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J VET Diagn Invest 1994 6: 480
DOI: 10.1177/104063879400600413

The online version of this article can be found at:
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>> Version of Record - Oct 1, 1994

What is This?
The salient features of a porcine reproductive and respiratory syndrome (PRRS) epizootic include respiratory disease followed by reproductive failure. The respiratory component is usually recognized first in finishing pigs or in the breeding herd as a mild flu-like disease with a high morbidity for all ages and high mortality in younger pigs. In breeding herds, acute maternal reproductive failure often follows the onset of respiratory disease. PRRS is characterized by a sudden increase in early farrowings, late-term abortions, still-born and mummified fetuses, weak neonates with high mortality, late returns to estrus, and repeat breeders. No consistent histopathology has been observed in fetal tissues from epizootics or following experimental infections of susceptible dams. In this report, we describe fetal histopathology associated with an experimental PRRS virus (PRRSV) infection.

Eight pregnant gilts underwent surgery at different stages of gestation: 2 at 34 days, 1 at 45 days, 3 at 49 days, 1 at 65 days, and 1 at 85 days gestation (Table 1) (Lager KM, Mengeling WL, unpublished). All fetuses in 1 uterine horn (identified as principal fetuses) were exposed to a well-characterized PRRSV isolate by transuterine injection of virus into their amniotic fluids. All fetuses in the contralateral uterine horn (identified as control fetuses) were exposed to a sham inoculum in a similar fashion. The gilts were necropsied 17-31 days postsurgery, and their fetuses were recovered for study. Virus isolation was attempted on sera from live fetuses, thoracic fluids from dead fetuses, and tissue composites from each fetus (Lager KM, Mengeling WL, unpublished). Tissue samples for histopathologic examination were collected from lung, kidney, liver, and spleen and placed in neutral buffered 10% formalin. Tissues were processed routinely, cut into 8-µm sections, and stained with hematoxylin and eosin (HE). Sections of lung, liver, and kidney were mounted in embedding medium, frozen, and sectioned on a cryostat. A fluorescein isothiocyanate-labeled monoclonal antibody raised against the nucleocapsid protein of PRRSV was used for direct staining of tissue sections as previously described.

PRRSV was recovered from 34 of 35 live principal fetuses and from 1 of 12 dead principal fetuses at the time of necropsy. Gross lesions in the dead fetuses consisted of mild to severe autolysis and mummification. Gross lesions observed in some live principal fetuses consisted of mild to severe cutaneous petechial hemorrhages and hemorrhagic and friable internal organs. In litter 7, all principal fetuses had died and were mummified at the time of necropsy. Two control fetuses located next to the uterine body had recently died and were beginning to mummify. Virus was recovered from the live control fetus adjacent to the dead control fetuses, implying that virus had spread from the principal to the control uterine horn. Lesions comparable to those of the principal fetuses were present in this live control fetus infected with virus. Histopathologic evaluation was not attempted on any mummified fetuses; however, sections of lung tissue from all dead fetuses were examined for porcine parvovirus antigen by immunofluorescence as previously described. No porcine parvovirus antigen was observed in any dead fetus, and no cytopathic agents were recovered from the dead fetuses other than the previously described PRRSV isolation in 1 dead fetus. The cause of death for the 11 fetuses for which no agent was isolated is unknown; however, we presume their deaths resulted from exposure to PRRSV and subsequent fetal infection. Litter 4 had 2 fetuses located next to the uterine body from which PRRSV was recovered. The location of the infected control fetuses in litters 4 and 7 suggest that intrauterine spread of virus probably occurred.

The most consistent and severe microscopic lesions were present in lung tissues from fetuses inoculated between 45 and 49 days of gestation, most notably litters 3 and 6 (Table 2). All principal fetuses from litters 3 and 6 had pulmonary

Table 1. Virus isolation results* for porcine fetuses.

