

Serosurvey for viruses associated with reproductive failure in newly introduced gilts and in multiparous sows in Belgian sow herds

Serologische vensters voor virussen die reproductiestoornissen kunnen veroorzaken bij recentelijk geïntroduceerde gelten en bij multipare zeugen in Belgische zeugenbedrijven

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ABSTRACT

A serosurvey for viruses associated with reproductive disorders was conducted in 25 conventional Belgian farms. Serum antibody titers for porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), porcine enteroviruses (PEV) and swine influenza viruses (SIV) were determined in gilts and sows. All the animals were seropositive for PCV2 and >95% were seropositive for all 4 embryopathogenic PEV serotypes. Consequently, special preventive measures appear to be unnecessary for these viruses. In 1 farm, non-vaccinated gilts were found to run a risk of developing PPV-induced reproductive disorders. Vaccination against PPV could exclude this risk. In 10 farms, gilts seronegative for one or more specific SIV subtypes were introduced into a herd that had previously been infected with the same subtypes. Vaccination of gilts against SIV may prevent reproductive disorders in gilts and respiratory problems in their offspring. In 1 farm, newly purchased gilts that were possibly shedding PRRSV were introduced into a PRRSV seronegative sow herd. Serological screening prior to purchase or vaccination of the sows could have resolved this dangerous situation.

SAMENVATTING

In 25 conventionele Belgische zeugenbedrijven werden bij gelten en zeugen antistoftiters bepaald tegen virussen die geassocieerd worden met reproductiestoornissen, meer bepaald het porcien reproductief en respiratoir syndroom virus (PRRSV), porcien circovirus type 2 (PCV2), porcien parvovirus (PPV), porcien enterovirussen (PEV) en de varkensinfluenzavirussen (SIV). Alle dieren waren seropositief voor PCV2 en >95% was seropositief voor de 4 embryopathogene PEV-serotypen. Het lijkt bijgevolg onnodig om specifieke preventieve maatregelen te nemen tegen deze virussen. In 1 bedrijf riskeerden niet-gevaccineerde gelten reproductiestoornissen door PPV te ontwikkelen. Vaccinatie tegen PPV zou dit risico uitsluiten. In 10 bedrijven werden gelten die seronegatief waren voor 1 of meerdere SIV-subtypen, geïntroduceerd in een zeugenpopulatie die voordien geïnfecteerd was met de overeenkomstige subtypen. De vaccinatie van gelten tegen SIV zou reproductiestoornissen bij gelten en ademhalingsstoornissen bij hun nakomelingen vermijden. In 1 bedrijf werden aangekochte gelten die mogelijk PRRSV verspreidden, geïntroduceerd in een PRRSV-negatieve zeugenpopulatie. Een serologische screening vóór de aankoop of vaccinatie zou deze gevaarlijke situatie kunnen oplossen.

INTRODUCTION

Different viruses are able to cause reproductive failure in gilts and sows. Viruses such as Aujeszky's disease virus (ADV), classical swine fever virus (CSFV), porcine reproductive and respiratory syn-

drome virus (PRRSV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV) and porcine enteroviruses (PEV) of the serotypes 1, 3, 6 and 8 (Dunne *et al.*, 1971) can infect embryos, fetuses and/or placentas via transmission through semen or via the blood of viremic gilts and sows (Pensaert *et al.*, 2004; Guérin and

Pozzi, 2005; Maes *et al.*, 2008). Subsequent replication of these viruses in embryos, fetuses and/or placentas will cause a return to estrus, abortion, mummification, premature birth, late birth, stillbirth or weak-born piglets. In Belgium, ADV and CSFV have been eradicated, but enzootic PRRSV, PCV2, PPV and PEV are highly prevalent in the pig population due to virus persistence on farms. Swine influenza viruses (SIV) do not replicate in embryos, fetuses or placentas, but epidemic SIV infections are frequently associated with high fever, which can also lead to reproductive failure. The SIV subtypes H1N1, H3N2 and H1N2 are highly prevalent in Belgian pig farms due to frequent circulation of these viruses in Belgian pig farms (Labarque *et al.*, 2004b).

