Elimination of porcine reproductive and respiratory syndrome virus from a seedstock breeding farm and a supplying boar stud by a modified test and removal method

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To eliminate porcine reproductive and respiratory syndrome virus (PRRSV) from a supplying boar stud, samples of serum and semen from 118 boars were assessed three times a month by an indirect fluorescent antibody (IFA) test to detect antibodies, and by a nested reverse transcriptase-PCR (nRT-PCR) to detect the genome of PRRSV. The boars detected as persistently infected carriers were culled. A PRRSV-negative population of boars was established after three months and no semen positive for the virus was detected for six months. Subsequently, a three-step plan was introduced to eliminate PRRSV from the seedstock breeding farm during three parity cycles on the farm over 15 months, each step taking five months. In step 1, umbilical cords taken from piglets at birth and serum samples taken from their dams at the start of weaning were subjected to IFA and nRT-PCR analysis. The sows with PRRSV detected in serum by nRT-PCR were regarded as carrier sows and culled. The rates of detection of PRRSV were reduced from 5 per cent to 2·5 per cent in the sera of the sows, and from 14·8 per cent to 1·8 per cent in the umbilical cords of the piglets. In step 2, the sows that had farrowed the piglets with PRRSV detected by nRT-PCR in their cords were considered to have transmitted the infection and removed. During step 2, the virus detection rates in umbilical cords by nRT-PCR were reduced, but not completely eliminated. In step 3, 10-week-old nursery pigs with antibodies to PRRSV in their serum by IFA and ELISA were culled. The three steps established the PRRSV-negative state of the multisite farm containing the breeding and nursery farm, and the PRRSV-negative state of both the multisite farm and the supplying boar stud was evaluated by monthly monitoring over at least one parity cycle of the farm for five months.

PORCINE reproductive and respiratory syndrome virus (PRRSV) is a member of the family Arteriviridae (Cavanagh 1997). The virus can induce chronic, persistent infections in individual pigs and pig herds for long periods, and is transmitted both vertically and horizontally (Christianson and others 1993, Christopher-Hennings and others 1995, Wills and others 1997, Bierk and others 2001). Various control programmes have been developed to eliminate the virus from infected farms, but no single programme is satisfactory for controlling it in all types of herds; programmes including partial depopulation (Dee and others 1997), segregated early weaning (Rajic and others 2001), vaccination with nursery depopulation (Dee and others 1998), and test and removal (Dee and Molitor 1998, Dee and others 2000) have been described, and the test and removal technique has been applied successfully to some herds. However, the limitations of this procedure include the requirements for labour on the testing day, the diagnostic costs, and the removal of productive sows from the herd; owing to these difficulties it is not an economically viable option for many producers.

Currently available diagnostic tests to detect PRRSV exploit virus isolation and the reverse transcriptase-PCR (RT-PCR). Virus isolation in cell culture is labour-intensive, less sensitive than RT-PCR, and dependent on the presence of viable virus in the sample (Bautista and others 1993). In contrast, RT-PCR is a highly sensitive, specific and rapid procedure. Serological tests, such as ELISA, have been used to detect the antibodies that develop nine to 11 days after infection, and remain in persistently infected animals well after the virus is eventually cleared (Wills and others 1997). A false result with ELISA has been cross-checked by indirect fluorescent antibody (IFA) test as a second serological test (Yoon and others 1992, Batista and others 2004).

In this study, PRRSV was first eliminated from an artificial insemination (AI) centre producing semen for the subject breeding farm by serial tests of semen and serum for virus detection and serological tests. Subsequently, the elimination of persistently infecting PRRSV from a multisite farm was attempted through a three-step programme based on a test and removal strategy.

MATERIALS AND METHODS

Herd history

The AI centre providing semen to the breeding farm contained 118 boars housed in individual stalls; they included Duroc, Landrace, and Yorkshire boars, which ejaculated twice a week.

The seedstock breeding farm contained 620 sows housed in a three-site production system. The farms used a forced ventilation system. In general, 20 to 25 sows and replacement gilts were farrowing each week in the farrowing facility, which consisted of three rooms, each with eight stalls. At the start of weaning, usually 21 to 23 days after farrowing, the pigs were sent to a nursery farm. After four to five weeks in the nursery when they weighed about 20 kg, they were sent to site 3, a growing-finishing farm where they spent eight weeks. The farrowing room was completely cleaned and sanitised before the next group moved in. The herd had been infected with PRRSV, but its performance data (Table 1) show that it had not recently been severely affected by clinical disease, and that there had been no history of severe clinical disease or vaccine use in the herd. Clinically, the production of weak piglets and the preweaning mortality were too low to relate to disease (Table 1). An estimate of the seroprevalence and infection rates of PRRSV in the seedstock breeding herd was obtained from a nested RT-PCR (nRT-PCR) after an IFA test of 113 random serum samples taken from 83 sows, 22 replacement gilts, and eight boars on the farm. The sows in the seedstock breeding herd were 22·1 per cent positive for PRRSV, and 37·2 per cent of the animals had antibodies to the virus; the replacement

