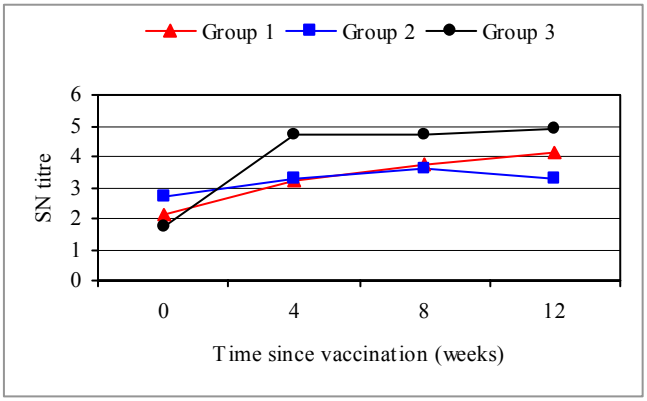
Group	Vaccine	Dose	Route	n
1	Porcilis® Begonia ID	0.2 ml	ID	10
2	Vaccine X	2 ml	IM	10

All three vaccines used were modified live vaccines (>$10^{5.5}$ $TCID_{50}$ per dose).
The adjuvant in both Porcilis® Begonia (Intervet) vaccines was dl-alpha-tocopherol acetate (Diluvac® Forte, Intervet), while vaccine X contained an oil adjuvant.
The ID administration in group 1 at farm A and farm B was done using a needle-less device for ID application of liquids (IDAL®, Intervet).
The Idexx HerdChek® anti-PRV-g1 test was performed to measure gE antibody titres. A serum neutralization (SN) test was performed to measure antibody titres against the Aujeszky virus. The mean titres were determined at each time of sampling.
Statistical analysis (SAS 6.12) was carried out on the results from farm A using Analysis of Variance (repeated measure) and from farm B using the Mann-Whitney U test.

Results and Discussion

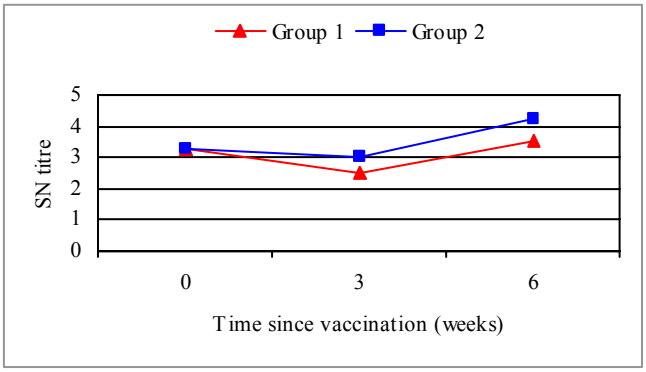
After vaccination, no adverse reactions were observed.
All samples were negative for gE antibodies, so no field infection took place during the time of the study.
There was no significant difference in the mean SN titres between the groups in farm A (P>0.05) (Figure 1).

Figure 1: Mean SN titres (log_2) of each group in farm A



There was also no significant difference in mean SN titres between the groups in farm B (P>0.05) (Figure 2).

Figure 2: Mean SN titres (log_2) of each group in farm B



Conclusion

Intradermal administration of a modified live Aujeszky vaccine gives similar SN titres to when intramuscular administration is used.

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INTRADERMAL APPLICATOR FOR LIQUIDS "IDAL®"

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Introduction

Methods of food production, from both vegetable and animal sources, have altered with the changing perceptions and needs of the consumer. For example, in animal production, there is a marked reduction in the use of antibiotics, growth promoters, and other substances with the propensity to compromise human health or the environment. As part of this trend, most of the world's important consumer markets demand ever more safe and healthy meat (4). The informed consumer now expects to know about the meat-producing animal's welfare, its living conditions, and the way its health is maintained throughout its lifetime. With respect to meat quality there is a distinct preference for animals which have never been injected.

To comply with these needs several needleless injection systems have been developed, not only to address the problem of local vaccination reactions, but also to reduce the potential for the iatrogenic transmission of infectious agents, PRRS virus (1) for example, at the time of vaccination.

Objectives

To offer the pig sector a needleless vaccinator capable of delivering a liquid into the thickness of the skin. This particular vaccinator has been named the "IDAL®" – IntraDermal Applicator for Liquids.

Material and Methods

The IDAL® vaccinator (Figure 1) and its operation:

1. The equipment consists of a vaccinator, two rechargeable Nickel Metal Hydride (NiMH) batteries, and a battery charger.
2. The batteries can be fully charged in an hour. A fully charged battery can deliver at least 500 doses.
3. The vaccinator is calibrated to deliver 0.2 ml of vaccine into the skin (intradermal).
4. The equipment has therefore been designed for use with concentrated antigens.
5. The vial containing the reconstituted vaccine is inserted into the upper part of the vaccinator.
6. The dosing mechanism is powered by the battery, which is an integral part of the device, so the operator is not hampered by connecting wires or tubes.
7. An electronic sensor detects the density of the liquid currently in the system. It can distinguish between a vaccine and the special IDAL® rinsing liquid. In the presence of the latter, the LED display on the front of the device reads "NOVA" (meaning "NO Vaccine in the system"). When the system contains vaccine, the display shows four dashes (- - - -).
8. The system is activated when the trigger is pulled.
   For the sake of safety, a dose is only delivered when the vaccinator is pressed firmly against the animal's skin.
9. Being an intradermal applicator, it can be used anywhere on the surface of the animal.
10. As one dose is delivered, the system automatically loads the next.
11. At the end of a vaccination session, the interior of the system must be cleaned with the special rinsing liquid. The outside can be washed under running water. Also the device may be autoclaved, at 100°C (max) for 60 minutes (max).
12. Normal maintenance consists of replacing the O-rings in the head of the vaccinator, every 10,000 doses.
13. The device has two automatic dose counters which can be read on the LED display – number of doses delivered in the current session, and total doses delivered.

Figure 1

IDAL®

Figure 2

Intradermal vaccination

Results

The IDAL® has been used around the world with live vaccines against PRRS and Aujeszky's Disease (3) with good results. It is reported to be easy to operate (Figure 2).

It has just begun to be used in Mexico, where results are expected to be in line with experiences reported previously.

Successful intradermal vaccination against *Mycoplasma hyopneumoniae* (2) has been reported as well.

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POST-WEANING MULTISYSTEMIC WASTING SYNDROME (PMWS) AND MYCOPLASMA, AN ALTERNATIVE APPROACH WITH HYORESP®.

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Keywords: PMWS, PCV-2, *Mycoplasma hyopneumoniae*, vaccination

Introduction and objective

Mycoplasma hyopneumoniae is the causative agent for enzootic pneumonia, an important cause of respiratory disease and retarded growth in pigs. Vaccination of young piglets is a common method of control of enzootic pneumonia. However, vaccination of young piglets is believed by many to be an important triggering factor for Post-weaning Multisystemic Wasting Syndrome (PMWS) [1], a major cause of loss in the pig farming industry worldwide. Porcine Circo Virus type 2 (PCV-2) is thought to be the main cause of this disease. This study evaluates an alternative vaccination strategy at a farm where PMWS was believed to be exacerbated by *Mycoplasma* vaccination of young piglets. This study assesses the effects of a *Mycoplasma* vaccination of sows combined with a one shot *Mycoplasma* vaccination of older piglets.

Material and methods

The study was conducted between 2002 and 2004 at a breeding farm with 3,000 sows, at a weaned piglet location with 4,800 pigs and at two fatteners farms (Farm A and B) with 1,300 and 1,800 pigs respectively. All the piglets and fatteners came from the same breeding herd. No piglets of other origin were introduced during the study.
Based on changes in mycoplasma vaccination strategies, the period between January 2002 and April 2004 could be divided into three different phases:
Phase 1: January to August 2002, severe PMWS problems and *Mycoplasma* vaccination at young age with a commercial *Mycoplasma* vaccine (different from Hyoresp®)).
Phase 2: September to December 2002, severe *Mycoplasma* problems; no *Mycoplasma* vaccination.
Phase 3: January 2003 to April 2004: Sow and piglet *Mycoplasma* vaccination with Hyoresp®.
The presence of PMWS was diagnosed by the existence of clinical symptoms, histology and the proven presence of PCV-2 antigen. The presence of *Mycoplasma* was established by means of serology and the presence of typical lung lesions. Lung and pleural lesions at slaughter were assessed by grading system from 0-2 whereby 0 means “not affected”, 1 means “slightly affected” and 2 means “severely affected”. Growth was measured by weighing at introduction and at time of marketing. *Mycoplasma* vaccination was carried out with a commercial vaccine (Hyoresp®). At the start of phase 3, all sows were vaccinated twice, with a 4-week interval (January and February 2003). Four weeks before farrowing, a one-shot booster vaccination was administered during each production cycle. The piglets were vaccinated once at the age of 7-8 weeks.