In Belgium, young breeding gilts are usually infected after weaning with the viruses that are circulating on a farm. This is the case for PRRSV, PCV2 and PEV (De Meurichy *et al.*, 1976; Labarque *et al.*, 2000; Mateusen *et al.*, 2002; Meerts *et al.*, 2004). The immune response that eliminates this primary infection will protect these gilts against reproductive disorders caused by re-infection during pregnancy. However, their immune system will not always have been primed before these gilts are introduced into the sow herd. Especially when purchased specified pathogen free (SPF) replacement gilts are not properly vaccinated or microbiologically adapted before introduction into a conventional sow herd and before first insemination, these gilts may suffer from viral primary infections during first gestation, causing severe reproductive problems. The same problems may arise when home-bred replacement gilts are raised in isolation, putatively preventing their infection with the viruses that are circulating among the sows on the farm. PPV and SIV may be exceptions to these general rules. In Belgium, gilts are usually vaccinated twice against PPV and other infectious agents such as *Erysipelothrix rhusiopathiae* at the ages of 6.5 and 7.5 months, and then inseminated at the age of 8 months. Just before insemination, the gilts are introduced into the sow herd. Maternally derived antibodies (Ab) generally protect gilts against PPV infection until 5 months of age (Paul *et al.*, 1982). If a farmer does not vaccinate the gilts against PPV, gilts have to be naturally infected with PPV in the 3-month period between the loss of maternally derived Ab and insemination. If not, natural PPV infection will occur during their first gestation and will cause reproductive losses in these gilts. The 3 SIV subtypes are highly prevalent on Belgian farms. Moreover, a previous study on Belgian farms found the seroprevalence of these 3 subtypes to be higher in sows than in gilts (Labarque *et al.*, 2004b). Taking into account that there is no good cross-protection between the different subtypes (Van Reeth *et al.*, 2006), the non-vaccinated gilts may not have been adequately primed before their introduction into the sow herd.

The introduction of young gilts into a herd of pregnant sows may not only cause problems for the gilts, but may also severely affect gestation in the sow po-

pulation. This is the case when the gilts introduced are sub-clinical carriers of pathogens for which the sow population is seronegative. This risk may be especially great with PRRSV, because young gilts may be sub-clinical PRRSV carriers for several months after infection (Allende *et al.*, 2000; Wills *et al.*, 2003) and viral shedding can be reactivated by transport stress and immunosuppression (Albina *et al.*, 1994).

Thus the introduction of gilts into sow herds, without knowing the serological status of the gilts and sows, may cause reproductive problems in the gilts or sows due to the transmission of wild-type endemic and/or epidemic viruses between the gilts and the sows. In order to address this issue, serological profiles for PRRSV, PCV2, PPV, PEV and SIV were determined in the gilts and in the sows in 25 conventional Belgian sow herds.

MATERIALS AND METHODS

Study design

Blood was collected by field veterinarians in 25 Belgian farms representative of conventional Belgian sow farms in terms of herd size, housing, management and husbandry practices, sanitary status (A3 or A4), hygienic measures (quarantine for purchased animals) and vaccination schemes, and without a recent history of reproductive problems. Five farms were located in the province of West Flanders, 3 in East Flanders, 6 in Antwerp, 6 in Flemish Brabant, 3 in Liège, 1 in Namur and 1 in Luxembourg. Farm size ranged between 124 and 540 sows. In 20 farms, pregnant sows were kept in individual pens; in 5 farms, they were kept in groups. Fourteen farms used a traditional one-week management system, 9 farms a 3-week system, 1 farm a 4-week system and 1 farm a 5-week system. Thirteen farms had home-bred replacement gilts, whereas 12 farms purchased replacement gilts. Blood samples (n=10) from home-bred replacement gilts were taken at 5-6 months of age, before vaccination against PPV. Blood was collected from the same gilts just before introduction into the sow herd. Blood samples (n=10) from newly purchased replacement gilts were taken when the gilts arrived in the farm. During the following 4- to 7-week quarantine period, the gilts were vaccinated against PPV. Just before introduction into the sow herd, new blood samples were collected from the same gilts. Blood samples (n=10) from sows with at least 3 parities, representative of the sow population of the farm, were collected on all farms.

