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Case Report

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) IN SLOVENIA: EVALUATION OF SEROLOGY

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Summary: Between 1995 and 2003, 27,925 pig sera were tested for PRRS virus antibodies using the Herd Check ELISA test (IDEXX) to determine the PRRS status of pig herds in Slovenia. The prevalence of seropositive samples was low and annually ranged from 1 to 7 % and the average hovered between 2 and 3 %. No clinical signs associated with a PRRS viral infection have been reported by any of the farms. Under field conditions, false positives with this assay may run up to 3 %. Based on the absence of clinical signs, the recorded levels of false positives associated with the assay and the persistently low frequency of serological signs, the presence of PRRS can not be determined.

Key words: PRRS; serology; monitoring

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is characterized by the reproductive failure of sows as well as respiratory distress in piglets and growing pigs and has a significant economic impact on the pig industry (1).

The disease was first seen in the United States and Canada in 1987 and was first reported in Europe in Lower Saxony in June 1990. Since then it has been reported in other parts of Europe – the United Kingdom, France, Spain, Denmark, Luxembourg, Italy, Malta, Poland – and in Japan, Korea, the Philippines and South America (2). Only Australia, Sweden and Switzerland have documented their virus-free status (2, 3, 4). The PRRS virus is currently classified as a member of the newly established order of *Nidovirales*, family *Arteriviridae*, genus *Arterivirus* (5). Clinical signs such as an increase in stillbirths, pre-weaning mortality and in abortions, as well as the development of respiratory diseases, the presence of lesions during post-mortem examinations and histological findings could indicate the presence of PRRS. This can be confirmed by immunofluorescence or by immunoperoxidase and by a reverse polymerase chain reaction. Virus isolation can also be performed (6). The detection of serum antibodies to the virus is the most commonly used method to confirm a diagnosis and to monitor the disease (5).

Serological methods used to determine PRRS include the following: ELISA tests, immunoperoxidase monolayer assays (IPMA), indirect fluorescent antibody tests (IFA) and virus neutralisation tests (VNT) (6, 7). Slovenia first detected animals that were seropositive to PRRS, in a quarantine station in 1994. Every pig at the station was immediately destroyed. Prior to that event PRRS had been unknown in Slovenia, although there had been no clinical or serological monitoring for the disease. In 1995, the Slovenian Government introduced strict guidelines governing the monitoring of PRRS. It became compulsory for farmers to test their breeding-age pigs for the disease on an annual basis. These tests are fully subsidized by the government (8, 9).

Material and methods

Sera: Blood was taken from every boar and sow of breeding age from each piggery with more than 1,000 breeding sows as well as from a selection of small farms. According to the figures, the number of

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Table 1: Sera samples tested for PRRS between 1995 and 2003

Year	No. of samples tested	Positive	Negative
1995	4,537	53	4,484
1996	4,943	170	4,773
1997	4,395	107	4,288
1998	3,176	91	3,085
1999	4,352	89	4,263
2000	1,932	32	1,900
2001	1,471	97	1,374
2002	1,582	30	1,552
2003	1,537	70	1,467

samples varies each year, however, a total of 27,925 samples were tested between 1995 and 2003.

Test: The Herd Check PRRS (IDEXX Laboratories Inc, Westbrook, Maine, USA), which is an enzyme immunoassay for the detection of antibodies to PRRS in swine serum using PRRS and normal host cell (NHC) antigens, was used to test all the sera. The PRRS and NHC antigens are coated on alternating columns on a microplate. The test sample is introduced into the coated wells and upon incubation antibodies specific to PRRS form a complex with the viral antigen coating. The coating of NHC antigens is used to assess whether immunoglobulins against tissue-culture components, present in vaccines, are contributing to the test results. After washing away any unbound material from the wells, an anti-porcine horseradish-peroxidase conjugate is added which binds to any porcine antibody attached to the wells. In the final step of the assay, unbound conjugate is washed away and enzyme substrate and a chromogen are added to the wells. The extent of any host-cell contribution to the total signal is assessed by relating the PRRS activity to the NHC reactivity. For the assay to be valid, the positive-control mean for PRRS, which is determined by the formula PC:PRRS minus NC:PRRS, must be greater than or equal to 0.150. The presence or absence of PRRS antibodies is determined by calculating the S/P ratio. If the S/P ratio is less than 0.4, the sample is classified as negative for PRRS. If the S/P ratio is greater than or equal to 0.4, then the sample is classified as positive for PRRS antibodies.

