Serological study on bovine viral diarrhoea virus infection in pig population in Poland between 2008 and 2011

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Received: April 04, 2014 Accepted: August 27, 2014

Abstract

In total, 14 608 pig sera, collected between 2008 and 2011, were tested with ELISA using antibodies specific for bovine viral diarrhoea virus (BVDV). All doubtful and positive samples were retested by virus neutralisation test (neutralising peroxidase-linked assay). The BVDV seroreagents were detected in 11 (68.75%) out of 16 provinces, the seroprevalence varied from 0.1% to 1.04% (average 0.31%). The obtained results indicate that the prevalence of BVDV infection in pig population in Poland is low.

Keywords: swine, bovine viral diarrhoea virus, seroprevalence, Poland.

Introduction

Bovine viral diarrhoea virus (BVDV) with classical swine fever virus (CSFV), and border disease virus (BDV) belong to the genus Pestivirus, family Flaviviridae. This results in a high level of homology in the scope of nucleotide sequences, synthesised amino acids, and antigenic structure (12, 23). Cattle and small ruminants are natural hosts for BVDV and BDV, respectively. However, both viruses could infect swine, including wild boar (14). The Pestivirus genus is of a significant epizootiological and economic importance, especially in relation to classical swine fever that is included in the OIE listed diseases (26). A high homology rate between CSF and BVD viruses, interspecies crossing, and infection of pigs with BVDV could create a serious diagnostic problem (25, 36, 37, 43). Cattle is considered as the main reservoir and source of BVDV for pigs (7, 8, 10, 15, 16, 19, 38, 41). Lenihan and Collery (15) found that 27.8% BVDV antibody positive sows had contact with cattle, while 3.6% seropositive sows were without contact, and 4.5% of five-six months old pigs have had little or no contact with cattle. According to De Smit et al. (7, 8) 59% of seropositive sows came from mixed farms (cattle and pigs on the same premises) or from farms where ruminants were kept in close vicinity of pig holding. Loeffen et al. (19) evaluated several risk factors associated with the presence of swine and ruminants on the same farm or in close vicinity of pig holding. They found that sows on mixed farms had a 3.4 higher chance to have a BVDV-seropositive status than in the herds without cattle. Moreover, if there are more than 60 ruminant herds within a radius of 3 km of the sow herds, the possibility of having a BVDV-seropositive status is 2.1-2.6 times higher than in the case of sow herds with less than 60 ruminant herds in the neighbourhood. The infection of swine may take place by an indirect contact, contaminated bedding, or vaccine and slurry. BVDV infecting the animals mentioned above regardless of age or physiological status (e.g. pregnant sows) may cause clinical symptoms nearly identical with those observed in the pigs infected with CSFV of low or moderate pathogenicity (24, 41). The intravital diagnosis of this disease is impossible and the suspicion of the CSF must be confirmed or excluded using laboratory methods (32). The differential diagnosis of BVDV and CSFV infections plays an important role with regard to the epizootiological and economic impact. The diagnosis
of CSF, according to the compulsory law regulations, introduces stamping out pig herds infected or suspected to be infected with CSFV, and in the world scale, an export ban of live pigs, pork meat, and pork meat products. In contrast, the diagnosis of pigs infected with BVDV has only an epizootiological importance without economic consequences (27). Thus, an appropriate differential diagnosis using laboratory methods is essential. However, in spite of many years that have passed since BVD has been diagnosed in swine, sometimes there are problems in the rapid and reliable differential diagnosis of infections caused by BVDV and CSFV. The laboratory methods mentioned above are not always sufficiently sensitive and specific to allow the unambiguous diagnosis (17, 32, 36, 37).

Taking into account the possibility of facing the above-mentioned diagnostic problems, and the fact that our country is free from CSF since 1994, and in Poland seroprevalence of BVDV in pigs was not currently known, a decision was made to undertake this study.

Material and Methods

Sera. A total of 14 608 swine sera from the whole territory of Poland were examined between 2008 and 2011 in the frame of monitoring of classical swine fever, by the regional Veterinary Diagnostic Laboratories of the Veterinary Inspection. After collection the sera were delivered to the Department of Swine Diseases of the National Veterinary Research Institute (Pulawy).

ELISA. POURQUIER® ELISA BVD/MD/BD P80 Antibodies Screening (Institut Pourquier, France) was used according to the manufacturer’s manual. This test detects antibodies against a viral protein p80, common in ruminant pestiviruses. Therefore, the manufacturer of this assay recommends verifying the doubtful and/or positive results by serological test more specific for BVDV antibodies.