Assay procedures

PRRSV- and PCV2-specific Ab in serum were determined by an immunoperoxidase monolayer assay (IPMA), as described previously (Wensvoort *et al.*, 1991; Labarque *et al.*, 2000). Lelystad virus (Wensvoort *et al.*, 1991) was used as antigen for the PRRSV IPMA, and Stoon-1010 (Meehan *et al.*, 1998) for the PCV2 IPMA. PPV-specific Ab and SIV H1N1-,

H3N2- and H1N2-subtype-specific Ab were determined by hemagglutination inhibition (HI) tests, as described previously (Palmer *et al.*, 1975; Joo *et al.*, 1976). The respective antigens were Weybridge 590/63 (PPV) (Wrathall *et al.*, 1984), Sw/Belgium/1/98 (H1N1), Sw/Flanders/1/98 (H3N2) and Sw/Gent/7625/99 (H1N2) (Van Reeth *et al.*, 2003a). Seroneutralization (SN) tests (Dunne *et al.*, 1971) were performed to determine anti-PEV Ab specific for serotypes 1, 3, 6 and 8, the serotypes that are associated with reproductive disorders (Derbyshire, 1999). The antigens were PS34, O2b, PS37 and PS27, respectively (Derbyshire, 1999).

Analysis of results

For every farm, the numbers of positive and negative serum samples were compared between 3 groups: (i) gilts before vaccination, (ii) gilts at introduction into the sow population, and (iii) multiparous sows. Antibody titers were expressed as the reciprocal of the last dilution that resulted in a positive reaction in an IPMA or that inhibited hemagglutination in an HI test or that neutralized the virus in an SN test. The detection limits of the assays, which were equal to the reciprocal of the first dilution that was used in the assay, were $4^{1.7}$ (10) for PRRSV and PCV2, $2^{3.0}$ (8) for PPV, $2^{3.3}$ (10) for SIV and $2^{1.0}$ (2) for PEV.

Statistical analysis

The 95% confidence intervals of the within-herd seroprevalences were calculated as described by Daniel (1990).

RESULTS

Porcine reproductive and respiratory syndrome virus

For PRRSV, complete sets of serum samples were available for 19 farms.

In 10 farms (8 with purchased replacement gilts and 2 with home-bred replacement gilts), the gilts and sows were vaccinated against PRRSV. In all of these 10 farms, the gilts were seropositive and they were introduced into a population of seropositive sows.

In 9 farms (7 with home-bred replacement gilts and 2 with purchased replacement gilts), the gilts and sows were not vaccinated against PRRSV. In 6 of these 9 farms (5 with home-bred replacement gilts and 1 with purchased replacement gilts), the gilts were seropositive and they were introduced into a population of seropositive sows. In 2 of the farms with home-bred gilts, the serum samples from the gilts and the sows were negative. In 1 farm, non-vaccinated seropositive purchased gilts (10 positive serum samples, corresponding to 73-100% within-group seroprevalence) were introduced into a population of sows for which the 10 serum samples were negative (corresponding to 0-27% within-group seroprevalence). The distribution of the number of PRRSV positive serum samples per