Results

Number of samples positive for PRRS antibodies was low in all years. The seroprevalence to PRRS is from 0.01 to 0.07. From all tested sera in this period 2.6 % were positive to PRRS. The only evidence of PRRS is Slovenia is based on serology.

Discussion

Serologic tests are very important tools in control and prevention programmes. The United States uses the ELISA, as we do in Slovenia. Under experimental conditions the test is essentially 100 % specific and 100 % sensitive when testing serum from acutely infected swine. In general, the range of the S/P ratio for false negatives is about 0.2 to 0.4. Under field conditions, false positives may run up to 3 % with this assay (10).

The testing of sera from 9 PRRSV-infection-free farms at the University of Minnesota's Veterinary Diagnostic Laboratory found that 0.89 % of the sera tested positive to PRRS. Adult animals had a higher prevalence of false positive results than younger animals (1.3 %). Twenty-four of the positive sera from the ELISA were retested using an immunofluorescence assay (IFA); only one sample tested positive and the herd history subsequently confirmed this sample as negative (11). In developing and validating an ELISA test for the detection of antibodies directed against the European or the North American strain of the PRRS virus, swine sera from countries free of PRRS (New Zealand and Switzerland) where used to gauge its specificity. This ELISA test established that out of

a total of 277 sera tested, 266 were negative, 6 were doubtful and 5 were positive (12). The testing and removal protocol for PRRS, detected 74 false positives (approx. 2.2 %) out of the 3,408 samples tested, which were taken from the farms during the monitoring periods (13).

The prevalence of PRRS antibodies detected in Slovenia ranges from 1 to 7 %, with the average between 2 and 3 %. As only pigs of breeding age are tested, the percentage of false positives can be higher than when only younger animals are tested. The herd history of Slovenia's eight largest pig farms indicates that there have been no typical signs of PRRS since monitoring began in 1995. These farms are now governed by strict biosecurity guidelines; replacement animals for the breeding herds usually come from the same farm and if they are imported they are quarantined and tested for PRRS with only seronegative pigs being introduced to the farm.

Despite nine years of serologically testing adult animals without clinical signs of PRRS, it is still unclear if our large pig herds are PRRS free. Only further testing and continued vigilance will provide any certainty in this regard.

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PRAŠIČJI REPRODUKCIJSKI IN RESPIRATORNI SINDROM (PRRS) V SLOVENIJI: VREDNOST SEROLOGIJE

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Povzetek: V obdobju od 1995 do 2003 smo pregledali 27.925 prašičjih serumov s testom ELISA Herd Check proizvajalca IDEXX Laboratories Inc, Westbrook, Maine, ZDA, in sicer na prisotnost protiteles proti virusu PRRS, da bi ugotovili stanje glede te bolezni na farmah prašičev v Sloveniji. Število pozitivnih vzorcev je bilo med 1 in 7 %, večinoma pa med 2 in 3 %. Na nobeni od farm ni bilo kliničnih znamenj, ki bi kazale na okužbo z virusom PRRS. Pri uporabi istega testa ELISA so ugotovili od 2 do 3 % lažno pozitivnih reaktorjev. Ker pri nas niso bila opisana klinična znamenja bolezni ter glede na opisan odstotek lažno pozitivnih reaktorjev v svetu in dolgoletno nizko prevalenco serološko pozitivnih reaktorjev ostaja status slovenskih farm prašičev v zvezi s PRRS še vedno nejasen.

Ključne besede: PRRS; serologija; monitoring