Results

Weaned piglets

The percentage of un-saleable weaned piglets decreased from 10.3% during phase 2 to 3.7% during phase 3 (Fig. I). Growth per day increased with 13 gram to 402 gram per day during phase 3 (Fig. II).

Fig. I. Changes in the proportion of unsaleable weaned piglets during Phases 1, 2 and 3

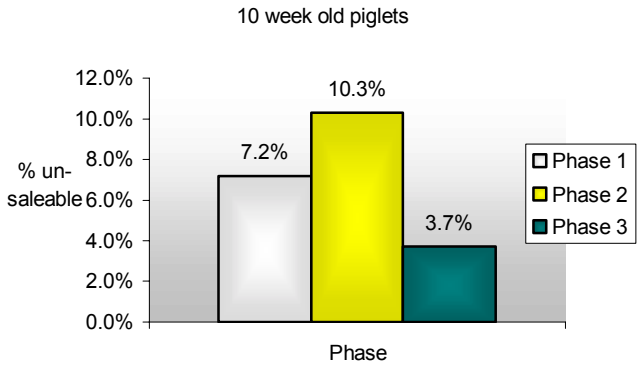
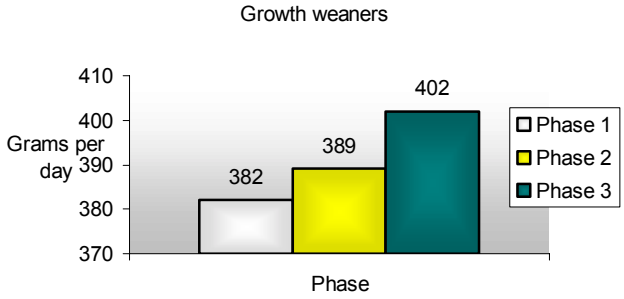


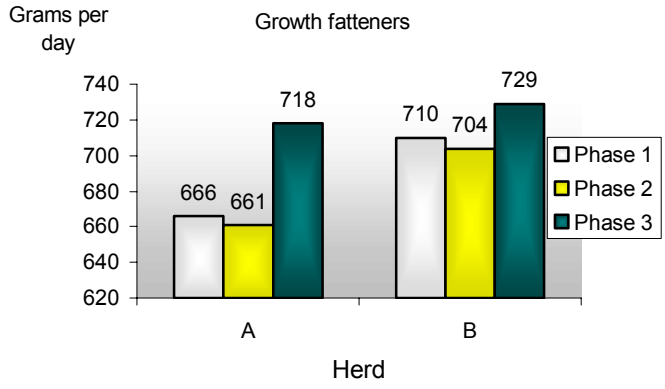
Fig. II. Growth rate measurements of weaner pigs during Phases 1, 2 and 3



Fatteners

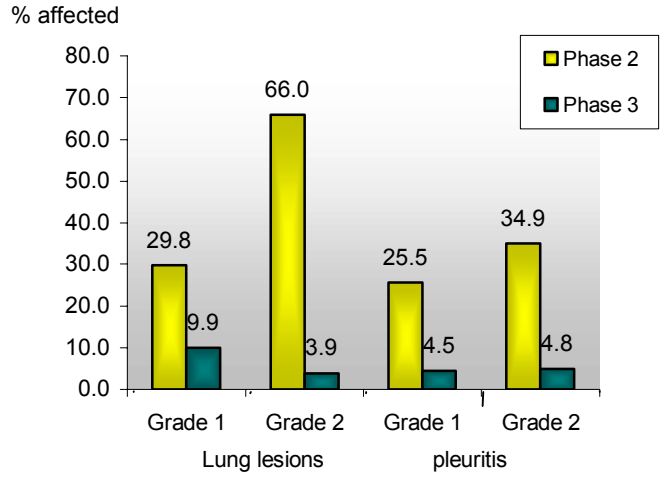
At farm A and B, growth per day increased 57 gram and 25 gram respectively to 718 and 729 gram per day respectively during phase 3 (Fig. III).

Fig. III. Growth rate measurements of fatteners during Phases 1, 2 and 3.



The percentage of lung lesions decreased from 95.8% during phase 2 to 13.8% during phase 3. The majority of the observed lesions during phase 2 were scored as grade 2 lesions, while, during phase 3, the majority of the lesions were scored as grade 1 lesions. The percentage of pleuritis decreased from 60.4% during phase 2 to 9.3% during phase 3 (Fig. IV).

Fig. IV. Assessment of lung lesions of pigs slaughtered during Phase 2 and Phase 3



Discussion

Vaccination of young piglets against *Mycoplasma* has been shown to exacerbate PMWS in some farms. Cessation of vaccination can alleviate PMWS symptoms and reduce mortality. However, this can result in a flare-up of mycoplasmal pneumonia. One possible solution to the problem would be to delay the vaccination for *Mycoplasma* by increasing the period of passive immunity by vaccinating sows prior to farrowing with a mycoplasma vaccine. Sows vaccinated against *Mycoplasma* do have higher serum antibody titers and their piglets do have a higher and more uniform maternal immunity status[2]. Vaccination of pregnant sows with Hyoresp does not have negative effects on gestation[3]. The results of this study showed that such a strategy can be used in farms where PMWS is aggravated by early piglet vaccination for *Mycoplasma*.

Conclusion

At this farm, the problems with PMWS disappeared after cessation of *Mycoplasma* vaccination at a young age. PMWS did not recur following sow vaccination combined with a one shot piglet vaccination at a later age using Hyoresp®. The *Mycoplasma* problems of phase 2, which occurred after stopping *Mycoplasma* vaccination, were eliminated by means of a *Mycoplasma* vaccination for sows combined with a one-shot piglet *Mycoplasma* vaccination with Hyoresp®. The growth per day increased, while the percentage of lung lesions and pleuritis decreased.

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Pharmacology

EFFECT OF TREATMENT WITH METACAM® IN SOWS WITH MMA SYNDROME ON THE VIABILITY AND GROWTH OF PIGLETS BORN WITH A LOW BIRTH WEIGHT

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Introduction and objectives

The mastitis-metritis-agalactia (MMA) syndrome is a frequent disease in sows during the first 12 hours to 3 days after farrowing and is caused principally by the enterobacteriaceae, particularly *E. coli*. It may present clinically with fever, anorexia, vaginal discharge and mastitis (1), but can also develop in a sub-clinical form, affecting one or more mammary glands. Its immediate consequence is a deterioration in the milk producing capacity of the sow, leading to hypogalactia or, in extreme cases, to total agalactia. As the intake of a sufficient quantity of colostrum during the first 24-48 hours of life of the piglets is a requirement for good health and low mortality during the neonatal period, hypogalactia of the sow during the hours following farrowing can reduce the passage of colostral antibodies to the piglets, affecting their level of immunity and predisposing them to diarrhoea (2), with the consequent increase in mortality and reduction in growth.

Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) which, in combination with antibiotic treatment, has been shown to be effective in the MMA syndrome, as it leads both to an earlier recovery of the clinically affected sows (3), and to a reduction in the mortality of the piglets and an improvement in their growth when used systematically on farms with a history of the disease (4). In a recent study (5), the treatment of sows with meloxicam significantly increased the number of piglets with the optimal desired weight at weaning (> 5 kg) in comparison with another reference NSAID, suggesting greater efficacy in re-establishing milk production in the sow. To investigate this subject further, the objective of the present study has been to evaluate the effect of post-farrowing treatment of the sows with meloxicam on the viability and growth of the low birth weight piglets on a farm with a history of clinical and sub-clinical MMA.

Materials and methods

A comparative field study was performed in Vic, Barcelona, between December 2004 and January 2005 on a farrow-to-finish farm with a history of clinical and sub-clinical MMA. Recently farrowed sows (less than 6 hours), without induced or assisted farrowing, and which did not present symptoms of the disease were selected for the study.

In summary, 16 sows were randomly assigned to two treatment groups and were treated with 0.4 mg/kg of meloxicam (Metacam®; Boehringer Ingelheim; 2 ml/100 kg bodyweight, IM) or placebo (normal saline; 2 ml/100 kg bodyweight, IM). In addition, each sow received basic treatment consisting of an intramuscular injection of long-acting amoxycillin (15 mg/kg bodyweight) and oxytocin (10 IU/sow). In total, 183 piglets were individually weighed at birth (d0) and at 18 days of life (d18) and the mortality was recorded during this period. The overall results of the 2 treatment groups, including all the piglets, were compared, followed by a comparison of the low birth weight piglets, analysing the results by groups using the following criteria: the 25% of piglets with lowest weight (< 1,258 g); the 20% of piglets with lowest weight (< 1,209 g); the 15% of piglets with lowest weight (less than 1,152 g); the 10% of piglets with lowest weight (< 1,080 g). The growth data were analyzed by comparison of the means using Student's t distribution method and the mortality was analysed by the χ^2 test, using Microsoft Excel for Windows. The significance level was taken as $p<0.05$.