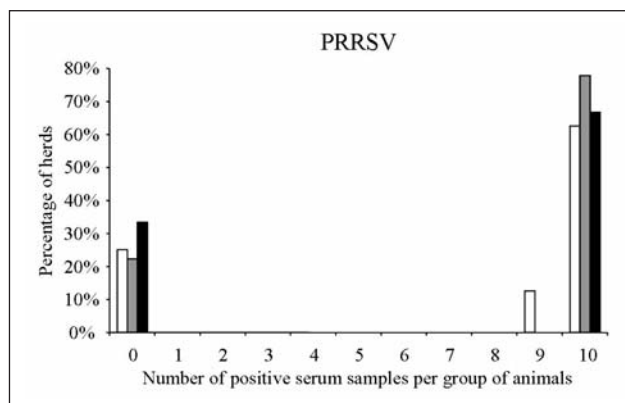


Figure 1. Distribution of the number of positive serum samples per group of animals for porcine reproductive and respiratory syndrome virus (PRRSV) in 9 conventional Belgian sow farms that did not vaccinate against PRRSV. In each group of animals, 10 serum samples were taken. White bars: young gilts (Gilts 1); grey bars: the same gilts at the moment of introduction into the sow population (Gilts 2); black bars: sows with at least 3 parities (Sows).

group of animals in these 9 farms that did not vaccinate against PRRSV is given in Figure 1.

Porcine circovirus type 2

None of the farms vaccinated against PCV2. All serum samples were positive for antibodies against PCV2.

Porcine parvovirus

Data was obtained from 23 farms.

In 20 farms (11 with purchased replacement gilts and 9 with home-bred replacement gilts), the gilts and sows were vaccinated against PPV. In all of these 20 farms, the gilts were seropositive and they were introduced into a population of seropositive sows.

In 3 farms with home-bred gilts, the gilts and sows were not vaccinated against PPV. In these 3 farms, the gilts were seropositive at the time of introduction into the population of seropositive sows. In 1 of these 3 farms, the gilts were seropositive from the first blood sampling on. In a second farm, the gilts were seronegative at the time of the first blood sampling and seropositive at the time of the second blood sampling. In the third farm, the gilts were seropositive at the time of the second blood sampling, but the blood samples from the first blood sampling in these gilts were not available.

Porcine enteroviruses

Data was obtained from 19 farms (10 with purchased replacement gilts and 9 with home-bred replacement gilts). The distribution of the number of PEV positive serum samples per group of animals in these farms is given in Figure 2. The respective percentages of serum samples positive for all 4 PEV serotypes in