Virus neutralisation test (VNT). Samples positive and/or doubtful in ELISA were examined for BVDV antibodies by neutralising peroxidase-linked assay (NPLA) to verify ELISA results according to the technical annex of Commission Decision (2). The sera were diluted from 1:5 up to 1:640 and then incubated for 1 h at 37°C (±2°C) with NADL strain of BVDV at a dose of 100 TCID₅₀/50µL and supplemented with MDBK continuous cell line. After 2-3 d of incubation in a CO₂ incubator (5% ± 1%) at 37°C (±2°C), the culture was fixed and stained according to the abovementioned technical annex.

Results

Results of serological examination by ELISA are shown in Table 1 and Fig. 1. The data indicated that serologically BVDV positive pigs were detected in 14 (87.5%) out of 16 provinces examined. Positive and/or doubtful animals were not only detected in the Świętokrzyskie (SW) province. The percentage of seroreagents ranged from 0.08% in the Podkarpackie (PK) province to 1.89% in the Warmińsko-Mazurskie (WM) province with an average of 0.73% (Table 1) within the whole period of examination.

Table 1. Results of BVDV antibody detection using ELISA

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of sera</th>
<th>Tested</th>
<th>Positive (%)</th>
<th>Doubtful (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolnośląskie (DS.)</td>
<td>1090</td>
<td>4 (0.37%)</td>
<td>3 (0.27%)</td>
<td>7 (0.64%)</td>
<td></td>
</tr>
<tr>
<td>Kujawsko-Pomorskie (KP)</td>
<td>1251</td>
<td>15 (1.2%)</td>
<td>12 (0.96%)</td>
<td>27 (2.16%)</td>
<td></td>
</tr>
<tr>
<td>Lubelskie (LB)</td>
<td>717</td>
<td>1 (0.14%)</td>
<td>2 (0.28%)</td>
<td>3 (0.24%)</td>
<td></td>
</tr>
<tr>
<td>Lubuskie (LU)</td>
<td>436</td>
<td>-</td>
<td>3</td>
<td>3 (0.69%)</td>
<td></td>
</tr>
<tr>
<td>Łódzkie (LO)</td>
<td>558</td>
<td>1 (0.18%)</td>
<td>4 (0.71%)</td>
<td>5 (0.89%)</td>
<td></td>
</tr>
<tr>
<td>Małopolskie (MP)</td>
<td>1133</td>
<td>5 (0.44%)</td>
<td>6 (0.53%)</td>
<td>11 (0.97%)</td>
<td></td>
</tr>
<tr>
<td>Mazowieckie (MA)</td>
<td>768</td>
<td>11 (1.34%)</td>
<td>1 (0.13%)</td>
<td>12 (1.56%)</td>
<td></td>
</tr>
<tr>
<td>Opolskie (OP)</td>
<td>446</td>
<td>5 (1.12%)</td>
<td>3 (0.67%)</td>
<td>8 (1.79%)</td>
<td></td>
</tr>
<tr>
<td>Podkarpackie (PK)</td>
<td>1214</td>
<td>1 (0.08%)</td>
<td>1 (0.08%)</td>
<td>2 (0.16%)</td>
<td></td>
</tr>
<tr>
<td>Podlaskie (PD)</td>
<td>748</td>
<td>6 (0.8%)</td>
<td>5 (0.67%)</td>
<td>11 (1.47%)</td>
<td></td>
</tr>
<tr>
<td>Pomorskie (PO)</td>
<td>569</td>
<td>4 (0.7%)</td>
<td>3 (0.53%)</td>
<td>7 (1.23%)</td>
<td></td>
</tr>
<tr>
<td>Śląskie (SL)</td>
<td>1210</td>
<td>17 (1.4%)</td>
<td>6 (0.5%)</td>
<td>23 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>Świętokrzyskie (SW)</td>
<td>255</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Warmińsko-Mazurskie (WM)</td>
<td>1323</td>
<td>25 (1.89%)</td>
<td>4 (0.3%)</td>
<td>29 (2.19%)</td>
<td></td>
</tr>
<tr>
<td>Wielkopolskie (WI)</td>
<td>2183</td>
<td>10 (0.46%)</td>
<td>25 (1.14%)</td>
<td>35 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>Zachodno-Pomorskie (ZP)</td>
<td>607</td>
<td>2 (0.33%)</td>
<td>15 (2.47%)</td>
<td>17 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14608</td>
<td>107 (0.73%)</td>
<td>93 (0.64%)</td>
<td>200 (1.37%)</td>
<td></td>
</tr>
</tbody>
</table>
An average percentage of pigs infected with BVDV in 15 (93.75%) out of 16 examined provinces (Fig. 1) was 1.37% and ranged from 0.16% in the Podkarpackie (PK) province to 2.8% in the Zachodnio-Pomorskie (ZP) province (Table 1).