Veterinary Record (2008) **162,** 333-337

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TABLE 1: Herd performance of the seedstock breeding farm in 2005

	Average data
Farrowing rate (%)	88·1
Live pigs born per litter	11·4
Litters per sow in herd	2·47
Weaners per litter	9·9
Weaners per sow in herd	21·6
Preweaning mortality (%)	8·4
Postweaning mortality (%)	3·7
Average parity	3·2

TABLE 2: Criteria for the elimination of porcine reproductive and respiratory syndrome virus (PRRSV) from the boars at the artificial insemination centre in relation to the results of three monthly tests

Semen nRT-PCR			Serum IFA			Serum nRT-PCR				
1	2	3	1	2	3	1	2	3	Decision	Pattern	Result (number of boars)
+	+	NT		+ or –				+ or –	Remove	1	0
+ or –	+			+ or –			+ to –		Remove	2	2
	–			Ab titre ↑,	+		+ to –		Retain	3	2
+ or –	–	–		Ab titre ↑,	+		–		Remove	4	1
	– to +			Ab titre ↑			–		Remove	5	1
	+ to –			Ab titre ↓,	–		–		Retain	6	13
	–			Ab titre ↓,	–		–		Retain	7	16
	–			–			–		Retain	8	83

Pattern 1 Boars that were persistently semen nested reverse transcriptase-PCR (nRT-PCR)-positive in the first and second tests (continual semen shedding with or without viraemia and antibody). These animals were removed after the second test
Pattern 2 Boars that were viraemic once, and semen nRT-PCR-positive at the next test. These animals were removed
Pattern 3 Boars that were viraemic once, but always semen nRT-PCR-negative. These animals were retained
Pattern 4 Boars that were semen nRT-PCR-positive once, and continually indirect fluorescent antibody (IFA)-positive or whose antibody titres increased. These animals were removed
Pattern 5 Boars that began to shed PRRSV after the first test, and whose antibody titres increased. These animals were removed
Pattern 6 Results of semen nRT-PCR tests changed from positive to negative, and their antibody titres decreased. These animals were retained
Pattern 7 Boars had been infected, but only traces of antibody remained and were cleared in the third month. These animals were retained
Pattern 8 Boars were not infected. These animals were retained
NT Not tested, Ab Antibody, + Positive, – Negative, ↑ Increased, ↓ Decreased

gilts had a higher positive rate for PRRSV (31·8 per cent) and antibodies (59 per cent).

Sample collection and preparation

Samples of semen and serum were obtained from the AI centre boars for three months. The interval between the samples being taken was on average three weeks (from one to five weeks). Each semen sample (1 ml) was separated into seminal plasma and pellets of cells by centrifugation at 600 *g* for 20 minutes.

The umbilical cords from the weakest one or two piglets of each farrowing sow were collected; about 20 to 25 were taken weekly from the piglets of the sows of each farrowing group. Samples of serum were taken from the dams of the piglets one week before the end of lactation. The umbilical cords were frozen and thawed three times, minced, and the suspensions were clarified by centrifugation at 2400 *g* for 30 minutes and used for the extraction of viral RNA

TRIzol LS (Gibco BRL) was used to extract RNA from 250 µl of the supernatant fractions of the samples of semen, umbilical cords and serum. Initially, equal volumes of the serum, semen or umbilical cord fractions from three animals were pooled to extract viral RNA. When a pooled sample from three animals was positive for PRRSV, the three contributing animals were assessed individually to identify the animal(s) responsible for the positive reading.

Virus detection and serological tests

The nRT-PCR was used to amplify ORF7 and the flanking regions of ORF6, using the following primers: N21, 26 for the RT-PCR (the first round of the nRT-PCR), and N23, 25 and N22, 24 for the second round of the nRT-PCR corresponding to the RT-PCR (Kono and others 1996). The products were visualised by agarose gel electrophoresis, followed by ethidium bromide staining. The apparent size of each RT-PCR and nRT-PCR product was compared with DNA markers of known sizes (Bioneer). Samples with RT-PCR and nRT-PCR products with 651 and 349 base pairs were classified as PRRSV-positive.

