COMPARATIVE STUDIES OF BORDER DISEASE AND CLOSELY RELATED VIRUS INFECTIONS IN EXPERIMENTAL PIGS AND SHEEP

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SUMMARY

A pestivirus originally isolated from weaner pigs was shown to be capable of infecting weaners experimentally, but without inducing significant signs of disease. When inoculated into pregnant sows and ewes in early gestation, both the porcine virus and an antigenically similar ovine border disease isolate could induce congenital infections in both species.

KEYWORDS: Pestivirus; sheep; pig; congenital infection.

INTRODUCTION

It is well recognized that ruminant strains of pestivirus [bovine viral diarrhoea virus, (BVDV), and ovine border disease virus, BDV] occasionally infect pigs where they may lead to diagnostic confusion with the related virus of classical swine fever (CSFV) (Terpstra & Wensvoort, 1988; Liess & Moennig, 1990). A pestivirus designated '87/6' was isolated in England in 1987 from weaner pigs with haemorrhagic lesions leading to suspicion of swine fever. The virus was subsequently shown to lack the antigenic markers characteristic of CSFV as defined by Edwards et al. (1991). Monoclonal antibody typing (Edwards & Sands, 1990; Paton et al., 1994), virus neutralization tests (Roehe, 1991; Edwards, unpublished data) and partial genetic sequencing (Rohe et al., 1992) have aligned the 87/6 virus most closely with the BDV group of pestiviruses. This paper describes the response of young pigs, pregnant sows and ewes to experimental infection with the virus. Parallel comparative studies were carried out using an antigenically similar BDV isolate from sheep.

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MATERIALS AND METHODS

Viruses

The non-cytopathogenic pestivirus strains '87/6' (porcine origin) and '137/4' (sheep isolate) have been described (Roehe et al., 1992). Animal inocula comprised the first or third culture passage in the PK-15 cell line for 87/6 and second culture passage in bovine turbinate cells for 137/4.

Virus isolation

Tissue suspensions were inoculated onto PK-15 or bovine turbinate cell monolayers in Leighton tubes. After 4 days' incubation at 37°C the cells were fixed in acetone for immunofluorescent labelling using a pestivirus-specific conjugate.

Serology

Virus neutralization tests were carried out on doubling dilutions of sera in microtitre plates using both the test strains and various reference strains of pestivirus as antigen, at a dose of 100 TCID$_{50}$ per well. Growth or neutralization of the non-cytopathogenic viruses was assessed by an immunoperoxidase labelling system (Holm Jensen, 1981).

Experimental animals

Weaner pigs and sows were obtained from the institute's specific pathogen free herd. Ewes were supplied from a border disease-free flock maintained at the institute. All animals were tested serologically negative for BDV neutralizing antibody before starting the experiments. The animals were housed in isolation pens, and those receiving different strains of virus were kept totally separate from each other. After inoculation, the animals were examined clinically every day for 15 days. Thereafter they were blood sampled at periodic intervals for antibody determination. In the case of pregnant sows and ewes, in order to avoid ingestion of colostrum the foetuses were removed by caesarian section under terminal anaesthesia 2 days before the date due for parturition. After clinical assessment, the offspring were also killed with barbiturate, and necropsied. Fetal development was assessed by comparison with normal parameters (Wrathall, 1972; Richardson et al., 1976) for body weight, crown-rump length, brain and cerebellum weight, and presence of growth arrest lines in long bones as seen on radiographic plates.

Inoculation schedules

Experiment 1. Four weaner pigs were inoculated intranasally with $10^{5.8}$ TCID$_{50}$ of first passage virus 87/6. Two were killed for pathological and virological study at 7 and 14 days, respectively, after inoculation. The others were retained up to 35 days for evaluation of their serological response.

