1. Introduction

Ruminant pestiviruses (bovine viral diarrhoea virus (BVDV) and Border disease virus (BDV)) are closely related to classical swine fever virus (CSFV) and all belong to the genus of pestiviruses (Van Rijn et al., 1997; Becher et al., 2003). Natural hosts for BVDV and BDV are cattle and small ruminants, respectively, but both are able to infect swine as well (Wrathall et al., 1978; Terpstra and Wensvoort, 1988; Walz et al., 2004; Wieringa-Jelsma et al., 2006). In swine, only CSF is notifiable to the OIE and can cause massive economic damages as was shown in recent outbreaks in Western Europe, in the Netherlands in 1997–1998 (Elbers et al., 1999), the UK in 2000 (Sandvik et al., 2000), and Germany in 2006.

Cross-reactions between pestiviruses occur, both regarding protective immunity and in diagnostic tests. The presence of BVDV and BDV in a swine population may thus affect the transmission of CSFV, but also the diagnosis of a CSFV infection. During 1997–1998 outbreak of CSF in the Netherlands, the
presence of BVDV and BDV in the swine population caused considerable problems in the serological diagnosis of CSF (De Smit et al., 1999).

A new development in the past decade has been the introduction of the first generation of marker vaccines against CSF. These marker vaccines are E2-subunit vaccines, generating only antibodies against E2 after vaccination (Van Rijn et al., 1999). Detection of infected animals in a vaccinated population is based on detection of antibodies against E\textsuperscript{ms}. In 2003 a serological test to detect antibodies against E\textsuperscript{ms} was evaluated by 15 national reference laboratories for CSF in the EU, under the guidance of the community reference laboratory in Hanover, Germany. This test was approved, thus allowing for the first time the use of marker vaccines in Europe (Blome et al., 2006). A setback of this test is, however, that it also detects antibodies against E\textsuperscript{ms} from ruminant pestiviruses. New generations of marker vaccines may still rely on the detection of E\textsuperscript{ms}-antibodies and will therefore face the same problem of cross-reactivity in diagnostic tests (Van Gennip et al., 2000; Koenig et al., 2007).

Within the framework of surveillance and eradication of CSF, including the possible use of the above mentioned marker vaccines, it is important to have information on the prevalence of ruminant pestiviruses in a country or region where it is applied.

In this study, the seroprevalence of ruminant pestiviruses in sows and finishing pigs in the Netherlands was determined. Furthermore, several risk factors, associated with the presence of swine and ruminants on the same farm or in the immediate surroundings, were evaluated.

2. Materials and methods

2.1. Sampling

In order to estimate the seroprevalence against BVDV and BDV in sows and finishing pigs in the Netherlands with reasonable precision, the following preconditions were used: an a priori expected herd prevalence of 2% (finishing pigs) to 5% (sows), a maximum allowable error in the prevalence estimate of approximately 2%, a 95% confidence in the estimate, and a population size of approximately 3000 finishing herds and 6000 sow herds. A random sample size of at least 178 finishing herds and 424 sow herds are required to meet these criteria (Thrusfield et al., 2001). Samples for the prevalence study were collected in the framework of the Dutch swine vesicular disease (SVD) surveillance program. This surveillance program, part of the Animal Disease Farm Inspection Regulation (ADFIR), ran under government supervision between 1993 and 2004 and after that under the supervision of farmers organisations. Every farmer with more than four pigs was obliged to submit between 5 and 12 samples every 4 months, depending on herd size (Hunneman et al., 1996). Blood sampling was carried out by the general practitioner. A total of 6020 serum samples taken from 616 sow herds and 1890 serum samples from 189 finishing herds were selected by a stratified (by region) random process out of this daily pool of samples and used for detection of antibodies against ruminant pestiviruses.