The presented data should be carefully considered because the ELISA results may be false positive and/or doubtful leading to misinterpretation of the epizootic situation. As it was mentioned in the previous section, each positive/doubtful result should be verified with the use of other serological methods, more specific for BVDV antibody detection. Similar recommendations are put forward by the Commission Decision (2) with regard to differential examinations of swine infected with pestiviruses.

Out of 107 positive and 93 doubtful samples in ELISA only 169 sera were verified by the neutralisation peroxidase-linked antibody test (NPLA). This limitation resulted from poor quality of the samples (haemolysis, cytotoxicity) and in several cases too small volume (<20 µL) of the sera due to their previous use in the ELISA.

The comparative results obtained by the NPLA and ELISA are presented in Table 2 and Fig. 2. An average percentage of swine infected with BVDV was 0.31% and ranged from 0.1% in the Dolnośląskie (DS) province to 1.04% in the Mazowieckie (MA) province (Table 2). The presence of seroreagents was detected in 11 (68.75%) out of 16 provinces (Fig. 2).

Table 2. Comparative results of BVDV antibody detection using ELISA and NPLA

<table>
<thead>
<tr>
<th>Province</th>
<th>tested (total)</th>
<th>tested by ELISA*</th>
<th>tested by NPLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
<td>doubtful</td>
</tr>
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</tr>
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<td>4</td>
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<td>Małopolskie (MP)</td>
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<td>4</td>
<td>5</td>
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<td>20</td>
</tr>
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<td>Zachodnio-Pomorskie (ZP)</td>
<td>607</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>14 608</td>
<td>90</td>
<td>79</td>
</tr>
</tbody>
</table>

* The number of samples used in confirming examinations by NPLA

Fig. 1. Prevalence of BVDV infection in pigs in Poland based on ELISA results
Discussion

Results of the study should be compared to the results of similar investigations conducted in Poland in the 1980’s and 1990’s of the 20th century. According to Pejsak et al. (29) in farms with mixed production (swine and cattle together) the virus neutralisation test revealed that the average percentage of infected swine was 3.53% (from 1.08% to 6.25%). Similar results, i.e. higher rate of BVDV seroprevalence in pigs on mixed farms, was also reported by Deng et al. (5), De Smit et al. (7, 8), Gatto et al. (10), Lenihan and Collery (15), Loeffen et al. (19), and Tao et al. (38). At that time, the presence of seroreagents was found in all provinces in which the samples were collected (29). On the other hand, in pigs reared in farms with no contact with cattle, and located in three out of four examined provinces, the average rate of animal infection was 0.3% (29), which was very similar to the results obtained in the present study conducted 20 years later.

A subsequent work, which demonstrated the presence of BVDV infection in the Polish swine population included the use of the ELISA in monitoring classical swine fever (28). It was found that an average percentage of seroreagents (17 out of 49 provinces) to BVDV was 0.4% (from 0.18% to 5.0%). These results do not vary substantially from those demonstrated in the present work. A higher percentage of seroreagents equal to 0.7% was reported by Rypula (32).

The presented data is definitely lower than those demonstrated during the last decades in different parts of the world. Studies on BVDV/BDV seroprevalence in swine are rare and mostly difficult to compare because of different sampling schemes, different age categories of tested animals, and different tests applied (19). Studies on seroprevalence of ruminant pestiviruses in wild boar were conducted in Germany (3, 34), the USA (20), Croatia (31, 44), and the Czech Republic (33) and revealed that the percentage of seropositive animals did not exceed 8%.

The problem of BVDV infections in domestic pigs, and connected with these difficulties in differential diagnosis of CSF have been recognised in mid 1970-ties of the last century (15, 16, 35). Results of serological investigation showed that the BVDV seroprevalence in that time varied from 3.6% to 27.8% in the Republic of Ireland (15), in Germany amounted 42.3% (16), and in Australia fluctuated between 1.6% in animals younger than 12 months of age to 43.5% in animals older than 12 months of age (35). In the next years, the seroprevalence of 9.16% in Germany (1) and 4.4% in France (30) was reported. Jensen (13) showed that in Denmark 6.4% seroreagents were found in pigs exported to Germany. Seropositive pigs were also found in Norway – 2.2% (18), in the Republic of Ireland – 3.2% (21), in the Netherlands – 43.3% (42) and 19.8% (40), in 1994 and 1997 respectively. In Northern Ireland (UK), BVDV antibodies were detected in 1 (0.14%) out of 680 tested pig serum samples (11).