Results and discussion

The overall the mortality up to day 18, including all the piglets, was of 4.49% in the meloxicam group and 14.89% in the placebo group (Table 1); these differences, however, were not statistically significant. The average daily weight gain (ADG) was significantly higher in the meloxicam group than in the placebo group (235 versus 219 g/day; p=0.038).

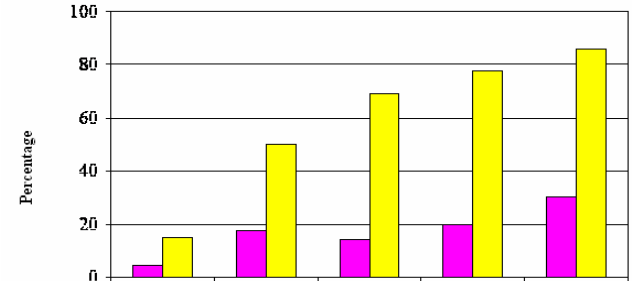
On analysis of the effect of treatment with meloxicam in low birth weight piglets, a positive tendency was observed which was inversely proportional to the weight at birth (Figures 1 and 2), and a statistically significant difference was also observed in the mortality in the piglets with a birth weight of less than 1,209 g (p=0.014). These results confirm that treatment with meloxicam increases milk production in the sows and that this greater availability of milk gives rise to a benefit principally in the low birth weight piglets.

Table 1. Mean production data in response to treatment.

	Meloxicam	Placebo
Number (no.) of sows	8	8
No. of piglets d0	89	94
Mean no. of piglets/sow d0	11.12	11.75
Mean no. of piglets/sow d18	10.62	10,00
Mortality (%)	4.49	14.89
ADG (g/day) d0 to d18	235[a]	219[b]

[a,b] The different superscripts indicate statistical differences.

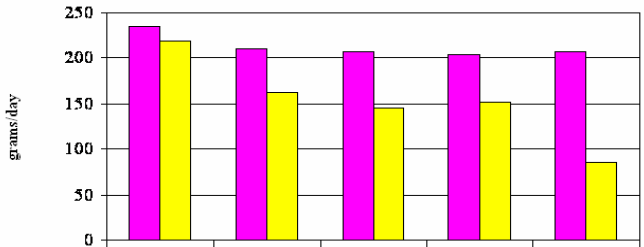
Figure 1. Percentage mortality in the overall group and in low birth weight piglets[1].



	All	<1,258	<1,209	<1,152	<1,080
Meloxicam	4.49	17.39	14.29[a]	20	30
Placebo	14.89	50	69.23[b]	77.78	85.71

[1]The 25% of piglets with lowest birth weight (<1,258 g); the 20% of piglets with lowest birth weight (<1,209 g); the 15% of piglets with lowest birth weight (<1,152 g); the 10% of piglets with lowest birth weight (<1,080 g). [a,b] The different superscripts indicate statistical differences.

Figure 2. Mean daily weight gain in the overall group and in the low birth weight piglets[1].



	All	<1,258	<1,209	<1,152	<1,080
Meloxicam	235[a]	210	207	204	207
Placebo	219[b]	162	145	152	86

[1]The 25% of piglets with lowest birth weight (<1,258 g); the 20% of piglets with lowest birth weight (<1,209 g); the 15% of piglets with lowest birth weight (<1,152 g); the 10% of piglets with lowest birth weight (<1,080 g). [a,b] The different superscripts indicate statistical differences.

In this study, the postpartum treatment of sows with meloxicam on a farm with a history of MMA significantly increased the average daily weight gain of the piglets from birth to weaning, and significantly reduced the mortality in the low birth weight piglets.

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Effects of beta-glucans on ADG, ADFI, FCR, FCG and Rate of sick of Growing Pigs

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Introduction and Objectives

Beta-glucans is a natural polysaccharide fiber produced from yeast (3). Influences of beta-glucans on growth performance in weaning pigs were reported elsewhere (1). They were concluded that supplement nursery pig diets with 0.025% beta-glucans had increased growth performance. However other authors found that 0.015 and 0.03% beta-glucans had no effects on ADG of pigs. However, feed intake was increased in this study (2). The mechanisms of beta-glucans on improving growth performance of pigs might be the reduction of pro-inflammatory cytokines which has the effect on central nervous system contributing to increased feed intake (2).

On those studies the experiments on weaning pigs were performed. In this study the experiment in growing pigs in commercial farm on which has scarcely information about the effect of beta-glucans on ADG, ADFI, FCR and FCG and rate of sick were performed.

Material and Methods

Two hundred forty crossbred growing pigs weight between 25 to 30 kilograms from a commercial pig farm in Khon Kaen province were used. The farm was considered to have high pathogen load. The ration of pigs was divided into 3 treatments which were T1-basal diet composed of rice-soybean meal containing antimicrobial additive normally used on this farm (CTC 300 ppm, Amoxycillin 300 ppm and Halquinol 120 ppm) T2-basal diet without antimicrobial additive but 500 ppm of beta-glucans (Alphamune from Alphamar) and T3-basal diet with antimicrobial additive normally used on farm but substitute 60% Halquinol with 500 ppm beta-glucans. Feed intake was calculated per pen. Bodyweights were recorded on the day that pigs leave nursery barn and on the day pigs enter finishing barn. Sign of sick on individual pig was recorded daily.

Results and Discussion

Alphamune™ was used as the source of beta-glucans at the doses of 500 ppm. However, this product contains 26% of beta-glucans which were equal to 0.013% in feeds. ADG, ADFI, FCR and FCG of the pigs from the three different feeding groups during the experimental periods are presented in Table 1. No apparently difference of ADG, ADFI and FCR and FCG were observed among the treatment. However, FCG of pigs in T1 was 0.96 and 0.34 baht higher than in T2 and T3 accordingly.

Abbreviation:
ADG=average daily gain, ADFI=average daily feed intake, FCR=feed conversion ratio, FCG=feed cost per gain

Table 1: ADG, ADFI, FCR and FCG (means±STDV) of Pigs treated with three different feeding groups. Mean values sharing the same letter were not significantly different.

	T1	T2	T3
ADG(kg/d)	0.74±0.06[a]	0.71±0.02[a]	0.71±0.05[a]
ADFI(kg/d)	1.28±0.12[b]	1.22±0.11[b]	1.24±0.04[b]
FCR(kg/kg)	1.74±0.09[c]	1.71±0.13[c]	1.75±0.07[c]
FCG(baht/kg)	23.76±3.14[d]	22.80±1.32[d]	23.42±1.33[d]

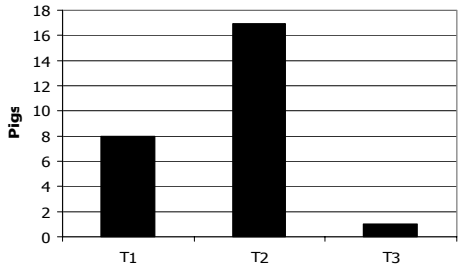

Figure 1: Morbility rate along the experimental period

There are clear differences of sick rate among the treatments. Eight, 17 and 1 pig were sick in treatment 1, 2 and 3 consecutively (Fig 1). There was no death of the pigs along the period of the experiment. Cough was the often sign of sick pigs. Morbility rate was very low in treatment 3 comparing to treatment 1 and 2. These pigs were injected with appropriate drugs and recovery was soon observed. It is speculated that this should be the effect of beta-glucans coincide with antibiotic. Beta-glucans alone do not give enough appropriate protection to the pigs from cough in this study. However, it might be the immuno-modulatory effect of beta-glucans that involved.

When FCG and morbility rate was taken into account, combination of antibiotics with beta-

glucan in feed of growing pigs was suitable as demonstrated in Table1 and Figure 1.

In conclusion, the dose of beta-glucans supplement in the diet of growing pigs on their growth performance should be further elucidated.

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Induce farrowing in sows by using Dinoprost (Lutalyse®): studies on behavioral changes, timing of farrowing, duration of parturition, total born litter size and stillbirth rate

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Introduction

The use of Prostaglandins ($PGF_{2\alpha}$) for the induction of farrowing has been successfully and widely used worldwide in pig farms, including in Thailand. The advantages are to improve batch farrowing and also induce farrowing on daytime in which the farmers are convenient to supervise farrowing of sows, prompt assistance may be given to sows which may have difficulty. This is result in safer delivery of the piglets and thereby reduces piglets mortality. The aim of the present study was to study the efficacy of different doses and route of administration of Dinoprost (Lutalyse®)

Material and Methods

Twenty multiparous sows (Yorkshire x Landrace) were housed individual pens in a large enclosed fan-ventilated house under natural daylight and temperature varied between 25-32 ºC. They were selected for induction of parturition on days 113 to 114 of gestation. The average gestation period of sows on this farm was 115 days. Sows were fed with commercial feed and water was *ad libitum*. Parturition was induced by one of the following treatments:

- 10 mg $PGF_{2\alpha}$ (Dinoprost, Lutalyse®), administered IM, group A (n=10)
- 5 mg $PGF_{2\alpha}$ (Dinoprost, Lutalyse®), half of the manufacturer's commenced dose, administered IM at perivulva area group B, (n = 10)

Sows were observed for the sign of parturition at 24 h after injection of $PGF_{2\alpha}$ (from 7 am to 9 pm). The following parameters were evaluated: time from treatment to the onset of farrowing, expulsion intervals, duration of parturition, total number of piglets born (stillborn, mummy and born alive), umbilical cord morphology, meconium staining and bodyweight of each piglet.