In nRT-PCR-positive samples, the supernatants of minced umbilical cords were used to isolate PRRSV by using MARC-145 cells (Kim and others 1993). The nRT-PCR was used to identify the presence of the virus before the cytopathic effects were visible. Only the PRRSV isolates identified by cytopathic effects or nRT-PCR were continually isolated for five passages.

The serum samples were also assessed by the IFA method (Yoon and others 1992). The antibody titres were assessed in 96-well plates containing MARC-145 cells (Kim and others 1993) by using a four-fold dilution series (16 to 256). Positive signals from the 16-fold diluted samples were considered positive. Positive serum samples established by the IFA were subjected to a commercial ELISA (IDEXX).

Elimination of PRRSV from the AI centre and seedstock breeding farm

Monthly tests were performed on samples of serum and semen from boars at the AI centre for three months, using IFA to detect PRRSV antibodies and nRT-PCR to detect PRRSV nucleic acid, to identify virus carriers (Yoon and others 1992, Kono and others 1996). The decision to remove or retain boars was based on eliminating PRRSV from the AI centre through the results of three sets of tests on serum and semen over a period of three months (Table 2).

During the elimination programme, the seedstock breeding herd was closed to the introduction of any replacement animals. The elimination programme consisted of three steps. In step 1 carrier sows with active PRRSV that were able to transmit the virus to other sows by horizontal transmission were removed. Sows were removed or retained on the basis of the results of serum nRT-PCR; if they were serum nRT-PCR-positive they were removed (Table 3). After all these carrier sows had been removed, the objective of step 2 was to eliminate any opportunity for transplacental infection. The sows whose piglets had umbilical cords which were nRT-PCR-positive were removed. Two of them were examined post-mortem. Selected tissues, including tonsil, lung, heart, liver, spleen, kidney, iliac lymph node, sternal lymph node and mandibular lymph node, were analysed by nRT-PCR, RT-PCR and virus isolation for the presence of PRRSV. The aim of step 3 was to eradicate the virus from all the sows and nursery pigs by the serological monitoring of 10-week-old animals. Ten-week-old nursery pigs from the breeding farm that were IFA or ELISA-positive were removed.

RESULTS

Elimination of PRRSV from the AI centre

Four of the 118 boars were determined to be carriers of PRRSV and removed for three months on the basis of the criteria for the elimination of PRRSV at the AI centre (Tables 2, 4). Two of the four removed boars that were serum nRT-PCR-positive and semen nRT-PCR-positive at the next test had pattern 2, one that was semen nRT-PCR-positive at the first test and continually serum antibody-positive had pattern 4, and the other boar that was semen nRT-PCR-positive after being negative, newly shedding PRRSV, had pattern 5 (Table 2). The negative

TABLE 3: Criteria for the removal of sows from the breeding farm and six patterns observed during step 1

Umbilical cord	Serum			
nRT-PCR	IFA	nRT-PCR	Decision	Pattern
+	+	+	Remove	1
+	+	–	Retain	2
+	–	+	Remove	3
–	+	+	Remove	4
–	+	–	Retain	5
–	–	–	Retain	6

Pattern 1 and 3 Sows were viraemic or persistently infected. These animals were removed
Pattern 2 Sows were infected during 80 to 90 days of gestation, and the virus was cleared by the immune system. These animals were retained
Pattern 4 Sows were infected due to stress of breeding and farrowing, and were viraemic. These animals were removed
Pattern 5 Sows were infected by porcine reproductive and respiratory syndrome virus but the infection was not a significant threat. These animals were retained
Patterns 2 and 5 were monitored carefully until the antibody titres had decreased and cleared
Pattern 6 Sows were not infected. These animals were retained
nRT-PCR Nested reverse transcriptase-PCR, IFA Indirect fluorescent antibody test, + Positive, – Negative

herd of boars was established from the first three months up to the present time.

Elimination of PRRSV from the seedstock breeding farm

Step 1: Removal of carrier sows by monitoring umbilical cords and sera On average, 136 umbilical cords and 143 sera were collected from the sows every month for five months. A total of 14 sows with nRT-PCR-positive serum among 178 animals with IFA-positive samples (most of them with titres less than 64) were removed from the farm. The results of the IFA corresponded in 98·8 per cent of cases with the results of the ELISA; there were some IFA-positive samples that were ELISA-negative, but no IFA-negative samples were ELISA-positive. After the completion of step 1, the positive rates of PRRSV and antibody from the umbilical cords and sera were slightly reduced (Table 5).