Experiment 2. Eight sows were inoculated at between 34–49 days' gestation with either $10^{5.8}$ TCID$_{50}$ of third passage strain 87/6, or $10^{5.0}$ TCID$_{50}$ of strain 137/4.
Table I
Inoculation schedule for pregnant sows and ewes with pestiviruses of porcine and ovine origin

<table>
<thead>
<tr>
<th>Animal and no.</th>
<th>Age</th>
<th>Gestational age at inoculation (days)</th>
<th>Route of inoculation</th>
<th>Strain of inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow 307</td>
<td>1 year</td>
<td>46</td>
<td>Intranasal</td>
<td>87/6</td>
</tr>
<tr>
<td>Sow 305</td>
<td>1 year</td>
<td>37</td>
<td>Intranasal</td>
<td>87/6</td>
</tr>
<tr>
<td>Sow 163</td>
<td>1 year</td>
<td>34</td>
<td>Intramuscular</td>
<td>87/6</td>
</tr>
<tr>
<td>Sow 164</td>
<td>1 year</td>
<td>38</td>
<td>Intramuscular</td>
<td>87/6</td>
</tr>
<tr>
<td>Sow 303</td>
<td>1 year</td>
<td>49</td>
<td>Intranasal</td>
<td>137/4</td>
</tr>
<tr>
<td>Sow 302</td>
<td>1 year</td>
<td>37</td>
<td>Intranasal</td>
<td>137/4</td>
</tr>
<tr>
<td>Sow 304</td>
<td>1 year</td>
<td>41</td>
<td>Intramuscular</td>
<td>137/4</td>
</tr>
<tr>
<td>Sow 111</td>
<td>4 years</td>
<td>45</td>
<td>Intramuscular</td>
<td>137/4</td>
</tr>
<tr>
<td>Sow 4</td>
<td>1 year</td>
<td>NA</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>Ewe 357</td>
<td>22 months</td>
<td>41</td>
<td>Intranasal</td>
<td>87/6</td>
</tr>
<tr>
<td>Ewe 361</td>
<td>22 months</td>
<td>39</td>
<td>Intranasal</td>
<td>87/6</td>
</tr>
<tr>
<td>Ewe 813</td>
<td>20 months</td>
<td>45</td>
<td>Intramuscular</td>
<td>87/6</td>
</tr>
<tr>
<td>Ewe 779</td>
<td>20 months</td>
<td>41</td>
<td>Intramuscular</td>
<td>87/6</td>
</tr>
<tr>
<td>Ewe 329</td>
<td>21 months</td>
<td>62</td>
<td>Intranasal</td>
<td>137/4</td>
</tr>
<tr>
<td>Ewe 330</td>
<td>21 months</td>
<td>60</td>
<td>Intranasal</td>
<td>137/4</td>
</tr>
<tr>
<td>Ewe 1482</td>
<td>21 months</td>
<td>62</td>
<td>Intramuscular</td>
<td>137/4</td>
</tr>
<tr>
<td>Ewe 709</td>
<td>21 months</td>
<td>62</td>
<td>Intramuscular</td>
<td>137/4</td>
</tr>
<tr>
<td>Ewe 2661</td>
<td>22 months</td>
<td>NA</td>
<td>NA</td>
<td>none</td>
</tr>
</tbody>
</table>

NA=not applicable.

virus. The routes and timing of inoculation are detailed in Table I. One non-inoculated sow was kept and processed as a negative control.

Experiment 3. Eight ewes were inoculated at between 39–62 days' gestation with one or other of the same inocula as in experiment 2. Details are again in Table I. A further ewe was kept and processed as a negative control.

RESULTS

Porcine isolate in weaner pigs (experiment 1)
The pigs showed slight dullness, some shivering, and reduced appetites from days 6 to 11 post inoculation, and thereafter returned to clinical normality. No skin lesions were visible and the rectal temperature remained within normal limits throughout. No significant gross or histological lesions were observed in the pigs killed at 7 and 14 days. In the pig killed at day 7, virus was isolated from tonsil, parotid lymph node, lung, spleen, pancreas, ileum and muscle; other tissues (salivary gland, mandibular lymph node, thyroid, thymus, mediastinal lymph node, heart, brain, liver, kidney, mesenteric lymph node, bladder, urine and rectal swab) were negative. No virus was isolated from the tissues of the pig killed on day 14. In the
Table II
Virus and antibody status of foetuses following infection of the dam in early gestation with border disease-like pestiviruses