2.2. Serological tests

Blood samples were pre-screened in a pan-pesti NS3-ELISA (Prionics AG). Positive samples in the NS3-ELISA were tested in an E\textsuperscript{ms}-ELISA (Idexx Chekit®-Marker), and virus neutralisation test’s (VNT’s) against one CSFV strain (Brescia), two BVDV strains (NADL, a BVDV-1a strain, and Osloss, a BVDV-1b strain) and two BDV strains (Frijters and Moreduin).

The NS3-ELISA contains two monoclonal antibodies directed against different highly conserved epitopes on the non-structural protein NS3 of pestiviruses. The test has a relative sensitivity and specificity of approximately 98%, respectively 99%, compared to a VNT (Kramps et al., 1999). The test was considered positive if the inhibition percentage was above 50%.

The E\textsuperscript{ms}-ELISA was originally developed as a companion test to E2-subunit vaccines against classical swine fever, but also detects antibodies directed against E\textsuperscript{ms} from BVDV and BDV (Flogel-Niesmann, 2001). The test was considered positive if the inhibition percentage was above 40%.

The VNT was carried out as described by Terpstra et al. (1984). VNT’s were validated by back-titration of the virus stock used in the test and titration of two control sera per virus species of known titre and variation. Results of the test were only valid if all were within range. If there was a response against more than one strain in the VNT, the sample was categorised as having antibodies against the strain with the highest antibody titre. The other titres were regarded as a result of cross-reaction and therefore neglected. For BVDV only BVDV-1-strains of two different subgroups were included in the VNT, as BVDV-2 appears to be highly prevalent only in North America (Fulton et al., 2000; Evermann and Ridpath, 2002) and seems relatively rare in other continents (Wolfmeyer et al., 1997; Sakoda et al., 1999). Within each species and subgroup, the strains should be representative enough to detect antibodies against most or all strains within that subgroup, be it that the height of titres may vary (Wensvoort et al., 1989; Dekker et al., 1995; Patel et al., 2005).

2.3. Risk factors

From the herds in the study population, the following information was obtained:

- herd size of the pig herd;
- number of goats, sheep and cattle housed on the same premises as the pig herd;
- number of goats, sheep and cattle housed within a radius of 3 km from the pig herd;
- number of herds with pigs, goats, sheep or cattle within a radius of 3 km of the pig farm;
- nearest distance to herds with pigs, goats, sheep or cattle in the neighbourhood.

2.4. Statistical analysis

95% confidence intervals for proportions were calculated according to the efficient-score method (corrected for continuity) described by Newcombe (1998).
Statistical Analysis System (SAS/STAT, version 8, 1999) was used for frequency counts and univariate analysis. Differences in the presence of risk factors between pig herds with or without antibodies against BVDV were analyzed with multivariate linear logistic regression (PROC LOGISTIC, SAS 1999). Summary odds ratios (sORs), adjusted for other risk factors in the model, with accessory 95% confidence limits (CIs) were calculated to measure the strength of the association. In terms of disease causation, an OR = 1 indicates no relationship, whereas an OR > 1 or OR < 1 are indicative of increased or reduced risk (protection) risk, respectively (Dohoo et al., 2003).

3. Results

In sows, 309 out of 6020 samples were positive in the NS3-ELISA. From these 309 samples, 126 were positive in the E\textsuperscript{ms}-ELISA and 152 in at least one of the VNT’s, mainly those samples that has inhibition percentages of >80% in the NS3-ELISA (Table 1, Fig. 1). From these 152 positive samples in the VNT, 151 had titres against strain Osloss, 124 against NADL, 60 against Moredun, 67 against Frijters and 32 against Brescia. In 142 of these cases the titres against Osloss were the highest (ranging from 10 to 15,360) and in 7 samples titres against NADL were the highest (ranging from 15 to 1280). All the other titres against NADL (ranging from 10 to 2560) were lower than the titre against Osloss in the same sample (on average almost seven times as low). Except for 2 highest titres against strain Moredun (titres of 15 and 20) and 1 against Frijters (titre of 10), all titres against these strains and against strain Brescia were considered the result of cross-reaction. Titres against Osloss were on average 50, 61 and 130 times higher than against strains Frijters, Moredun and Brescia, respectively. In sows, the seroprevalence against BVDV was estimated at 2.5% on the animal level, and 11% on the herd level (Table 2), mainly against BVDV-1b. It was concluded that none of the samples contained BDV specific antibodies.

