Short communication

Identification of viral pathogens in aborted fetuses and stillborn piglets from cases of swine reproductive failure in Spain

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Abstract

The objective of the present study was to determine the presence of recognised abortifacient viruses such as porcine reproductive and respiratory virus (PRRSV), Aujeszky’s disease virus (ADV), porcine parvovirus (PPV) and porcine circovirus type 2 (PCV2), in tissues from aborted fetuses and stillborn neonates in cases of late reproductive failure in swine. A total of 293 specimens (fetuses aborted in the last third of gestation and stillborn piglets) from 100 different cases of late-term abortions and premature farrowing from 15 different Spanish provinces were studied. PRRSV was detected in 9/100 cases by RT-PCR. Only 1/100 cases analysed (corresponding to a late-term aborted fetus with a negative PRRSV RT-PCR result) was positive for PCV2 by PCR. Neither ADV (monitored by viral isolation plus antigen detection) nor PPV (monitored by ELISA antigen capture test) infection was identified. The results suggest that PRRSV is one of the most important infectious agents, if not the most relevant one, associated with fetal infection leading to abortion or premature farrowing in Spain. Moreover, other viral pathogens such as ADV, PPV and PCV2 seem to have a minor impact on reproductive disease.

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Reproductive disease during the third trimester of gestation in sows is usually manifested by abortion or premature farrowing. Two major classes of late reproductive failure are recognised in swine (Straw et al., 1999). A first category is represented by infectious agents that cause primary infection of the reproductive tract (considered responsible for 30–40% of abortions, mummified fetuses, and stillbirths). A second group includes toxins, environmental and nutritional stresses, and systemic disease in the sow (of infectious and non-infectious origin) and is considered responsible for the rest of the late reproductive problems.

A large list of viruses should be included in the first category. Porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky’s disease virus (ADV), porcine parvovirus (PPV), enteroviruses (EV), hog cholera virus (HCV) and encephalomyocarditis virus (EMCV) have been associated with fetal death due to a direct attack on the fetus and/or the placenta (Christianson, 1992; Holler, 1994). On the other hand, in recent years, porcine circovirus type 2 (PCV2), considered the causal agent of postweaning multisystemic wasting syndrome (PMWS), has also been linked to reproductive failure, with a direct effect on the fetus (West et al., 1999; O’Connor et al., 2001; Sanchez et al., 2001).

The objective of the present study was to determine the presence of recognised abortifacient viruses known to be present in Spain such as PRRSV, ADV and

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PPV, as well as PCV2, in tissues from aborted fetuses and stillborn neonates from late reproductive failure cases in swine.

A total of 293 specimens (fetuses aborted in the last third of gestation and stillborn piglets) from 100 different cases of late-term abortions and premature farrowing, submitted to the Veterinary Diagnostic Service of Laboratorios Hipra S.A. between January and October 2002, were included in this study. One to 13 specimens were submitted per case. All reproductive failure cases corresponded to different conventional farms \((n=100)\) with various production systems (including farrow-to-finish, farrow-to-wean and multiple site herds), which were located in 15 Spanish provinces.

After a complete necropsy, several tissue samples and thoracic fluid from each specimen were collected. Samples were then handled as individual tissues for histopathological examination (fixed by immersion in buffered formalin), or pooled (one pool per farm), homogenised (10\% w/v in PBS) and frozen at \(-80\, ^\circ\text{C}\) for retrospective virus detection.

Histopathological studies were performed on myocardium, lung, spleen, liver and kidney tissues from all 293 sampled animals. They consisted of a haematoxylin/eosin stain to detect potential lesions under light microscope, and an in situ hybridisation test (ISH) to detect PCV2 using a previously published protocol (Ronell et al., 1999).

Several virus detection tests were applied to pooled-homogenised tissues. For the detection of PRRSV RNA, pooled samples of thymus, lung and thoracic fluid from each case were analysed by RT-PCR using a previously described technique (Sitges et al., 2000). For PCV2 DNA detection, samples of lung, spleen, liver, kidney, thymus and heart from each case were investigated by a previously published PCR method (Quintana et al., 2002). The presence of ADV was analysed in a pool containing brain and lung tissues from each case by isolation of the virus in the continuous cell line PK-15, as described elsewhere (Wittmann and Rziha, 1989); confirmation was by immunoperoxidase monolayer assay (IPMA) on the cell culture using a polyclonal antibody to ADV. Finally, PPV antigen detection was performed on pools of liver and intestine tissues corresponding to 29 cases (which included 87 specimens), using the ELISA test Ingezim Parvo Porcino Kit (Ingenasa) following the manufacturer’s instructions.