Step 2: Removal of transplacental infection by monitoring umbilical cords A total of 25 sows whose piglets had virus-positive umbilical cords by nRT-PCR and their littermates were removed from the farm during five months of step 2. In the third month of step 2, the detection rate of virus-positive umbilical cords was significantly higher than at the end rate of step 1, and was maintained for several months. The viruses were detected only by nRT-PCR, and not by RT-PCR or virus isolation (Table 6). The tissue samples of the two sows examined postmortem by nRT-PCR, RT-PCR and virus isolation were negative for the virus.

TABLE 5: Results from step 1 of the elimination programme at the pig breeding farm, this involved the removal of carrier sows by monitoring umbilical cords and sera

	Umbilical cords		Sera
	nRT-PCR	IFA	nRT-PCR
Step 1	Number +ve (%)	Number +ve (%)	Number +ve (%)
1st month	20/135 (14·8)	49/121 (40·5)	6/121 (5·0)
2nd month	5/124 (4·0)	38/134 (28·4)	3/134 (2·2)
3rd month	1/154 (0·6)	58/185 (31·4)	2/185 (1·1)
4th month	3/101 (3·0)	24/156 (15·4)	0/156
5th month	3/166 (1·8)	9/119 (6·7)	3/119 (2·5)

nRT-PCR Nested reverse transcriptase-PCR, IFA Indirect fluorescent antibody test

TABLE 4: Results of serial tests of serum and semen at the artificial insemination centre

	Serum		Semen
Duration	IFA (%)	nRT-PCR (%)	nRT-PCR (%)
1st month	16/108 (14·8)	4/108 (3·7)	15/79 (19)
2nd month	22/109 (20·2)	0	2/90 (2)
3rd month	5/17 (29·4)	0	0/13
After the programme	NT	NT	0/159

NT Not tested, IFA Indirect fluorescent antibody test, nRT-PCR Nested reverse transcriptase-PCR

Step 3: Elimination of PRRSV from all sows and nursery pig herds A pilot test of step 3 applied in the last month of step 2 revealed that 20 sera taken from 10-week-old nursery pigs were ELISA, IFA and nRT-PCR-negative for the virus. Consecutive tests showed that the sera from the 10-week-old nursery pigs were negative for both PRRSV and PRRSV antibody for at least up to eight weeks after the collection of the first sample.

DISCUSSION

For the effective control and possible eradication of PRRSV it was necessary to target the mechanisms underlying the persistence and dissemination of the virus among the adult pigs, and those controlling transplacental infection. Accurate diagnosis was also essential because the eradication process depended on the testing of samples of semen, serum, and umbilical cords by an antibody test and a nucleic acid test, and removing animals on the basis of the results of the tests. The sera of animals that tested positive by IFA were re-examined by ELISA to compare with the IFA results, and the nRT-PCR was used to detect the nucleic acid of PRRSV. The samples for the nRT-PCR were initially pooled and, when positive, individual animals were tested. Pooling samples reduces the cost, but always has a diluting effect, so that the results may underestimate the real prevalence of PRRSV infection. In this study, two or three samples of equal volume from each suspension were pooled to reduce the dilution effect to the minimum.

In this elimination programme boars from the AI centre providing semen to the breeding farm were the first target for the eradication of the virus, because the presence of PRRSV in the semen carries the possibility of venereal transmission (Gradil and others 1996). The method was based on the specific characteristics of PRRSV in semen and the boars' serostatus. Using a nRT-PCR, PRRSV was detected in the serum of adult boars for a short period and in the semen of experimentally infected boars for up to 92 days after infection (Christopher-Hennings and others 1995). In the initial phases of an infection, the serological results are negative, even though virus is being shed in the semen, and boars are likely to remain serologically-positive long after PRRSV is no longer being shed. Moreover, the virus is shed in semen intermittently, particularly in the chronic phase of the infection

TABLE 6: Results from step 2 of the elimination programme, this involved the removal of transplacental infection by monitoring umbilical cords

		Umbilical cords	
Step 2	RT-PCR	nRT-PCR (%)	Virus isolation
1st month	0/154	2/154 (1·3)	0/2
2nd month	0/114	8/114 (7·0)	0/8
3rd month	0/147	8/147 (5·4)	0/8
4th month	0/103	7/103 (6·8)	0/7

RT-PCR Reverse transcriptase-PCR, nRT-PCR Nested RT-PCR

(Prieto and Castro 2005). Therefore, the decision whether boars should be excluded or retained was based on the results of at least two nRT-PCR and IFA tests on semen and sera over a period of three months. Because the boars were confined in individual stalls, the risk of horizontal transmission would have been fairly low during the elimination programme, and it was therefore possible to use the observation whether the immune system of a boar would clear the virus or not to decide whether it should be removed.