<table>
<thead>
<tr>
<th>Litter no</th>
<th>Fetal age†</th>
<th>Principal</th>
<th>Control</th>
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<tr>
<td>1</td>
<td>34:55</td>
<td>7:8</td>
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<tr>
<td>2</td>
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<td>49:66</td>
<td>5:5</td>
<td>2:5</td>
</tr>
<tr>
<td>5</td>
<td>49:68</td>
<td>5:5</td>
<td>0:3</td>
</tr>
<tr>
<td>6</td>
<td>49:70</td>
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</tr>
<tr>
<td>7</td>
<td>65:86</td>
<td>0:5</td>
<td>1:4</td>
</tr>
<tr>
<td>8</td>
<td>85:106</td>
<td>3:3</td>
<td>0:6</td>
</tr>
</tbody>
</table>

* Number of fetuses infected with virus: number of fetuses (dead or alive) in uterine horn.
† Day of gestation principal fetuses inoculated: day of gestation for necropsy of dam.

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Received for publication October 8, 1993.
lesions and 3 of 5 and 4 of 5 principal fetuses in litters 4 and 5, respectively, had lesions. The lesions were similar among pigs but differed in severity. The most severely affected pigs had marked, focally extensive hemorrhage into the mesenchymal interstitium surrounding bronchial buds, large bronchi, and blood vessels (Fig. 1). Within these areas of hemorrhage, lesions were also present in bronchial buds. There was marked segmental to circumferential necrosis of smooth muscle cells and mesenchymal spindle cells subjacent to the

<table>
<thead>
<tr>
<th>Litter no.</th>
<th>Fetal age‡</th>
<th>Principal</th>
<th>Control</th>
<th>Lesion severity‡</th>
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<tr>
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<td>Dead</td>
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<td>45:66</td>
<td>4:4</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
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<td>49:66</td>
<td>3:5</td>
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<tr>
<td>5</td>
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<td>4:5</td>
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</table>

* Number fetuses with lesions; number of fetuses (dead or alive) in uterine horn.
† Day of gestation principal fetuses inoculated; day of gestation for necropsy of dam.
‡ Pulmonary lesions were scored individually for each pig on a scale of 0–4 (4 = most severe) and reported as the average value of affected pigs in litter.
§ Fetuses were mummified.

Figure 1. Lung; fetal pig. There is marked, focally extensive hemorrhage, bronchial bud degeneration and necrosis (arrows demarcate the extent of the lesion). HE.

Figure 2. Lung; fetal pig. Bronchial bud in which there is necrosis of the smooth muscle cells surrounding the airway (arrow). There is marked congestion and hemorrhage. HE.
epithelial cells of bronchial buds (Fig. 2). Many of these buds were moderately dilated and contained small amounts of necrotic cell debris. In some of these airways, there was moderate, focally extensive necrosis of the epithelium (Fig. 3). These airways were often markedly distended and contained various amounts of necrotic cell debris. Associated with the necrotic bronchial buds were moderate numbers of mononuclear cells within the peribronchial interstitium. There were no obvious organisms or inclusion bodies. Pulmonary lesions were scored subjectively with predetermined criteria: 0 = minimal hemorrhage and congestion; 1 = mild, focally extensive hemorrhage that was perivascular involving several blood vessels; 2 = moderate, focally extensive hemorrhage that was perivascular and involving > 5 blood vessels along with multifocal necrosis of elongate cells surrounding bronchial buds; 3 = marked, focally extensive hemorrhage that was perivascular, interstitial, and intra-airway throughout the lesion and necrosis of elongate cells and epithelial cells of bronchial buds; and 4 = severe hemorrhage throughout the section along with necrosis of bronchial bud epithelial cells and surrounding elongate cells. Replicating virus was identified in all litters, indicating that fetuses could support virus replication from early gestation through term. However, the lack of lesions in fetuses inoculated at times other than 45-49 days of gestation suggests that there is a time of susceptibility during midgestation (Table 2). A less striking and consistent feature in fetuses from litters 3-6 was multifocal renal cortical hemorrhage. Random renal glomeruli from many of these fetuses had dilated and congested glomerular capillaries and numerous red blood cells in the Bowman’s space between parietal and visceral cells. In addition, there were mild multifocal areas of hemorrhage within the cortical interstitium. Similar areas of hemorrhage within both the glomeruli and interstitium were also present in control fetuses, but to a lesser degree. Litters 3-6 had an average of 5.1 areas of renal hemorrhage per section of kidney in fetuses from inoculated horns, compared with 0.38 area of hemorrhage per fetus from control horns. In addition, 18 of 22 fetuses from principal uterine horns that were alive at the time of necropsy had renal lesions, and 4 of 20 fetuses from control uterine horns had lesions. Several spleens from fetuses of both principal and control uterine horns had expansion of the red pulp by blood.