In addition, sows were observed for mothering g behaviour immediately after $PGF_{2\alpha}$ injection for 1 h. The behavioural observations including posture (kneel, lie belly, lie side, sit, stand), nesting behaviour (grasping behaviour, paw floor, paw wall, root floor, root wall, step) and others behaviours (inactive, defecate, urinate, drink, chew fixure, object scratch, rear leg scratch, shake head) were also recorded.

Results and Discussion

Times from treatment to the onset of farrowing are shown in Table 1. Zootechnical data relative to sows and piglets are shown in Tables 2, 3 and 4.

Table 1. Time from treatment to the onset of farrowing in which was divided into three groups.

Hours to farrowing	Groups (10 sows in each group)	
	A full-dose	B half-dose
<24 hr	4	4
24-30 hr	6	6
>30 hr	0	0

Table 2. Onset of farrowing, farrowing duration, expulsion interval, litter size, total born, presentation and piglet weight.

Variables (means ± SD)	Groups (10 sow in each group)	
	A full-dose	B half-dose
Hours to farrowing	26hr 20min (n=8)	25hr 6min (n=9)
Farrowing duration	5hr 37min (n=8)	5hr 3min (n=9)
Expulsion Interval	30.58±13.22	18.63±11.34
Litter size	11.4±3.44	12.2±1.98
Total piglets born	114	122
* liveborn	106(92.98%)	115(94.26%)
* stillborn	7(6.14%)	7(5.74%)
* mummy	1(0.88%)	0
anterior presentation	83	86
posterior presentation	20	27
Piglets weight	1.54±0.16	1.53±0.24

Table 3. Degree of meconium-stained on liveborn and stillborn piglets (altogether 226 piglets)

Birth	Grading of stain	Groups	
		A full-dose (n=103)	B half-dose (n=113)
Liveborn	negative	34	39
Liveborn	mild	38	55
Liveborn	moderate	21	12
Liveborn	severe	3	0
Stillborn	negative	3	3
Stillborn	mild	3	2
Stillborn	moderate	1	2
Stillborn	severe	0	0

Table 4 Morphology of umbilical cord from 226 piglets

The present data confirmed the observation in that a half dose of $PGF_{2\alpha}$ (Lutalyse®) administered at perivulva region was as effective for inducing farrowing as the full recommended dose injected at the neck area (IM). For the other criteria relative to the sows or to the piglets no significant difference was observed between groups. Further more, the present study show that different doses (i.e. half and full doses) of $PGF_{2\alpha}$ (Lutalyse®) have differential effects on mothering behaviour (e.g. nesting, restless) in sows. Sows given the 10 mg $PGF_{2\alpha}$ (full dose) IM displayed maximal level of mothering behaviour whereas lower level of mothering behaviour occurred in sows given 5mg of $PGF_{2\alpha}$ (half dose) in term of individual sow responses.

Umbilical cord morphology	Groups	
	A full-dose (n=103)	B half-dose (n=113)
normal	79 (76.70%)	101(89.38%)
edema	0	0
congestion	21(20.39%)	12 (10.62%)
hemorrhage	3 (2.91%)	0

In conclusion, the present data showed that induction of parturition using half dose of $PGF_{2\alpha}$ can be as effective as using full dose, and a decreased in the restless behaviour were also observed.

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Induce farrowing in sows by using Cloprostenol (Preloban®): studies on behavioral changes, timing of farrowing, duration of parturition, total born litter size and stillbirth rate

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Introduction

The use of Prostaglandins ($PGF_{2\alpha}$) for the controlled induction of farrowing has been successfully and widely used worldwide in pig farms including Thailand. The advantages are to improve batch farrowing and to induce farrowing in daytime whice is easy to supervise farrowing in sows, prompt assistance may be given to sows which may have difficulty.This is safer delivery of the piglets and thereby reduces piglets mortality. The aim of the present study was to the efficacy of different doses and routes of administration of R – Cloprostenol (Preloban®).

Material and methods

Twenty multiparous sows (Yorkshire x Landrace) were housed individual pens in a large enclosed fan-ventilated house under natural daylight and temperature varied between 25-32 ºC. They were selected for induction of parturition on days 113 to 114 of gestation. The average gestation period of sows on this farm was 115 days. Sows were fed with commercial feed and water was *ad libitum*. Parturition was induced by one of the following treatments:

- 75 µg $PGF_{2\alpha}$ (R-Cloprostenol, Preloban®),administered IM, group A (n = 10)
- 37.5 µg $PGF_{2\alpha}$ (R-Cloprostenol, Preloban®), halfof the manufacturer's recommended dose, administered IM at perivulva area, group B (n = 10)

Sows were observed for the sign of parturition at 24 h after injection of $PGF_{2\alpha}$ (from 7 am to 9 pm). The following parameters were evaluated: time from treatment to the onset of farrowing, expulsion intervals, duration of parturition, total number of piglets born (stillborn, mummy and born alive), umbilical cord morphology, meconium staining and bodyweight of each piglet.

In addition, sows were observed for mothering behaviour immediately after $PGF_{2\alpha}$ injection for 1 h. The behavioural observations including posture (kneel, lie belly, lie side, sit, stand), nesting behaviour (grasping behaviour, paw floor, paw wall, root floor, root wall, step) and others behaviours (inactive, defecate, urinate, drink, chew fixure, object scratch, rear leg scratch, shake head) were also recorded.

Result and Discussion

Times from treatment to the onset of farrowing are shown in Table 1. Zootechnical data relative to sows and piglets are shown in Tables 2, 3 and 4.

Table 1. Time from treatment to the onset of farrowing in which was divided into three groups.

Hours to farrowing	Groups (10 sows in each group)	
	A full-dose	B half-dose
<24 hr	8	7
24-30 hr	2	2
>30 hr	0	1

Table 2. Onset of farrowing, farrowing duration, expulsion interval, litter size, total born, presentation and piglet weight.

Variables (means ± SD)	Groups (10 sows in each group)	
	A full-dose	B half-dose
Hours to farrowing	25hr 1min (n=4)	25hr 41min (=5)
Farrowing duration	3hr 29min (n=4)	4hr 35min (n=5)
Expulsion Interval	23.32±12.01	22.69±11.81
Litter size	12.6±2.95	11.2±2.69
total piglets born	126	112
* liveborn	122(96.83%)	109 (97.32%)
* stillborn	4(3.17%)	3 (2.68%)
* mummy	0	0
anterior presentation	66	49
posterior presentation	37	24
Piglets weight	1.59±0.26	1.53±0.17

Table 3. Degree of meconium-stained on liveborn and stillborn piglets (altogether 180 piglets)

Umbilical cord morphology	Groups	
	A full-dose(n=110)	B half-dose (n=70)
Normal	93 (84.55%)	61 (87.14%)
Edema	1 (0.91%)	0
Congestion	15 (13.63%)	9 (12.86%)
Hemorrhage	1 (0.91%)	0

Table 4 Morphology of umbilical cord from 180 piglets

Birth	Grading of stain	No. of piglets	
		A full-dose (n=110)	B half-dose (n=70)
Liveborn	negative	46	29
Liveborn	mild	52	34
Liveborn	moderate	7	4
Liveborn	severe	2	0
Stillborn	negative	0	1
Stillborn	mild	3	2
Stillborn	moderate	0	0
Stillborn	severe	0	0

The present data confirmed the observation in that a half dose Cloprostenol (Preloban®) administered at perivulva region was as effective for inducing farrowing as the full recommended dose injected at neck area (IM). For the other criteria relative to the sows or to the piglets no significant difference was observed between groups. The mothering behaviour (e.g. nesting, restless) was not observed in both groups. However, the behaviours such as lie on belly and drinking, defecations, kneel and root wall can be observed. The reason for the efficacy of the lower dose Cloprostenol administered at perivulva area for inducing parturition was not clearly elucidated.

In conclusion, the present data showed that induction of parturition using half dose of Cloprostenol can be as effective as using full dose, which in turn, reduce a cost of induce farrowing.