Boars whose serum or semen was continually positive by IFA or nRT-PCR were classified as problematic carriers, because their immune system did not actively clear the virus. Boars which were viraemic would either have been acutely infected with insufficient time to generate antibodies for detection by IFA, or would have been carriers. In this case, the results obtained over a further period of three months were important, because some animals were no longer viraemic. Boars that were IFA-positive in serum and nRT-PCR-negative in semen had possibly been exposed to PRRSV, but were not actively viraemic; the virus could not have been transmitted by the day of sampling or the boar would have been a carrier; in this way the continual existence of antibody was related to virus infection. Monthly serial tests were also significant in this case, and only boars in the latter category were removed. Finally, boars negative for the virus and antibody were classified as uninfected, and retained.

In sows, PRRSV can be transmitted to naive animals which have been in contact for several months and the virus can be transmitted transplacentally during early or late pregnancy (Christianson and others 1993, Prieto and others 1997, Bierk and others 2001).

In the breeding farm, according to the estimate of the prevalence of PRRSV and antibodies before the project, the level of infection of the replacement gilts in pens was significantly higher than that of the sows in stalls. The plans for the elimination of PRRSV therefore targeted the sows that were confined in stalls.

The elimination programme for the seedstock breeding farm involved three steps. The objective of the first step was the removal of PRRSV carrier sows, which are persistently infected and consequently can transmit the virus. The viraemic sows were removed on the basis of the results of nRT-PCR tests of samples of serum and umbilical cords, and the results of IFA tests of serum were used only as supplementary data. During step 1, there were only minor changes in the rate of detection of PRRSV in the serum samples, but the rate of detection in the umbilical cords and the titre of antibodies in the sera were generally reduced, except in the third month. The lower sensitivity induced by pooling serum samples from three animals, or the reduction in the sensitivity of the nRT-PCR resulting from changes in the PCR materials, may have contributed to this discrepancy. Thirty of the 32 piglets with umbilical cords positive for the virus by nRT-PCR had negative sera. A comparison of the virus detection rates from the umbilical cords and serum samples in step 1 indicate that the cords were better samples for detecting carriers.

The aim of step 2 was to abolish any opportunity for transplacental infection. Sows whose piglets' umbilical cords were nRT-PCR-positive and were able to transmit the virus vertically were removed, together with their progeny, but sows whose piglets' cords were PRRSV-negative were classified as uninfected, and retained in the herd. The detection rates during the first month of step 2 were much higher than at the end rate of step 1, and were maintained for a few months. It is possible that the programme of removal by step 2 was more stringent than that of step 1. For example, PRRSV was detected in the sera of eight sows, which were removed, and in the umbilical cords of the piglets of 20 sows that were retained during the first month of step 1. Thus theoretically, 12 to 20 sows from this group may have had the virus in their umbilical cords. Another possible reason for the results would be the high sensitivity of the nRT-PCR, a positive result by nRT-PCR not necessarily indicating the presence of viable virus. No conclusions can be drawn from the negative results obtained from the two sows examined postmortem, owing to the small sample size.

In step 3, after the eradication of PRRSV from all the sows and their progeny, the aim was to eliminate virus infection in the nursery pigs by the serological monitoring of 10-week-old animals by ELISA and IFA. All the 10-week-old nursery pigs were negative for antibodies to PRRSV. The elimination of the virus from the breeding farm was therefore confirmed by the continual tests of step 3. However, to be certain that PRRSV had been eliminated from the breeding farm through steps 1 and 2, monthly tests using the protocol of step 3 during at least one parity cycle over five months were necessary.

In the light of the lack of clinical signs and gradual reduction in positive rates during the project, it appears that the PRRSV strains at the AI centre and breeding farm were not active and virulent. Further studies are required to establish the characteristics of these and other genetically diverse strains of PRRSV.

In conclusion, a PRRSV-negative boar herd has been established on the basis of serial tests of samples of semen and serum, and the herd has been continually monitored. Step 3 had yet to be completed, but the results obtained from steps 1 and 2 suggest that the programme had effectively eliminated PRRSV from a seedstock breeding farm. However, even if the programme proves to have been effective for detecting and eliminating PRRSV infections from the breeding farm in the long term, all three steps need to be completed, and the programme needs to be applied to other farms, before it is established as a successful method for eliminating PRRSV.

ACKNOWLEDGEMENTS

This work was supported by the Research Project on the Production of Bio-organs, Ministry of Agriculture and Forestry, Republic of Korea.

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