Viral antigen was observed only in tissues of fetuses that were positive by virus isolation methods. Although specific cell types containing virus antigen could not be distinguished, antigen was observed in randomly distributed foci throughout the lung and liver tissue. In the kidney, foci of antigen were localized in the renal cortex. Antigen was most easily seen in sections of lung (Fig. 4).

Diagnostic investigations of PRRS epizootics have not reported any consistent histopathologic findings in aborted fe-
tuses or neonates. Likewise, lesions have not been observed following experimental reproduction of PRRS maternal reproductive failure during late-term gestation. This report contains the first description of fetal lesions associated with natural or experimental PRRSV infection. Generally, microscopic lesions associated with viral infection were in fetuses exposed to virus between 45 and 49 days of gestation and examined 17-21 days later. Fetuses exposed to virus during early gestation (about 34 days) had replicating virus and were normal in appearance when examined 21 and 31 days post-exposure. In contrast, some fetuses exposed to virus during late gestation (65 and 85 days) had died and their deaths were attributed to PRRSV infection. In litter 7 (exposed at 65 days), all principal fetuses were mummified when examined 21 days later. Based upon the extent of mummification, they were estimated to have been dead at least 7-10 days, implying they had survived the surgical procedure and probably had supported virus replication prior to death. In litter 8 (exposed at 85 days), 2 fetuses exposed to virus were dead at the time of necropsy, 1 was undergoing mummification, and 1 had recently died. The remaining 3 principal fetuses were alive, had replicating virus, and were normal in appearance. Upon necropsy of the live principal fetuses, various amounts of thoracic fluid and edema of the abdominal viscera were observed. The gross lesions were not considered pathognomonic for PRRSV because similar lesions are frequently observed following experimental fetal infection with several virulent viruses (personal observation). The development of these lesions is thought to be a normal consequence of fetal infection prior to death of the fetus. Litter 8 was examined during late gestation at approximately the time when clinical signs of PRRS epizootics are usually observed, and it resembled field cases based upon fetal death with concomitant lack of microscopic lesions.

This report suggests that fetuses during mid to late gestation are more susceptible to the lethal effects of a PRRSV infection. One possible mechanism for this putative window of increased susceptibility may be that a lethal event occurs when some cell population(s) becomes infected during normal ontogeny. This proposed cell type may be separate from the population(s) of cells that supports viral replication during early gestation without obvious pathology. Studies are underway to test this hypothesis and other possibilities.

Acknowledgement. We thank Deb S. Adolphson for technical assistance. No endorsements are herein implied. Brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standards of the products, and the use of the names by the USDA implies no approval of the products to the exclusion of others that may also be suitable.

Sources and manufacturers

a. Tissue-Tek® O.C.T. Compound, Miles, Elkhart, IN.

References


Detection of the large latency transcript of pseudorabies virus by RNA-PCR and its potential in diagnosis

Andrew K. Cheung

Pseudorabies virus (PRV) causes a major disease in swine. Animals that recover from an infection usually become carriers of the virus in a nonproductive state, commonly known as latency. Infectious virus can be reactivated from latently infected carriers and can spread to susceptible animals. This latency-reactivation cycle enables PRV to perpetuate in the swine population. An understanding of the latency process would facilitate the development of intervention methods to eradicate PRV.

A DNA-based polymerase chain reaction (PCR) that detects the PRV genome has been developed to assess latency. However, the presence of PRV DNA is a necessary but not sufficient criterion for determining PRV latency because virus replication could be occurring at a very low level.

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Received for publication January 31, 1994.