<table>
<thead>
<tr>
<th>Species</th>
<th>Virus strain</th>
<th>No. of litters</th>
<th>Number of foetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virus isolated:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pig</td>
<td>87/6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Pig</td>
<td>137/4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Sheep</td>
<td>87/6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Sheep</td>
<td>137/4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Includes one aborted foetus for which serology could not be completed due to cytotoxicity of the serum.
†Represents two aborted foetuses, one mummified and one autolysed, for which serology could not be carried out.

remaining two pigs, a low titre (1/10) neutralizing antibody response to homologous virus was detected from day 14, rising steeply to 1/800 or higher from day 21 onwards.

Porcine and ovine viruses in pregnant sows (experiment 2)

No clinical signs or pyrexia were observed in the 2-week observation period post-inoculation. All sows yielded live litters at slaughter. Of the four inoculated with strain 87/6, only one (sow 305) showed abnormalities in the foetuses with one stillborn piglet showing severe limb malformation, and seven piglets having hyperextension of the digits. Of the four inoculated with strain 137/4, one sow farrowed 1 day before the intended slaughter and killed her apparently normal offspring. Two of the other three sows had one mummified pig in an otherwise normal looking litter, but so did the control non-infected sow.

Morphometric data suggested a slight impairment of development in a proportion of the piglets, as evidenced particularly by low values (P<0.05) for femur length, whole brain weight, and cerebellum weight, expressed in each case as a ratio to whole body weight. In addition, growth arrest lines were observed in long bone radiographs from a number of the piglets.

All the sows (except the negative control) seroconverted by between 14 and 28 days post-inoculation to the homologous infecting virus. The peak neutralization titres were approximately 1/1000. They remained seropositive up till the end of the study. Cross-neutralizing antibodies were detected at lower titre (≤1/100) to the other of the two experimental strains, with an even weaker heterologous response (≤1/30, and in some cases ≤1/10) to BVDV and CSFV strains. Among the foetuses, only two litters (sows 305 and 163) from those inoculated with strain 87/6 contained some piglets with neutralizing antibody. All four litters from the strain 137/4 group included a proportion of seropositive pigs. With only a few exceptions, those piglets which were seropositive had very high titres against the homologous virus, generally greater than 1/1000 and in some cases 1/10 000 or more.
Pestivirus was isolated from a range of tissues in a proportion of the foetuses. The data are summarized, together with the serological status of the foetuses, in Table II.

Porcine and ovine viruses in pregnant ewes (experiment 3)

No clinical signs were shown by the ewes during the post-inoculation monitoring period. No dramatic clinical signs were observed in the lambs of the ewes given strain 87/6. Although those lambs which were born alive exhibited tremors, similar signs were observed in the control lamb so a guarded interpretation was placed on this sign. The wool coats did not differ markedly from the control lamb, and no macroscopic lesions were observed at necropsy.

In the 137/4 inoculated group, more dramatic signs were observed. Two of the ewes (nos. 330 and 709) aborted at 105 and 139 days of gestation, respectively. The two lambs from ewe 709 showed hydranencephaly and cortical cavitation, as well as a pronounced hairiness in the wool coat. The live-born lambs of the other two ewes were weak, and showed signs of torticollis and clonic muscular spasms. No gross lesions were apparent at necropsy. Morphometric analysis indicated that all the lambs in the infected groups had experienced growth retardation compared with published figures for normal values (Richardson et al., 1976).

All the inoculated ewes seroconverted to the homologous infecting virus between days 14–21 post-inoculation. Cross-reacting antibodies to the heterologous virus, and to other strains of pestivirus, appeared later and at lower titre. The ewes remained seropositive for the remainder of the study. In the strain 87/6 group, all the lambs were seronegative at birth and virus was isolated from their tissues. In the 137/4 group, the two lambs of ewe 329 were seropositive, virus negative, while those of ewe 1482 were positive for pestivirus isolation, and seronegative. One of the aborted lambs of ewe 709 was also virus positive. The results are summarized in Table II.