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IN VITRO SUSCEPTIBILITY TEST OF MYCOPLASMA HYORHINIS TO ANTIMICROBIAL AGENTS

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Introduction and Objectives

Mycoplasma hyorhinis is one of the porcine mycoplasmas causes of the systemic disease in pig named Mycoplasmal polyserositis- arthritis. Lameness in the nursery and respiratory problems or Glasser's disease- like symptoms found in the farm can be caused by this pathogen, so *M. hyorhinis* (MHr) has to be taken into consideration when these symptoms occur. Control programme usually involve medication and management measures. The susceptibility of microorganisms to antimicrobial agents may vary from place to place due to different field isolates, what antibiotics and how the farmers use them in that area. MIC study of some MHr field isolates will be a useful data to select effective drug for treatment the disease in each pig industrial area and to compare the effective drugs in all over the world. So the objectives of this study were to determine the minimum inhibitory concentrations (MICs) for ten antimicrobial agents against 26 MHr fields isolates in Thailand.

Materials and Methods

The 26 local isolates of MHr which were isolated from pigs died by pneumonia and /or polyserositis, polyarthritis and convulsion from year 1999-2004 were inoculated in modified Hayflick's broth and subculture for 2 times until the microorganism could grow rapidly within 2 days in 3-4 ml broth. Stocked them at –80C while the small volume was used to determine the culture concentration by ten-fold dilution and count in agar plate.

Take MHr from the stock to make concentration at $5x10^5$ cfu/ml with modified Hayflick's broth and incubate at 35C for 2 hours. The MICs were performed by a microdilution method and the concentrations of the antimicrobial drugs were two-fold serial dilutions from 50-0.024 ug/ml (chlortetracycline, oxytetracycline and Tilmicosin) and 12.5-0.006 ug/ml (Aivlosin, Doxycycline, Josamycin, Lincomycin, Tiamulin, Tylosin and Valnemulin) Briefly, fill 25 ul of broth in all wells of the 96 well microtiter plate except for the first column, drop 25 ul of antibiotic stock solution to all wells in the first and the second column and dilute the antibiotic from the second column through the last column. Add 175 ul of prepared isolates, one isolate per two rows. Cover the plates and incubate at 37 C until the MHr can grow by inspection of color changing of the control culture tube. In this study, we read the result at 3 days incubation. Read the MIC value of each drug which could inhibit the MHr growth (2).

Results and Discussion

All isolates were highly susceptible to valnemulin, tiamulin, aivlosin, josamycin, lincomycin, doxycycline and tylosin, moderately susceptible to tilmicosin, oxytetracycline (OTC), and chlortetracycline (CTC). Valnemulin showed very highly activity against all MHr isolates. MIC 90 was less than 0.006 ug/ml, ten times lesser than in Hungary (3). The reason might because valnemulin is still not used in Thailand. Tiamulin was less active with MIC90 at 0.19 ug/ml which were 5 times lesser than in Hungary (3) but almost same as the test by Ter Laak et al. (4) and in Japan (1). The MIC90 for OTC and CTC were high compare to the data in Japan which were performed 9 years ago (1) and in USA. (5).

The way of using of antibiotics in each country may not the same so that results of the MICs range for certain drugs in each country were different. The year the tests performed are also shown the consistency of the effective drugs and trend of antibiotics resistance. MICs for Tylosin seem to be higher from time to time, however its MIC90 from many tests still lower than 2 ug/ml (1,4) except in Hungary (3). MICs90 for Josamycin and Lincomycin also not more than 2 ug/ml (1,4), however their MIC range are wide. In conclusion, all of the antimicrobial tested in this study could be used for treatment of *Mycoplasma hyorhinis* infection in the affected farm.

Table 1. MICS for *M.hyorhinis* determined by microdilution method

Drug	MIC (ug/ml) from MHr (n = 26)		
	range	50%	90%
Aivlosin	0.024-6.25	0.097	0.19
Chlortetracycline	6.25-50	25	25
Doxycycline	0.097-3.12	0.78	1.56
Josamycin	0.19-6.25	0.39	0.78
Lincomycin	0.19->12.5	0.78	0.78
Oxytetracycline	1.56-25	6.25	12.5
Tiamulin	0.024-0.39	0.097	0.19
Tilmicosin	0.19-50	0.78	3.12
Tylosin	0.39-6.25	0.78	1.56
Valnemulin	≤0.006	≤0.006	≤0.006

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THE EFFICACY OF COLISTIN AND BACITRACIN COMBINATION ON NUMBER OF ENTERIC BACTERIA AND SOME PRODUCTIVE TRAITS IN POST-WEANING PIGLETS

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Keywords: pig, post-weaning diarrhea, antibiotic, *E. coli*

Introduction

In pig, antimicrobial feed additive reduced the severity of gastrointestinal disorders caused by several pathogenic bacteria, often included *Escherichia coli (E. coli), Bachyspira spp., Salmonella spp.* and *Clostridium spp.* Prohibited the use of antibiotics as a feed additive for swine producer, due to the legislation, significantly influence growth rate, mortality and morbidity rate (Robertsson and Lundeheim, 1994). Although, the current trend worldwide is to reduce antibiotic used in animal production (Mateu and Martín, 2000), in practice, it appears impossible to raise animals under high productivity conditions without the use of antimicrobials, particularly in tropical countries. *E. coli* is the most common bacteria causing post-weaning diarrhoea in piglets. Colistin is an antibiotic widely used in the pig farm to control and treatment of diarrhea in pig (Mateu and Martín, 2000; Savoini et al., 2002; Tummaruk et al., 2004). It is well established that the over use of antibiotic resulted in drug resistance (Mateu and Martín, 2000). Due to the high frequency of using colistin in the pig farm, there is a need to find the procedure of using colistin effectively. Bacitacin methylene disalicylate (BMD) is an antibiotic recommended to used for treatment of diarrhea due to *Clostridium spp*. (Duquette et al., 1990) and have been reported in vitro to inhibit the resistance process of *E. coli* (Bineva and Karaivanov, 1984). The clinical study on the efficacy of drug synergist between colistin and BMD to control and treatment diarrhea in piglets has never been performed. Additionally, the economic purpose of using a combination of colistin and BMD is to reduce the cost of feed additive antibiotic.

Objectives

The objective of the present study was to evaluate the efficacy of drug combination between colistin sulfate and BMD in post-weaning piglets under field conditions.

Materials and methods

The study included 653 piglets with an average BW of 6.6±1.2 kg at weaning (21±2 days). The piglets were randomly assigned to 4 groups (A, B, C and D). Piglets in group A were served with colistin 150 g/ton in feed. Piglets in groups B and C were served with BMD 30 g/ton in combination with colistin 100 and 80 g/ton in feed, respectively.

Piglets in group D were fed without colistin or BMD (control group). The trial was carried out in 3 replications and included 174, 280 and 199 piglets in the first, second and third batches, respectively. Four piglets per group were randomly selected for fecal sampling at 3, 5 and 7 wk of age. Dilution/ spread-plating/ replica-plating techniques on selective media were used to obtain count of the microorganism. Productive traits analyzed included average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and body weight (BW) at 7 wk of age. The number of *Escherichia coli (E. coli)*, *Enterococcus spp* and *Lactobacillus spp* in feces of the piglets were also enumerated.

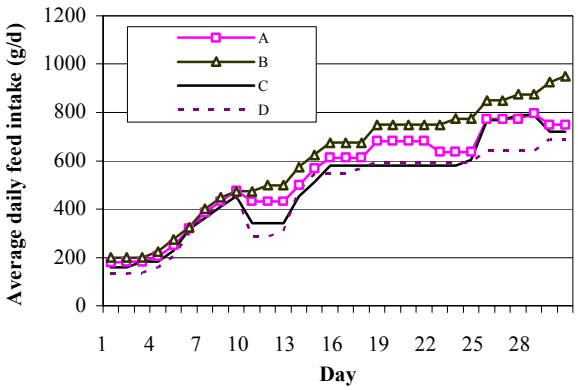

Fig. 1 Average daily feed intake (g/d) in piglets treated with colistin 150 ppm (A), colistin 100 ppm + BMD 30 ppm (B), colistin 80 ppm + BMD 30 ppm (C) and no colistin and BMD in feed

Results

The BW at 7 wk of age in groups D (16.1 kg) was lower than group A (17.6 kg; $P<0.001$), B (17.7 kg; $P<0.001$) and C (17.1 kg; $P<0.01$). ADFI of the piglets in group B (621.6 g/d) was significantly greater than group D (552.6 g/d). The number *E. coli*, *Enterococcus spp.* and *Lactobacillus spp*. counted (log_{10}) at weaning were 9.9, 7.8 and 11.1 CFU/gm, respectively. Number of *E. coli* counted were decreased after 2 and 4 wk of weaning in all group ($P<0.05$). *Enterococcus spp.* in group B decreased in 2 wk of weaning ($P<0.05$). At the 4th wk of weaning, *Lactobacillus spp.* in groups B and C was greater than group D ($P<0.05$).

Discussion

Colistin was the compound routinely used most often nowadays for prevention of post-weaning diarrhoea in piglets due to *E. coli*. The reasons are that colistin is easy to use and low rate of resistance among entero-bacteria (Catchpole et al., 1997; Mateu and Martín, 2000). In addition, colistin given orally is poorly absorbed in the gut, it is therefore that the resistance could be over come by increase in-feed doses of the antimicrobial or increase the efficacy by means of drug combinations.