The non-vaccination strategy of CSF eradication used in European Union supports the need to have very specific methods discriminating CSFV from BVDV infection in pigs. The significance of this was demonstrated by De Smit et al. (7) describing laboratory examination of 2.15 million serum samples for CSFV antibody during the CSF epizootic between 1997 and 1998 in the Netherlands. The authors reported that among serum samples positive for CSFV antibodies in ELISA, 26.5% were positive in pestivirus (BVDV/BDV) VNT. Furthermore, among samples negative for antibodies against CSFV in ELISA 11% of
the sera were positive in pestivirus VNT. A survey among 300 slaughtered sows and boars demonstrated that a total of 20% of the pigs were pestivirus seropositive and among them 39% had antibodies against BVDV. More details regarding laboratory experiences during the above mentioned CSF epizootic were presented in another paper of the same authors (8). From more than 135,000 sera collected from animals from herds infected with CSFV or suspected to be infected, which were tested by ELISA, 3.5% were positive in ELISA and out of them 16% had antibodies against pestiviruses. Furthermore, from 1.5 million serum samples collected during different serological screening programmes, which were found positive in the automated CSFV antibody ELISA, 35% were positive for BVDV antibody. These sera were mostly found in sows and varied from 0% to 60% depending on the pig farm. Similar investigation in the Dutch swine population was presented ten years later by Loeffen et al. (19). They found that in sows BVDV seroprevalence was 2.5% on the animal level and 11% on the herd level, and among finishing pigs – 0.42% and 3.2% respectively.

Infections of pigs with BVDV were also investigated in Western Hemisphere. Herd prevalence level of BVDV in North American swine herds has been reported to be anywhere from 2% to 43%, but in Ontario (Canada) swine herd prevalence of BVDV is negligible (22). Lately, in Brazil BVDV antibodies were found in 2.32% of finishing pigs slaughtered in a slaughterhouse located at the State of São Paulo (9). Moreover, amongst 412 serum samples collected from pigs originating from 20 small, traditional farms located in the Northeast region of Brazil, 4.13% were positive for BVDV antibodies and these pigs were found in 45% of the mentioned holdings (10).

Very interesting results concerning BVDV infection in swine were published by Chinese authors. Deng et al. (5) reported that in recent years BVDV seroprevalence in some Chinese pig herds was 20%-30%. Using RT-nPCR specific for BVDV detection in serum and tissue samples collected from pigs from 11 provinces in China, the authors revealed that the BVDV prevalence between 2007 and 2010 was on average 26.8% and oscillated from 23.1% in 2007 to 33.6% in 2009. Moreover, phylogenetic analysis of BVDV detected in 20 isolates out of 137 BVDV-positive samples showed that all of them could be classified as genotype 1 of BVDV (BVDV-1) and clustered into five subtypes (5). From these five subtypes, one was found to be a novel subgenotype BVDV-1q, not detected previously in China (4). Another group of Chinese researchers indicated that BVDV seroprevalence in pigs in Shanghai in 2007 and 2008 was 35.9% and 64.1% respectively (38). These findings strongly suggest an increasing rate of BVDV infection in pigs in the region, and indicate that BVDV-1 is the predominant genotype of BVDV strains in China (38). This statement was based on detection of such genotype of the virus in pigs where, in 2008, the seroprevalence of BVDV in five provinces in China was 16.3% (39). However, Tao et al. (38) were also able to isolate a genotype BVDV-2 from a pig blood sample, and this genotype was then confirmed by further genetic characterisation and phylogenetic analysis (39).

Finally, it should be emphasised that the low rate of BVDV infections observed in the Dutch and Polish swine populations is probably caused by continued specialisation of animal farms (19). In the past most farmers used to rear cattle, pigs, poultry, and other animals on the same premises. In the last decades, however, more farms decided to have only one type of animal production. This trend is observed not only in Poland but also in other countries, e.g. the Netherlands (19). Mixed farms, with many animal species living in close contact with each other have become rare. This is a very promising situation regarding differential diagnosis of BVDV and CSFV.

Acknowledgements: This work was supported by the Ministry of Science and Higher Education (grant No. R12 023 02). I would like to express my thanks to Mrs Renata Wydra for skilled technical assistance.

References