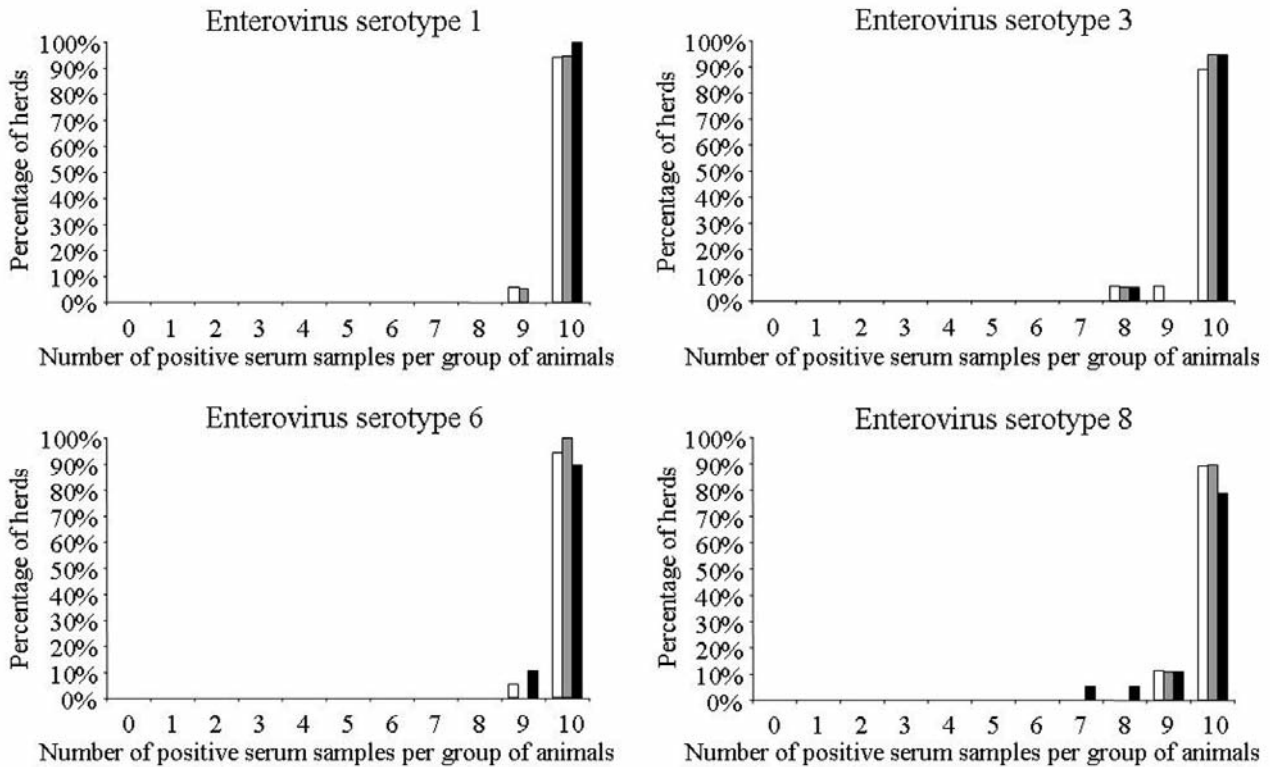


Figure 2. Distribution of the number of positive serum samples per group of animals for porcine enterovirus (PEV) serotypes 1, 3, 6 and 8 in 19 conventional Belgian sow farms. In each group of animals, 10 serum samples were taken. White bars: young gilts (Gilts 1); grey bars: the same gilts at the moment of introduction into the sow population (Gilts 2); black bars: sows with at least 3 parities (Sows).

each of the 3 overall groups were: 96% (all gilts sampled before vaccination), 97% (all gilts sampled at introduction into the sow population) and 95% (all multiparous sows sampled). All the other serum samples were positive for 3 serotypes, except for one single sow that was seropositive for only 2 serotypes.

Swine influenza viruses

For SIV, complete sets of serum samples were available for 19 farms.

In 3 farms with purchased replacement gilts, the gilts and sows were vaccinated against SIV. In 2 of these 3 farms, H1N1- and H3N2-seropositive (10 positive serum samples, corresponding to 73-100% within-group seroprevalence) but H1N2-seronegative gilts (10 negative serum samples, corresponding to 0-27% within-group seroprevalence) were introduced into a population of sows for which the 10 serum samples were positive for H1N1, H3N2 and H1N2 (73-100% within-group seroprevalence). In 1 of these 3 farms, H1N1-, H3N2 and H1N2-seropositive gilts were introduced into a population of sows seropositive for H1N1, H3N2 and H1N2.

In 16 farms (9 with home-bred replacement gilts and 7 with purchased replacement gilts), the gilts and sows were not vaccinated against SIV. On 10 of these farms (7 with home-bred replacement gilts and 3 with purchased replacement gilts), the gilts for which all 10 serum samples were negative for at least 1 subtype