The present study demonstrated that colistin combined with BMD enhanced ADFI and ADG of the post-weaning piglets. The use colistin and BMD were effective to control the severity of post-weaning diarrhea in piglets. Post weaning diarrhea was found most during 4-5 week of age. During this period, the ADFI of the piglets were reduced (Fig. 1). Piglets receiving drug combination of colistin 100 g/ton and bacitacin 30 g/ton after weaning seem to suffered from post-weaning diarrhea less than other groups, as indicated by the reduction of feed intake was less pronounce than the others.

Bacteriological examination revealed that both colistin and the combination between colistin and BMD could effectively control the number of *E. coli* over growth in the nursery piglets. Furthermore, drug combination between colistin and BMD significantly enhanced number of *Lactobacillus spp.* The high number of *Lactobacillus spp.* could inhibit the over growth of pathogenic bacteria in the intestine, increase

digestibility of the pig and have been used as probiotics in pig (Chesson, 1994).

Conclusions

Both colistin and the combination could improve ADFI, ADG, FCR and BW at 7 wk of age in the nursery pigs. The use of the drug combination agents significantly enhanced the number of beneficial bacteria, *Lactobacillus spp* in post weaning piglets.

Acknowledgments

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Efficiency of Moxidectin pour-on in the treatment of Sarcoptic mange in swine

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Introduction

Sarcoptic mange is often a severe disease. Intense, constant pruritus results in annoyance, irritability, decreased feed intake, anemia, decreased weight gain or weight loss, difficulties in estrus detection, lowered conception as well as reproductive problems.

Definitive diagnosis is base on history, physical examination, skin scraping, skin biopsy, and response to therapy. Mites are often very difficult to find in scraping. In swine, crusts and cerumen gathered from ear canal and pinna are the most consistent sources of mites (1).

Moxidectin pour-on was approved to control the endoparasites and ectoparasites in cattle (2) and also has an efficiency to against swine nematodes (3). The aim of the present study was to determine efficiency of moxidectin pour-on in treatment sarcoptic mange in swine.

Materials and methods

A farm was selected in which clinical signs of mange were evident: encrusted lesion in ears, dermatitis, and pruritus. This was confirmed by positive ear scrapings.

Thirty pigs were naturally infected and divided into 2 main groups; control group (5 pigs) and treatment group (25 pigs): control group were treated with normal saline, and treatment group were subdivided into five-dose of moxidectin 0.5% pour-on with five pigs per group. The doses were: moxidectin 0.5, 0.75, 1.00, 1.25, and 1.5 mg/kg body weight. Moxidectin was given twice with a 14-days interval (d0 and d 14, respectively). Each animal in the groups was monitored for clinical signs such as scratching index, average dermatitis score, and mite score on day 0, 7, 14, 21, and 28 after treatment. Ear scrapings were taken from the inside of the pinna of the ear an area of approximately 2 cm^2 with a curette. The material collected from each ear was examined microscopically for the presence of live and dead mites.

The scraping were collected into centrifuge tube and digested in 10 ml of the 10% potassium hydroxide solution overnight. On the following day mixed with vertex and centrifugation at 2,500 rpm for 15 minutes. The suspended was calculated the mite including adults, nymphs and larvae, and mite egg from day 0 to day 28 post-treatment were calculated from the means.

On the same days when scraping were collected, pigs were observed for 15 minutes for signs of rubbing and scratching. The severity of skin lesion and pruritus were scored from 0 to 3. The scheme used for scoring was as following – for skin lesion: 0= no active lesion, 1= slight lesion of encrustation, scaling or erythema, 2= a few localized moderately thick dry crusty lesions, 3= large, thick, grayish, rough and dry crusts with coalescence of lesion. Chi-square was used to statistically interpret the results.

Results and discussion

Table 1. The amount of pig was remained the live mite on day 7, 14, 21, and 28 after treatment.

Groups	days after treatment			
	d7	d14	d21	d28
Control	4/5	4/5	3/5	1/5
1	3/5	3/5	2/5	2/5
2	2/5	2/5	2/5	1/5
3	5/5	3/5	0/5	0/5
4	1/5	1/5	0/5	0/5
5	1/5	0/5	0/5	1/5

All of treatment group has shown the reduction rate of live mite on Day 28 was significantly ($p<0.005$) (Table 1). After secondary treatment, were begin decrease the skin lesion in the treatment group (2, 4, and 5 subgroups). On the day 28 after treatment, 3, 4, 4, 2, 2,and 3 pigs were not found skin lesion in group control, 1, 2,3 4, and 5 respectively. There was no significant difference between control and treatment groups in the data of scratching index.

2 pigs in group 5 had skin irritation after treatment on day 16.

Discussion

The field trial of moxidectin pour-on has shown the high efficacy against sarcoptic mange in treatment group (3, 4, and 5 subgroups). But the effective dose and not irritated skin is 1.00-1.25 mg/kg and give

two times interval 14 days. After secondary treatment, were begin decrease the skin lesion in the treatment group (2, 4, and 5 subgroups). Scratching index is not completely specific for mange, other cause of the pruritus observed in this study could account for the lack of a significant difference in scratching index (4).

Clinical observations, scratching observations, average dermatitis scores, and examination of ear scrapings have their limitations as indicators of sarcoptic mange infestation. However, their combined use allows an evaluation of the efficiency moxidectin pour-on for treated sarcoptic mange in swine by the results of the present study.

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Production

The effect of post-ovulatory insemination on reproductive performances in sows

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Introduction

In non-mated sows at oestrus, the endometrium with its large number of neutrophils in the subepithelial layer (5), has a potential to respond quickly to invading agents. After mating (natural mating or AI, during oestrus), a normal uterine response is an influx of polymorphonuclear cells into the uterine lumen (9). This inflammatory response to spermatozoa appears to be a normal physiological reaction in order to clear the uterus from excess spermatozoa and contamination associated with mating as well as to prepare an optimal uterine environment for the early embryos (10). Seminal plasma itself can also induce a transient inflammatory response, as shown in the gilt uterus (1). However, successful pregnancy in pigs is also associated with intrauterine immunosuppression, i.e. suppression of MHC molecules and T cell responses which allow embryos (semiallografts) to develop in the uterine lumen without being rejected (11).

In pigs, it has been shown that the optimal insemination time in order to achieve a good fertilisation rate is within 24 h before ovulation (9, 12). The ability of a pig oocyte to be fertilised has been considered to be as short as 8-12 h after ovulation (4). Therefore, insemination after ovulation results in impaired farrowing rate and litter size (13). In addition, in a study on the influence of pre- compared with post-ovulatory insemination (20-15 h and 15-20 h, respectively), on the distribution of spermatozoa in the oviduct, the accessory sperm counts on the zona pellucida and early embryonic development (6), we found that when sows were inseminated after ovulation (i.e., their plasma level of oestradiol is lower and progesterone higher compared with insemination before ovulation), fertilised oocytes and developed embryos were observed up to day 11 but no embryos were found at day 19. This may cause the sows return to oestrus with a prolonged interval (between 18-24 days=regular return or more than 24 days=irregular return). Furthermore, in an experimental model on uterine infection in gilts, it was shown that gilts inoculated with bacteria into the uterus at metoestrus had increased plasma progesterone levels, developed endometritis and showed vaginal discharge (3). However, this was not found in gilts inoculated at oestrus.

When sows are inseminated after ovulation, it can be expected that their plasma level of oestradiol is lower and progesterone higher compared with insemination before ovulation. This may cause disturbances not only in pregnancy outcome but also in the reaction of immune cells. Therefore, in relation to clinical findings and pregnancy outcome, the aim of the present investigation was to study the effect of post-ovulatory insemination on the subsequent oestrous cycle length in sows and the vaginal discharge syndrome.

Materials and Methods

Ten Large White sows with an average parity number of 3 were purchased from a commercial herd on the day of weaning. Prior to this study, the sows had shown a normal reproductive performance. Their body weights ranged from 242-276 kg. The sows were kept in individual crates and boars were housed in the same stable throughout the experimental period. The sows were fed twice a day.

Water was available ad libitum. Oestrous detection was performed by inspection of the vulva for reddening and swelling (prooestrus) as well as by control of the standing reflex (oestrus) in the presence of a boar. The oestrous detection was carried out twice daily. Ovulation was followed every 8 h by transrectal ultrasonography as described earlier (Kaeoket et al., 2002). Six sows (2 sows in gr. A and 4 sows in gr. B) were inseminated once at 15h after ovulation and another four sows (gr. C) at 30h after ovulation with a dose of semen (boar of proven fertility), containing 3 x 109 spermatozoa in 100 ml BTS (Beltsville Thawing Solution,10). On days 11-12 after standing oestrus (day of standing oestrus=day 1), two sows from gr. A were allocated to ovariohysterectomy (OVH) under general anaesthesia. The ovaries were examined for the corpus luteum (CL) and follicles. The uterine horns were equally divided into 3 parts and flushed twice with 20 ml of phosphate buffer saline (PBS). The fluid was collected in petri dishes and the embryos were examined in PBS under a stereomicroscope. Eight sows from gr. B and C (four from each group) were observed for the subsequent oestrus cycle (return to oestrus) and vaginal discharge (during prooestrus). The oestrous detection was carried out twice daily start on day 17 after standing.