Univariate analysis revealed several risk factors to be associated \((P < 0.20)\) with a BVDV-seropositive status of sow herds and finishing herds (Table 3). In the final multivariate logistic regression model two risk factors were significantly \((P < 0.05)\) associated with a BVDV-seropositive status of sow herds (Table 4), but no risk factors were significantly \((P < 0.05)\) associated with a BVDV-seropositive status of finishing herds. Sow herds with cattle present on the premises had a 3.4 higher odds of having a BVDV-seropositive status compared to sow herds without cattle present on the premises. Furthermore, if the number of sheep and/or goats herds in a radius of 3 km of the sow herds was higher than 60, the odds of having a BVDV-seropositive status was 2.1–2.6 times higher than for sow herds with less than 60 sheep and/or goat herds in the neighbourhood.

Table 1

<table>
<thead>
<tr>
<th>NS3-ELISA</th>
<th>E\textsuperscript{ms}-ELISA</th>
<th>VNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Inhibition</td>
<td>Positive</td>
<td>% Positive</td>
</tr>
<tr>
<td>≤50</td>
<td>5711</td>
<td>nd</td>
</tr>
<tr>
<td>&gt;50</td>
<td>309</td>
<td>126</td>
</tr>
<tr>
<td>50–60</td>
<td>57</td>
<td>1</td>
</tr>
<tr>
<td>60–70</td>
<td>69</td>
<td>2</td>
</tr>
<tr>
<td>70–80</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>80–90</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>&gt;90</td>
<td>126</td>
<td>114</td>
</tr>
</tbody>
</table>

nd: = not done.
4. Discussion

The seroprevalence of ruminant pestiviruses in swine is currently very low in the Netherlands, especially in finishing pigs. Because on average only 10 samples per herd were tested, herds with a low intra-herd prevalence may be missed. Thus, the seroprevalence on a herd level may be somewhat higher in reality. Risk factors suggest that direct introduction from mainly cattle, but possibly also small ruminants occurs. Further transmission among pigs and spread through live pig transports between pig farms cannot be ruled out based on our results. However, a recent transmission experiment in pigs (Wieringa-Jelsma et al., 2006), using a recent Dutch BVDV isolate originating from a pig, showed a very limited transmission of BVDV among pigs. The results from that transmission experiment suggested that BVDV would become extinct from the pig population if no repeated introductions from outside the pig population would occur.

The NS3-ELISA seemed to have a somewhat lower specificity in sows than what was previously determined for cattle (Kramps et al., 1999). With a cut-off value of 50% inhibition, we ultimately ended up with 157 false positive results, or a specificity of 97.3% compared to the VNT. Especially in the range of 50–70% inhibition, few NS3-positives could be confirmed by VNT. Neither could they by an E\textsuperscript{m}o-ELISA, that, in itself, was able to detect 126 out of 149 sows seropositive against BVDV in the VNT. Because of this, it is not expected that a relevant number of BVDV-infected sows was missed in the VNT because of the selection of strains that were used.

Prevalence studies of ruminant pestiviruses in swine are relatively rare and difficult to compare because of different sampling schemes, different age categories of tested animals, and different tests being used. In wild boar seroprevalences of 0–8% against ruminant pestiviruses were found in several countries over the last few decades (Dahle et al., 1993a; New et al., 1994; Schmitt, 1999; Zupancic et al., 2002; Roic et al., 2007). In domestic pigs seroprevalences were reported of 2.2% (BVDV strain NADL) in Norway (Loken et al., 1991), 6.4% (BVDV strain Ug59) in Denmark (Holm Jensen, 1985) and 3.2% (BVDV strain NADL), 0.2% (BVDV strain NADL) and 0% (BDV strain 137/4) in Ireland (O’Connor et al., 1991; Graham et al., 2001). Seroprevalences of up to 40% in breeding pigs are however mentioned as well (Terpstra and Wensvoort, 1988; Liess and Moennig, 1990).