were introduced into a population of sows for which at least 1 serum sample was positive for the corresponding subtypes. More specifically, H1N1-seronegative gilts were introduced into an H1N1-seropositive sow population in 3 farms (2 with home-bred gilts and 1 with purchased gilts), H3N2-seronegative gilts were introduced into an H3N2-seropositive sow population in 6 farms (4 with home-bred gilts and 2 with purchased gilts) and H1N2-seronegative gilts were introduced into an H1N2-seropositive sow population in 7 farms (5 with home-bred gilts and 2 with purchased gilts). In 1 farm with home-bred replacement gilts, H1N2-seropositive gilts (10 positive serum samples, corresponding to 73-100% within-group seroprevalence) were introduced into a sow population for which the 10 serum samples were negative for H1N2 (0-27% within-group seroprevalence). In 5 farms (4 with purchased gilts and 1 with home-bred gilts), the gilts introduced had the same immune status for all 3 subtypes as the multiparous sows. The distribution of the number of SIV positive serum samples per group of animals in the 16 farms that did not vaccinate against SIV and for which complete data was obtained is given in Figure 3.

DISCUSSION

This survey suggests that the vast majority of conventional Belgian gilts and sows are immune to PCV2 and PEV serotypes 1, 3, 6 and 8 due to natural infec-

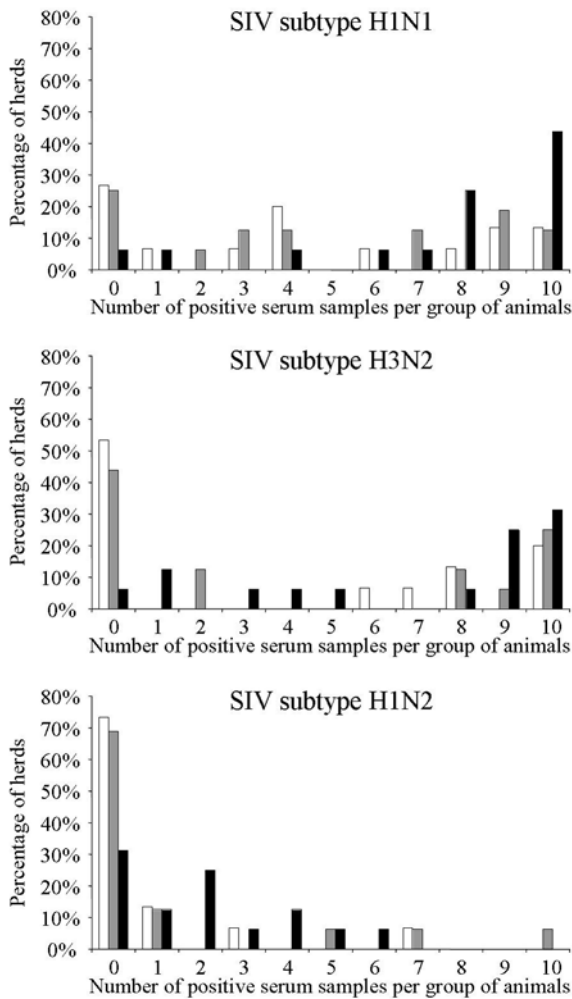


Figure 3. Distribution of the number of positive serum samples per group of animals for swine influenza virus (SIV) subtypes H1N1, H3N2 and H1N2 in 16 conventional Belgian sow farms that did not vaccinate against SIV. In each group of animals, 10 serum samples were taken. White bars: young gilts (Gilts 1); grey bars: the same gilts at the moment of introduction into the sow population (Gilts 2); black bars: sows with at least 3 parities (Sows).

tions. Consequently, we may assume that PCV2 and PEV infections are of little importance in terms of causing reproductive failure in conventional Belgian gilts and sows. Thus it seems unnecessary to vaccinate these animals against PCV2 for the purpose of preventing reproductive failure. Due to the small sample sizes, however, it could not be demonstrated that all Belgian gilts and sows are immune to PCV2 infection. Thus it is not impossible that on some farms small numbers of animals would benefit from PCV2 vaccination before insemination. It also seems unnecessary to immunize conventional gilts against PEV before insemination by bringing them into contact with feces from weaned piglets. However, the results from the present study also indicate that in some farms not all gilts and sows are seropositive for all 4 embryopathogenic PEV serotypes. This may suggest that small

numbers of gilts and sows may be vulnerable to PEV-induced reproductive failure.