Results and Discussions

The average weaning to oestrous interval was 5.0 $\pm$ 0.6 (means $\pm$ S.D.) days, with a range of 4-6 days. Ovulation took place at 33.3 $\pm$ 4.1 h after onset of the first oestrus after weaning, with a range of 28-36 h. The average number of CL in group B sows was 20.0 $\pm$ 5.0.

For the embryonic loss, two embryos were observed in one out of two sows from gr. A. All embryos had a spherical shape but differed in size (range 1-2mm) which was in agreement with the size of day 11-embryo reported by (2) and also (6). The size of CL and follicles were 8-9mm and 2-4mm, respectively. The number of recovered embryos on day 11 showed that AI 15 after ovulation led to increased rates of embryonic loss during early pregnancy. Low recovery rate in this group might be due to fertilisation failure or embryonic death before day 11 (6). Another reason could be that some embryos have remained in uterine horns after flushing.

For the oestrous cycle length, three sows in gr. B had a regular return to oestrus (i.e. standing on days 21, 21 and 23). Another sow had an irregular return to oestrus (i.e. standing on day 27). Four sows in gr. C had a regular return to oestrus (i.e. standing on days 22, 23, 23 and 24, respectively). A prolonged oestrus interval found in groups B and C sows might be due to the influence of early but disturbed development of embryos. It has also been shown that embryonic loss by experimental flushing of the blastocysts out of the uterine horns between days 12 and 13 resulted in a prolonged oestrus interval (14).

For the vaginal discharge (during prooestrus), two out of four sows from gr. B had shown vaginal discharge (i.e. thick, white in colour and yellow pus) on days 20 and 38 after standing oestrus. It had been earlier reported that no growth of bacteria was found when uterine samples for bacterial culture were taken from vaginal discharged sows (7). The reason might be that the extender used for semen dilution in those study contained antibiotic. However, the bacterial culture was not performed in the present study. The individual variation due to the sow uterine immune system may be explained the fact that another two sows in this group had no vaginal discharge. It has earlier been shown that gilts, inoculated with bacterial suspension into the uterus during metoestrus (12 h after the end of standing oestrus, the gilts having increased progesterone levels),

developed endometritis and showed vaginal discharge (3). Therefore, the vaginal discharge found in the present study may be, at least, explained by the increased level of progesterone and also low number of neutrophils in the endometrium at AI (i.e. post-ovulatory insemination) (7). However, no sow in group C (inseminated 30 h after ovulation) had shown a vaginal discharge. The underlying mechanism of the uterine immune system for these particular sows need to be elucidated.

Conclusion

Post-ovulatory insemination resulted in early embryonic loss, a subsequent prolonged oestrus interval and also a vaginal discharge syndrome in sows.

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BREEDING FOR FARM EFFICIENCY

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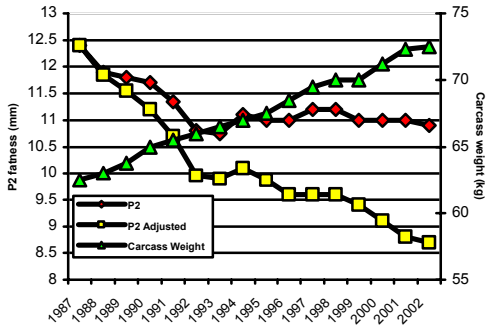
Within any genetic selection programme, it is important to adequately define the selection objective, before identifying the criteria to achieve this. An objective of breeding for farm efficiency, would historically have centred on breeding for leanness, and efficiency of growth. Whilst these criteria are still very important, it is no longer sufficient just to breed for farm efficiency, rather we have to widen the objective to define it as breeding for efficiency of the pork chain.

Selecting solely for efficiencies on farm without the realisation that producer sustainability is inter-dependent with processor efficiency could lead to the efficient production of a product that is not suited to the processing sector's needs.

The major factors influencing farm efficiency are feed conversion ratio (FCR) and growth rate of the pig together with prolificacy within dam lines. As production of lean meat is the primary objective of the producer and takes 3 to 4 times less energy to produce than fat tissue, by definition, selecting for efficient pigs will also lead to selecting for lean pigs.

Historically, genetic selection programmes has been very successful at improving leanness at commercial level. For example, in the UK, improvement over 15 years has resulted in an equivalent reduction in back fat at P2 of over 3.5 mm (**Error! Reference source not found.**)

Figure 2 – UK Grading Data 1987-2002



In reality, this has led to increases in the average slaughter weight with no accompanying increase in back fat. UK dead weight contracts for 90kg carcasses with a top grade of ≤ 14mm back fat allow increased efficiency of production with the extra 15kg deadweight (current average deadweight is around 75kg) returning £15 for £8 outlay (**Error! Reference source not found.**). This represents an 87.5% return.

Over the last 10 years, there has been an increase in accuracy of selection for leanness through the use of technologies such as real time ultrasound and image analysis software along with improved statistical analysis techniques such as BLUP (Best Linear Unbiased Predictor).

Increased return at 100p/kg
15kg * 100p/kg = £15.00
Associated Costs
15 kg dead weight = 20kg live weight
0.75 Kill Out %
2.5 FCR * 20 kg = 50 kg Food
50kg food (@ £120 Tonne) = £6
20kg live weight = 20 days (@1kg/day)
20 days (@10p labour etc. costs) = £2
Total Extra Costs = £6+£2 = £8
Figure 1 – Return from Heavier Pigs

Whilst there have been advances in the genetic potential for growth rate (conservative estimates of between 5-15g/year), the benefits of this selection have only been realised by a proportion of producers; the remaining producers' genetic potential being compromised by the health status within their herds. This emphasises the importance of maintaining a high health status to ensure the genetic potential of breeding stock is achieved.

Measurement of feed intake under commercial conditions is an important factor in a selection programme. With FCR having negative genetic correlations with growth and positive genetic correlations with back fat, selecting for lower FCR improves growth rate and increases leanness. Whilst, it is more efficient to select for lean growth with restricted feed intake (Cameron & Curran 1995), computerised *ad-libitum* feed recording systems (e.g. Hunday FIRE systems) allow feed intake and hence FCR to be measured on individual pigs whilst still enabling pigs to be penned in commercially applicable group systems.

Breeding for prolificacy within dam lines relies heavily on the use of BLUP estimated

breeding values (EBVs) together with high quality reproductive data from nucleus breeding farms and, as is the case at ACMC, further down the pyramid using data from purebreds at multiplication. Small but cumulative gains can be made in litter size by these methods although breed substitution can enable step gains to be made. Prolific Meishan sows can increase litter size of the commercial parent female by as much as 1 pig per litter when used within the dam lines at a level which has no detrimental effect on the slaughter pig characteristics (ACMC's own Meidam line). Additional advantages of the use of Meishan genes are additional teats and improved ease of re-breeding. This reproductive improvement is the equivalent of between 10 and 20 years of within line selection and is worth around £100 per sow by improving breeding efficiency (**Error! Reference source not found.**).

Margin over feed added value:
Slaughter weight 100 kilos
Feed: 6 to 100 kg – FCR 2.5 = Used 235 kilos
Feed cost per tonne average £125.00
Feed cost per pig sold = £29.38
Killing out 74%: Slaughter return £74.00
AESA £1.00 per kilo dead weight
Margin £44.62 per pig say **£42.00** per Pig
2.38 litters / sow / year with 1 Extra pig sold / litter
Increased Margin / Sow / year = **£100.00**
Figure 3 – Increase in Margin per sow from 1 pig extra

However, the objective is not just to gain efficiency on farm. Processor efficiency must also be improved and this equates to improving distribution and quality of the lean meat produced.

Unfortunately, there is not always synergy between the two objectives. For example, selecting against HAL-1843 using DNA marker technology will reduce the incidence of pale, soft, exudative (PSE) meat and reduce drip loss (1.2%), but will also reduce lean by 4% and longissimus dorsi muscle by 12cm^2 (Sellier 1998).

Intra-muscular fat (IMF), which has genetic correlation estimates with tenderness of up to 0.53 (Sellier 1998), is also reduced by selection for leanness. However, the low correlation of IMF with carcass fatness could potentially allow selection for lower carcass fat whilst maintaining or increasing IMF.

The RN gene associated with the Hampshire breed is responsible for an 8.1% reduction in Napole yield (Sellier 1998) yet is associated with tenderness in fresh pork. This makes RN a favourable gene to select for if the market requires fresh pork, yet an unfavourable gene to select for if the product is destined for further processing.