Three out of 293 specimens had catarrhal-purulent bronchopneumonia; no significant microscopic lesions were observed in the rest of the tissues examined. No PCV2 nucleic acid was detected by ISH in any of the tested samples.

PRRSV RNA was detected in 9/100 cases (9\%). Only one out of 100 pooled samples analysed (corresponding to a late-term aborted fetus with a negative PRRSV RT-PCR result) was positive for PCV2 by PCR. The obtained PCV2 amplicon was sequenced and its identity confirmed. Finally, neither ADV nor PPV infection was identified in any of the studied cases.

The present results indicate that PRRSV is one of the most important, if not the most relevant, agent associated with fetal infection leading to abortion or premature farrowing in Spain. The detection of PRRSV associated with reproductive problems was relatively expected, since this infection is enzootic and vaccination is not a systematic practice under field conditions in our country (Segalés, 2003). Based on our results, it is assumed that the involvement of this virus in reproductive failure was probably underestimated, since only aborted fetuses in the last third of gestation and stillborn piglets were studied. It is well known that PRRSV can also cause abortion without infection of the fetus (Christianson, 1992). No fetal lesions as described by Lager and Halbur (1996) were observed in the present study; however, this result was expected since lung, liver and heart lesions in fetuses from PRRSV aborted sows are relatively infrequent (Lager and Halbur, 1996).

The absence of ADV and PPV infected tissues was surprising, since they are enzootic in Spain. A possible explanation for these latter findings could be that most of the farms in Spain follow active vaccination programmes for the control of these viral infections. Vaccination for ADV is compulsory in Spain and this disease is included in the text of several European Council Directives dealing with animal health requirements applicable to intra-community trade and imports. Moreover, PPV vaccination is systematically applied on most, if not all, Spanish pig herds and, therefore, the lack of reproductive clinical signs associated with PPV could be explained by this fact. Obviously, it cannot be ruled out that a higher number of studied cases would have generated some positive results regarding these viruses; however, the results obtained allow us to speculate that reproductive failure due to PPV and ADV in Spain is probably of low incidence nowadays.

PCV2 was detected by PCR in one of the cases of reproductive failure studied; however, the aborted fetus did not show any of the typical lesions such as myocardial necrosis and fibrosis usually associated with this infection (West et al., 1999; O’Connor et al., 2001). Moreover, no PCV2 nucleic acid was detected in the myocardial or liver tissue by ISH, which suggests that, although PCV2 intra-uterine infection occurred in this case, its role in the reproductive failure and fetal damage is questionable. The low PCV2 detection rate in the specimens studied is in agreement with a preliminary study by Segalés et al. (2002); in this report, also from Spain, one single PCV2 positive sample out of 195 fetal submissions was detected by ISH. These authors suggested that the non-availability of myocardial tissue in 143 of these submissions could have been a handicap.
in detecting PCV2. However, in our study, heart tissue was systematically included for both PCR and ISH studies and the results remained very similar. As a result, the lack of evidence of association between PCV2 and cases of reproductive failure in Spain suggests that PCV2 is probably not an important abortifacient pathogen in a country where PMWS and PCV2 infection are widespread (Segalés et al., 2003). Therefore, the potential of this virus as a fetal pathogen in the field may be low, because most sows in breeding herds have high levels of antibodies against this ubiquitous agent, as previously suggested (Sánchez et al., 2001).

The bronchopneumonia lesions observed in three specimens were attributed to an intra-uterine bacterial infection; however, it was not possible to isolate bacteria (data not shown) or detect any virus in these particular cases.

No other viruses able to cause fetal damage such as HCV, PEV and EMCV were investigated in the present study. The two latter viruses have never been studied in Spain and, therefore, their presence is currently unknown. Consequently, we cannot rule out the existence of these abortifacient agents in the studied cases. On the other hand, Spain was free of HCV infection at the time the study was performed, which indicates that this virus was not related with the reproductive failure cases studied.

Data from the USA attribute 30–40% of abortions to infectious agents primarily affecting the reproductive tract (Straw et al., 1999). Our results point at an even lower percentage and, although only certain viral agents have been investigated (thus, no information on bacterial or chlamydial reproductive failure has been generated), the results of this study further support the fact that diagnosis of the cause of abortion, and other reproductive problems in swine, is seldom straightforward and frequently unsuccessful (Straw et al., 1999). In addition, our results confirm that a large percentage of reproductive wastage is probably not associated with an infectious aetiology (Holler, 1994).

In summary, the data presented indicate that PRRSV is apparently the most important viral infectious agent associated to late reproductive failure with direct effect of the fetus in Spain. Furthermore, other viral pathogens such as ADV, PPV and PCV2 seem to have a minor impact on reproductive disease. In fact, our results suggest that reproductive failure due to PCV2, ADV and PPV in Spain is a rare event.

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