BVDV- and BDV-seroprevalences in the Netherlands used to be much higher in the past than the ones found in the current study. In the late 1980s a seroprevalence of 20% (BVDV-strain Oregon) was found in Dutch sows and boars, testing more than 700 samples from the slaughterhouse

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sows BVDV+, n = 68</th>
<th>BVDV−, n = 548</th>
<th>P-value</th>
<th>Finishing pigs BVDV+, n = 6</th>
<th>BVDV−, n = 183</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median number of total pigs on farm</td>
<td>223 (45–1724)</td>
<td>406 (8–8600)</td>
<td>0.0006</td>
<td>275 (80–500)</td>
<td>384 (12–4000)</td>
<td>0.14</td>
</tr>
<tr>
<td>1 bovine on same farm</td>
<td>39 (57%)</td>
<td>145 (26%)</td>
<td>&lt;0.0001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Median # of pig farms in 3 km radius</td>
<td>37 (0–194)</td>
<td>37 (0–168)</td>
<td>0.10</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Median distance to closest cattle farm</td>
<td>191 (38–703)</td>
<td>226 (27–1659)</td>
<td>0.03</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Median # of cattle farms in 3 km radius</td>
<td>87 (0–1371)</td>
<td>73 (0–313)</td>
<td>0.002</td>
<td>115 (67–166)</td>
<td>81 (0–321)</td>
<td>0.11</td>
</tr>
<tr>
<td>Median # of bovines &lt;1 year old in 3 km radius</td>
<td>847 (0–1625)</td>
<td>787 (0–2193)</td>
<td>0.13</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Median # of bovines 1–2 years old in 3 km radius</td>
<td>1827 (0–30554)</td>
<td>1340 (0–24870)</td>
<td>0.02</td>
<td>2069 (1253–2951)</td>
<td>1592 (0–2357)</td>
<td>0.10</td>
</tr>
<tr>
<td>Median # of bovines &gt;2 year old in 3 km radius</td>
<td>2219 (0–3848)</td>
<td>2057 (0–5626)</td>
<td>0.17</td>
<td>3055 (1360–4249)</td>
<td>2374 (0–4661)</td>
<td>0.14</td>
</tr>
<tr>
<td>Median # of small ruminant farms in 3 km radius</td>
<td>60 (0–127)</td>
<td>46 (0–217)</td>
<td>0.001</td>
<td>73 (43–97)</td>
<td>49 (0–212)</td>
<td>0.15</td>
</tr>
<tr>
<td>Median # of goats in 3 km radius</td>
<td>ns</td>
<td>ns</td>
<td>467 (76–1413)</td>
<td>110 (0–3898)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Median # of other goats in 3 km radius</td>
<td>ns</td>
<td>ns</td>
<td>17 (4–26)</td>
<td>7 (0–828)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Median # of ewes in 3 km radius</td>
<td>538 (0–1661)</td>
<td>401 (0–9180)</td>
<td>0.03</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Median # of ewes with lam in 3 km radius</td>
<td>102 (0–549)</td>
<td>76 (0–3377)</td>
<td>0.07</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Median # of other ewes in 3 km radius</td>
<td>39 (0–2009)</td>
<td>31 (0–8078)</td>
<td>0.18</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns: not significant.

