ISOLATION OF PIG ROTAVIRUS IN FRANCE IDENTIFICATION AND EXPERIMENTAL INFECTIONS
G. Corthier, J. Cohen, R. Scherrrer

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Rotaviruses were detected in faeces from diarrheic pigs in various countries (Woode and Bridger, 1974; Rodger et al., 1975; Lece et al., 1976; McNulty et al., 1976). Few informations were available about such pigs infections in France. A preliminary serological survey in Brittany revealed a high percentage of sera containing anti-rotavirus antibodies (Corthier and Vautherot, 1979).

Rotavirus etiology in pig enteritis was not demonstrated yet in our country. It seems interesting to us to look for this virus in field occurring diarrhea and to test the pathogenicity of pig rotavirus isolates.

Abbreviations

ELISA : enzyme linked immunosorbent assay
PROF 7, PROF 16, PROF 26 : strains of pig rotavirus isolated in France.
HD piglet : hysterotomy derived piglet.
PBS : phosphate buffer saline.
SPF : specific pathogen free.

Material and Methods.

1. Source of infectious material.
Faecal samples originated from diarrheic pigs in 20 different farms of Brittany. Only one sample from diarrheic pigs at any age was taken per farm. OSU strain of pig rotavirus (Bohl et al., 1978) was kindly provided by Dr Bohl.

2. Experimental infections
New born piglets, obtained by aseptic hysterotomy, were held in isolation room containing no other animals. Piglets were fed four times daily with reconstituted cow milk (Ducluzeau et al., 1978). In our conditions, animals could be maintained healthy for three weeks at least.

For experimental infections, 4 to 24 h after birth, piglets were fed with a 5 ml of milk containing rotavirus prepared from fecal samples diluted 1/10 in PBS, clarified and passed through 0.45 μm millipore filter.
In protective feeding, piglets were fed with milk containing 10% of pig serum (anti OSU strain serum prepared on SPF pig serally injected with tissue culture virus). Normal foetal pig serum was given to controls.

3. ELISA.

Micro ELISA technique was previously described (Seigneurin et al., 1979; Scherrer et al., 1979). Immunological reagents were prepared from rabbits hyperimmunized with purified bovine rotavirus. Negative and positive controls were performed respectively with faecal samples collected from SPF pigs, faeces containing rotavirus as judged by electron microscopy or with virus suspensions obtained by infecting cell cultures with OSU strains.

Results.

1. Detection of rotavirus ex-pig in field samples.

Rotavirus antigens could be detected by ELISA in faecal materials originating from 3 out of 20 different farms. In three samples, particles having typical rotavirus morphology could be observed in electron microscopy (fig. 1). In positive samples, transmissible gastroenteritis virus was not observed in electron microscopy and virus isolation in tissue culture was not successful (3 serial passages).

Two rotavirus isolates PROF 7 and 16 were collected on diarrheic sows. The third isolate, PROF 26, originated from unweaned piglets.

Table 1. — Infection of HD piglets with rotavirus field isolates

<table>
<thead>
<tr>
<th></th>
<th>PROF 7</th>
<th>PROF 16</th>
<th>PROF 26</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infected piglets</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Contact controls</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Elisa titer* in feces collected at</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>1/2600</td>
<td>1/1200</td>
<td>1/900</td>
<td>&lt; 1/20</td>
</tr>
<tr>
<td>day 2</td>
<td>1/2600</td>
<td>1/450</td>
<td>1/900</td>
<td>&lt; 1/20</td>
</tr>
<tr>
<td>day 3</td>
<td>1/260</td>
<td>1/80</td>
<td>1/900</td>
<td>&lt; 1/20</td>
</tr>
<tr>
<td>Death (4 to 5 days post infection)</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>0/2</td>
</tr>
</tbody>
</table>

* a : average titer of the group

2. Experimental infections of hysterotomy derived piglets with field rotavirus isolates.

Bacteria-free isolates were inoculated orally to 6 piglets (2 per isolate and per cage). In each cage one piglet was kept as contact control (table 1). Diarrhea appeared in infected piglets one day post infection. The aspect of faeces during disease was similar to previous descriptions (Woode and Bridger, 1974): watery the first day, milky-like the 2nd and the 3rd day of diarrhea. Contact controls develop...
Ped disease later than infected piglets. All animals died 4 to 5 days post infection. Rotavirus antigen production appeared at the very beginning of disease and persisted till animal death (table 1)..

Uninfected piglets kept in a separate cage remained healthy during the whole experiment: Infection of piglets with OSU strain lead to similar disease.

3. Protective feeding.

Four piglets were infected with one field isolate (PROF 7) and fed either with normal foetal pig serum or antiserum against OSU strain (table 2). In the first group, diarrhea occurred as previously described. In the second group, as in uninfected piglets (kept in a separate cage), the animals remained healthy, no rotavirus antigens could be detected in faeces.

Discussion

We have been able to demonstrate the presence of rotavirus in pig diarrheic faeces by Elisa and electron microscopy. The 3 field isolates were pathogenic for HD new born piglets. Clinical signs and mortality are similar to previous descriptions in other countries (Tzipori and Williams, 1978; McNulty et al., 1976; Lecce et al., 1976).

Performing Elisa with anti bovine rotavirus conjugate do not implies a bovine origin of the virus isolates since this technique detects internal antigen common to rotavirus from several animal species.

An antigenic relationship exists between American pig rotavirus (OSU strain) and our French isolates as demonstrated by protective feeding experiments. This result underline ubiquitous nature of rotavirus.

Because of the limited number of specimens hitherto examined for rotavirus, it is not possible to estimate the economic impact of among pigs infection rotavirus in France. It is clear however that rotavirus disease occurs in pigs and may therefore play an important aetiologi- cal role.

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Acknowledgements.

We wish to thanks Dr Ravaud who provided us H-D piglets, Dr Aynaud for transmissible gastroenteritis detection assay and Dr Vannier and Dr Gosselin for providing field samples.

Summary

Rotavirus had been demonstrated in France, in diarrheic pig faeces by electron microscopy and ELISA. Field isolates were pathogenic for HD piglets and antigenically related to American OSU rotavirus as demonstrated by protective feedings experiments.

<table>
<thead>
<tr>
<th>Table 2. -- Protective feeding with anti rotavirus sera against diarrhea induced after infection with PROF 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection with rotavirus</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>anti OSU pig serum*</td>
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<tr>
<td>Foetal pig serum*</td>
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<tr>
<td>Control</td>
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<tr>
<td></td>
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<tr>
<td>Number of piglets</td>
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<td>day 2</td>
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<tr>
<td>day 3</td>
</tr>
<tr>
<td>Death (4 to 5 days post infection)</td>
</tr>
</tbody>
</table>

a : added in the milk ; b : average titer of the group.
References