Ultimate pH is genetically correlated:
-0.70 with drip loss
+0.45 with water holding capacity
-0.70 with cooking loss
-0.53 with reflectance
+0.49 with tenderness
+0.59 with overall acceptability
Figure 4 – Genetic Correlations between Ultimate pH and Meat Quality Trait

With the preceding examples, it is obvious that breeding programme must be in tune with the processors requirements to achieve delivery of the correct raw material.
Thankfully, there are traits which are correlated with both objectives, that is to say farm and processor efficiency. There is a negative correlation between tenderness and age at slaughter so increased growth rate increases tenderness of the meat (MLC Phase Feeding Trial), and ultimate pH is favourably correlated with many measures of meat quality. Ultimate pH ranges from 6.4 to 5.2 with 6.1 to 5.7 being the most desirable. Scores above 6.1 indicate DFD meat and scores below 5.5 indicate PSE meat. The aim would therefore be to remove the extremes of the population. The cost advantages of quality genetics can be substantial. With downgrading of quality product to commodity product due to PSE being worth 20p/kg (~£5.00/pig) and the reduction of 4% in yield due to drip loss being ~£3.44 on 86kg carcass (at £1.00p/kg). This equates to £8.44 per carcass suffering PSE meat.

The carcass yield and distribution of yield are also important traits for processors. With recent advances in grading technology such as the AutoFOM, it is now possible to identify the location of the lean in the carcass. This can allow accurate identification of animals which show the lean distribution profile in the carcass which best matches the processors needs.

By capturing the real value of the product for producer and processor alike, the pork chain as a whole can become more efficient. A well focussed breeding programme will ensure the supply chain is aligned and moving in the same direction towards the common goal of profitability for all.

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Identification of genes differentially expressed in McCoy cell monolayers infected with *Lawsonia intracellularis*

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Introduction

Ileitis is one of the most important infections in pig industries caused by Lawsonia intracellularis, an obligate intracellular bacterium. The disease is characterized by proliferation of intestinal mucosa enterocytes leading to gross thickening of the ileal wall. The pathogenesis in pigs is very similar to that in mouse. The Lawsonia intracellularis is cultured in mouse-originated McCoy cells. The purpose of this study is to investigate pathogenomics of enterocytes when McCoy cell monolayers are infected with Lawsonia intracellularis.

Materials and Methods

Cell and Bacteria

The murine fibroblast-like McCoy cell(ATCC CRL 1696) were grown in DMEM with 1% L-glutamine, amphotericine B(2.5 ㎍/ml) and 5% FBS without antibiotics, at 37℃ in 5% CO_2. The cells were trypsinized at weekly intervals and seeded at 5×10^5/ml to fresh containers.

Lawsonia Intracellularis strain PHE/KK421 was isolated from intestinal tissue of diarrheal pigs. *Lawsonia intracelluraris* were inoculated on one day-old cultures of the McCoy cell at 37℃ in 10% H_2-CO_2/N_2. After five days incubation, L. intracelluraris infection in McCoy cell was confirmed by indirect FA and PCR.

DEG-PCR (Polymerase Chain Reaction for Differential Expressed Genes)

Total RNAs were extracted 5days pi and reverse transcription was performed for 1.5 h at 42ºC in a final reaction volume of 20 ㎕ containing 3 ㎍ of the purified total RNA, 4 ㎕ of 5 × reaction buffer, 5 ㎕ of dNTPs (each 2 mM), 2 ㎕ of 10 μM dT-ACP1 (5'-CGTGAATGCTGCGACTACGATIIIIIT(18)-3'), 0.5 ㎕ of RNasin® RNase Inhibitor (40 U/㎕), and 1 ㎕ of Moloney murine leukemia virus reverse transcriptase (200 U/㎕). First-strand cDNAs were diluted by the addition of 80 ㎕ of ultra-purified water, and stored at -20ºC until use.

For the detection of differentially expressed genes, second-strand cDNA synthesis was conducted at 50ºC during one cycle of first-stage PCR in a final reaction volume of 20 ㎕ containing 3-5 ㎕ (about 50 ng) of diluted first-strand cDNA, 1 ㎕ of dT-ACP2 (10 μM, Seegene, Korea), 1 ㎕ of 10μM arbitrary ACP(Seegene, Korea), and 10 ㎕ of 2 × Master Mix (Seegene, Korea). The PCR protocol for second-strand synthesis was one cycle at 94ºC for 1 min, followed by 50ºC for 3 min, and 72ºC for 1 min. After second-strand DNA synthesis was completed, the second-stage PCR amplification protocol was 40 cycles of 94ºC for 40 s, followed by 65ºC for 40 s, 72ºC for 40 s, followed by a 5 min final extension at 72ºC. The amplified PCR products were separated in 2% agarose gel and stained with ethidium bromide.

Sequencing and analysis

The differentially expressed bands were extracted and cloned into TOPO TA cloning vector. The genes were sequenced and analyzed by BLASTX of the National Center for Biotechnology Information(NCBI).

Results and Disscussion

Five genes were differentially expressed compared with non-infected cell monolayer. Transcription was up-regulated for one gene and down-regulated for the other four genes (Fig.1.). Blast study in the GenBank revealed that the sequences of all transcripts were above99% homologous to those of tumor-related molecules and highly proliferative tissue(especially mammary gland).

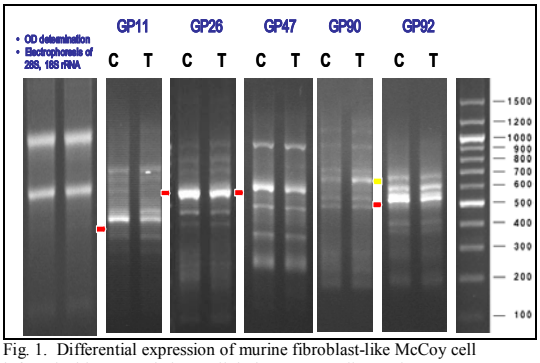

Fig. 1. Differential expression of murine fibroblast-like McCoy cell infected with Lawsonia intracellularis. McCoy cell monolayers were infected (T) and non-infected(C) with Lawsonia intracellularisits. RNAs were extracted 5 days pi and reverse-transcribed into cDNAs. The cDNAs were amplified by ACP primer sets (GP11, GP26, etc.,) and those PCR products were separated by electrophoresis and differential bands were indicated by color arrows.

Table 1. Identification of differentially expressed-genes of McCoy cells

Clone#	Identity	GenBank accession number	Homology	Functional role	Tissue Type
GP11	Mus musculus leucine-rich repeats and calponin homology (CH) domain containing 4	BC027789	99%(411/412)	A structural framework for the formation of protein-protein interactions and Cytoskeleton	Mammary tumor. C3(1)-Tag model. Infiltrating ductal carcinoma.
GP47	Mus musculus RIKEN cDNA 2810428I15 gene	BC028495	99%(492/496)	RIKEN cDNA 2810428I15	Mammary gland (lactating mouse)
	Mouse EST gene	BF020321 BI903560 BU898656 AA615479 AV061744 AW740742	99%(493/496) 99%(423/425) 99%(394/395) 99%(400/404) 96%(403/418) 99%(359/360)	Tumor-related gene	Lactating mammary gland Pooled lung turmors Type B spermatogonia Barstead mouse myotube MPLRB5 Small intestine Pooled mammary gland tumors
GP92	CaiC; Region: Acyl-CoA synthetases AMP-acid ligases II	BC021611	100%(325/325)	AMP-forming Lipid metabolism Secondary metabolites biosynthesis, transport, and catabolism]	Liver (Normal 5 month old male mouse)
	Mus musculus cDNA sequence	BC022709	100%(324/324)	CDNA sequence BC021611	Mammarytumor. Metallothionien-TGFalpha model.10monthold virgin mous
	Mouse EST gene	BE630489	99%(324/325)	Soares_mammary_gland_NMLMG Mus musculus cDNA clone IMAGE	Lactating mammary gland
GP 90	Mus musculus signal transducer and activator of transcription 5B	BC024319	99%(407/411)	Latent cytoplasmic transcription factors	Kidney, normal . 5 month old mail mouse
GP26	Mus musculus cysteine rich protein 61	BC066019	99%(378/379)	cell-cell and cell-matrix interactions	Brau\in, mouse 12.5dpc

These data suggest that Lawsonia infection may activate to turn on/off genes of turmor-related molecules as well as on highly proliferative tissue. *Lawsonia intracellularis* in pigs causes marked adenomatous proliferation of immature, infected enterocytes in the intestinal crypts (Rowland and Lawson. 1974). These informations indicate that pathogenesis of *Lawsonia intracellularis* may be related with tumorgenesis. To further investigate whether transcription of gene is affected by incubation time after infection, the expressions of the genes will be analysed by real-time PCR at day 1, 2, 3, 4, and 5 post infection. In conclusion these results may provide an insight into pathogenomics of *Lawsonia intracellularis* infection.

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