### Table 4

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Value</th>
<th>N</th>
<th>sOR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of cattle (1 or more animals) on premises</td>
<td>Yes</td>
<td>184</td>
<td>3.4</td>
<td>2.0–5.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>432</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of goat and/or sheep herds within a radius of 3000 m of sow herd</td>
<td>≥80</td>
<td>79</td>
<td>2.6</td>
<td>1.3–5.1</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>60–80</td>
<td>120</td>
<td>2.1</td>
<td>1.1–3.8</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>&lt;60</td>
<td>417</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Reference category.
Neglected, as shown by an incident in the Netherlands, however, still not a route of introduction to be fully determined, as almost no titres against BDV were found. This means that either BDV is not present in the small ruminant populations either or that transmission of BDV from small ruminants to swine is more difficult than assumed. In former times, farms used to have all kind of production animals on the same premises: cattle, poultry, pigs, small ruminants, et cetera. In the past few decades, however, more and more farms specialized in only one type of production animal. Furthermore, in those cases where more than one species is present on one farm, housing became such that the possibilities for direct contact became less and less. Mixed farms, where different species live in close contact with each other, exchanging infectious agents, have become rare.

The presence of small ruminants in the neighbourhood of swine herds has not been reported before as a possible risk factor for BDV infections in swine. Knowledge of seroprevalences against BDV in sheep is relatively scarce, but infections do occur. Seroprevalences of 6.7% (Bechmann, 1997) and 4.5% (Loken et al., 1991) were reported in Germany and Norway, respectively. As such, they may therefore be a possible source for BDV infections in swine as well and represent a risk factor if many herds are present in the neighbourhood. Whether small ruminants also pose a possible risk factor for BDV in swine, could not be determined, as almost no titres against BDV were found. This means that either BDV is not present in the small ruminant populations either or that transmission of BDV from small ruminants to swine is more difficult than transmission of BDV. For both hypotheses there is currently insufficient evidence.

Another reason for the current low BDV-seroprevalences may be that BDV contaminations of vaccines, which was also considered an important cause for BDV infections in swine (Vannier et al., 1988; Wensvoort and Terpstra, 1988; Liess and Moennig, 1990), is becoming less of an issue due to increased quality control systems. This is, however, still not a route of introduction to be fully neglected, as shown by an incident in the Netherlands several years ago with a bovine herpes virus vaccine in cattle (Barkema et al., 2001).

The presence of BDV and BDV antibodies in pigs at the time of a CSFV infection may affect the transmission of CSFV in and between infected herds (Wieringa-Jelsma et al., 2006). Transmission of CSFV within a herd may be reduced, to a level that an introduction of CSFV in a swine herd may result in a very small and self-limiting outbreak. Such an outbreak may go unnoticed and does not necessarily pose a threat for any other herd. However, CSFV infections will also result in less noticeable clinical symptoms, and in case transmission of CSFV still occurs, such herds would be detected with a certain delay, in the mean time posing a threat for other herds in direct or indirect contact.

More important is probably how ruminant pestiviruses may affect serological CSF surveillance. Currently the serological diagnosis of CSF relies on a parallel interpretation of up to three virus neutralisation tests (EU Commission Decision 2002/106/EG). A CSFV infection in an animal with antibodies against BDV may however result in higher titres against BDV than against CSFV (Wieringa-Jelsma et al., 2006), thus giving a false negative result for CSF. False positive results on the other hand will occur in situations where the E\textsubscript{nu}n-test is being used, which is primarily after vaccination with a marker vaccine. Serological confirmation of the CSF-result, and differentiation with ruminant pestiviruses is so far not possible. The presence of antibodies against ruminant pestiviruses in a population may therefore seriously hamper any eradication strategy that involves vaccination with marker vaccines, especially during the phase where freedom of disease needs to be proven again.

Based on this study, diagnostic procedures for the serological surveillance of CSF were adapted in the Netherlands. BDV is no longer routinely used in the virus neutralisation test, lowering the costs and increasing the efficiency of the diagnostic procedure. Furthermore, emergency vaccination plans for CSF in the Netherlands were adapted. In the current plan sows, who have a much higher seroprevalence than finishing pigs, will not be vaccinated against CSF, amongst others to avoid diagnostic problems due to the presence of antibodies against BDV in breeding pigs. As such, these are good examples on how knowledge of seroprevalence of ruminant pestiviruses affects the diagnostic procedure and may affect intervention measures during a future CSF outbreak.

Acknowledgement

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References