This study also shows that not all conventional Belgian pig farmers vaccinate their breeding stock against PPV. In our opinion, this is not a wise decision. In 1 of the farms that did not vaccinate against PPV, the gilts seroconverted between the first and the second blood sampling, which was only a few weeks before insemination. This may suggest that in this farm the gilts may sooner or later be PPV-seronegative upon introduction into the PPV-seropositive sow population. When these pregnant gilts are subsequently infected with PPV, this will presumably cause severe reproductive disorders in them and financial losses for the farmer. Vaccination of gilts and sows is a cost-effective method for controlling PPV-induced reproductive failure in pig herds suffering endemic PPV infection (Parke and Burgess, 1993; Gardner *et al.*, 1996).

This survey also indicates that the majority of the conventional Belgian gilts are seronegative for one or more subtypes of SIV, thus indicating that the vaccination of gilts against SIV may be of potential benefit for farmers. The seroprevalence of SIV in sows seems to be higher than in gilts, and therefore it seems wise to decide to vaccinate sows against SIV based on the results of serological analysis. Vaccination of completely SIV-seronegative animals with a currently registered H1N1- and H3N2-based commercial European SIV vaccine will confer good protection against the H1N1 and H3N2 subtypes, but not against the H1N2 subtype (Van Reeth *et al.*, 2003b; Van Reeth *et al.*, 2004). In gilts and sows seropositive for one or more subtypes, the administration of commercially available SIV vaccines will dramatically increase Ab titers to previously encountered subtypes and will even induce H1N2 cross-reactive Ab in sows that were previously infected with H1N1 and not with H1N2 (Van Reeth *et al.*, 2006). Vaccination of gilts and sows will also improve maternal immunity and the protection of young piglets against disease (Thacker, 2000). Farmers with gilts and/or sows seronegative for one or more SIV subtypes may thus benefit from vaccinating their gilts and/or sows against SIV.

Finally, this survey demonstrates that in some conventional Belgian sow farms wild-type PRRSV may be transmitted from purchased, infection-seropositive gilts to seronegative sows in gestation. Purchasing PRRSV-positive gilts has previously been recognized as a risk factor for the introduction of PRRSV into sow herds (Mortensen *et al.*, 2002; Pesente *et al.*, 2006). Therefore we advise suppliers of gilts and sow farmers to have their gilts and sows regularly screened for PRRSV. On the basis of these screening results, farmers can then either vaccinate non-immune animals or else buy gilts from a different supplier. This procedure will help farmers to prevent PRRSV infection of non-immune animals during gestation and thus avoid the serious reproductive problems caused by PRRSV. Unfortunately, there does not exist a PRRSV vaccine at present that can guarantee full clinical and virological protection against infections with any wild-type

PRRSV, especially when the wild-type virus and the vaccine virus are genetically different (Opriessnig *et al.*, 2002; Labarque *et al.*, 2004a; Cano *et al.*, 2007; Prieto *et al.*, 2007). This implies that the decision to use a certain PRRSV vaccine or to purchase gilts from a different supplier should always be made together with the farm veterinarian and, if necessary, together with a virological expert. In any case, regular serological screening of gilts and sows may be a good tool for reducing the risk of reproductive failure due to PRRSV in conventional Belgian sow herds. For this reason, Animal Health Care Flanders is investigating the feasibility of offering a PRRSV screening program as a standard service for Belgian sow farms, in addition to the already existing service of supplying sow farms with certified PRRSV-free boar semen (Vangroenweghe *et al.*, 2008). Due to high pig densities and short distances to neighboring pig herds in certain areas of Belgium, and due to the lack of marker vaccines and routine diagnostic tests adapted to marker vaccines, a national PRRSV eradication program is not feasible in Belgium at the present time, just as is the case in the neighboring European countries as well (Beilage and Bätza, 2007).

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