DISCUSSION

The aim of the studies was to determine whether the 87/6 virus, originally isolated from weaner pigs with lesions suspicious of swine fever, could infect experimental pigs and, if so, whether it would induce clinical signs and lesions. It was established in experiment 1 that weaner pigs were susceptible to acute infection with the virus as evidenced by the re-isolation of virus from tissues at 7 days post-infection, and the demonstration of seroconversion from day 14 onwards. Clinically the signs were very mild, and might have been missed altogether had the pigs not been under close daily examination. No lesions resembling those reported from the original field outbreak were detected.

A study of the history of the field outbreak suggested that disease was confined to one or two litters of weaners, and that spread of the infection in the rest of the herd was very limited, as assessed by serology. A similar pattern was observed with BVDV infections in Dutch pig herds, and was attributed to infection by the congenital route (Terpstra & Wensvoort, 1988). In the outbreak from which 87/6 strain was isolated, congenital viral infection of the affected litters was therefore
considered a strong possibility. Experiments 2 and 3 were set up to test the ability of the virus to cause congenital infection in pigs, to compare this with an antigenically similar virus of ovine origin, and finally to determine whether both viruses could also induce congenital infection in sheep.

The study has demonstrated that congenital infection in pigs can readily be induced by the porcine isolate, 87/6. However not all litters became infected, even though successful infections were established in the sows at a susceptible stage of pregnancy, as evidenced by seroconversion. The route of inoculation did not appear to play a significant role, as both intranasal and intramuscular routes induced both infected and non-infected litters. Furthermore we have shown that a border disease virus from sheep can equally successfully induce congenital infection in pregnant sows. With both viruses, the congenitally infected foetuses comprises a mixture, even within a single litter, of virus-positive and antibody-positive animals. This suggests some variability in the time at which individual foetuses became infected. Gross clinical or pathological lesions were observed only rarely in these piglets. A full clinical assessment however would have required full revival and nursing of the piglets, whereas for the purposes of this study they were killed shortly after delivery.

Finally, the porcine virus was demonstrated to be capable of inducing congenital infection in sheep, as all the lambs following infection of the ewe in early gestation were seronegative and virus positive. These would presumably, had they survived, have been specifically immunotolerant and persistently infected with the virus. The ovine isolate, as expected, induced severe signs in the inoculated sheep, including abortion in 2/4 cases with evidence of developmental damage to the nervous system of the foetuses. A true comparison of the signs and lesions induced by the two viruses in sheep cannot be made as, due to logistical reasons, the 137/4 inoculations had to be made at a slightly later stage of gestation.

Natural and experimental congenital border disease virus infections in pigs have been described before (Wrathall et al., 1978; Leforban et al., 1988; Vannier et al., 1988; Wensvoort and Tersptra, 1988). In these cases, however, the virus was either of known ovine origin (Wrathall et al., 1978; Leforban et al., 1988), or was from a suspected contaminated ovine cell culture source (Vannier et al., 1988; Wensvoort & Terpstra, 1988). The 87/6 strain has been classified as 'BDV-like' on the basis of its antigenic and genetic makeup (Edwards & Sands, 1990; Roehe et al., 1992) even though there was no history of contact with sheep in the pig herd where it was isolated. The origin of the infection in the pigs therefore remains obscure. The present study supports its classification as a BDV-like strain, as it has been demonstrated to behave similarly to a typical ovine BDV strain in infections of pregnant sows, and to be fully capable of inducing congenital infections of pregnant sheep.

ACKNOWLEDGEMENTS

We are grateful to Mr R. Bradley for carrying out the pathological examinations in experiment 1. The project was funded by the Ministry of Agriculture, Fisheries
and Food (UK). PMR was supported by grants from the British Council and the Brazilian National Research Board (CNPq).

REFERENCES


(Accepted for publication 11 March 1994)