Enzootic pneumonia in feeder pigs: Association with transmissible gastroenteritis virus infection

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Abstract
Infection with transmissible gastroenteritis virus (TGEV) was present in some pigs on arrival at a feeder pig farm and was well established three weeks later. TGEV infection preceded Mycoplasma hyopneumoniae infection, which was not detected until three weeks after arrival. Severe lesions of enzootic pneumonia were observed at the end of the fattening period.

A trial was subsequently done in six-week-old pigs in order to evaluate the potentiating effect of TGEV infection on experimental M. hyopneumoniae infection. The effects of mycoplasmal infection were aggravated when associated with TGEV infection as determined by the extent of pneumonia lesions observed two weeks later.

Résumé
Pneumonie enzootique chez les porcs charcutiers : association avec le virus de la gastro-entérite transmissible
L'infection à virus de la gastro-entérite transmissible (VGET) était présente chez quelques sujets dès l'arrivée des porcs dans une ferme de porcs charcutiers et était généralisée trois semaines plus tard. L'infection à VGET précédait l'infection à Mycoplasma hyopneumoniae qui était non décelable à l'arrivée des porcs mais présente trois semaines plus tard. Des lésions sévères de pneumonie enzootique étaient observées à la fin de la période d'engraissement. Un essai a subéquemment été effectué chez des porcs de six semaines afin d'évaluer le rôle potentiel d'une infection par le VGET sur une infection expérimen tale à M. hyopneumoniae. L'association du VGET au M. hyopneumoniae accroit les lésions normalement produites par ce dernier chez les porcs examinés deux semaines après l'infection.


Introduction
In a previous study we found that each of eight swine herds surveyed was infected with many viruses as determined by virus isolation and serology. Transmissible gastroenteritis virus (TGEV) infection was the only viral infection that could be related to the severity of the clinical signs of enzootic pneumonia, the pneumonia scores, and the feed efficiency. The only reported study on the sequential appearance of viral and mycoplasmal infections in feeder pig herds is one longitudinal survey on selected viral infections in two herds affected with respiratory diseases (1). The pathogenicity of some viruses in the respiratory tract in experimental pigs has been reported (2-7). Pneumonia of greater severity has been produced by simultaneous inoculation with Mycoplasma hyopneumoniae and a porcine adenovirus (8). No other experimental study has been reported on the potentiating effect of viruses on experimental mycoplasmal infection in swine.

In this investigation we examined a) the sequential appearance of TGEV, M. hyopneumoniae and their corresponding antibodies in one piggery with a persistent enzootic pneumonia problem, and b) the possible potentiating effect of TGEV on experimental infections with M. hyopneumoniae in six-week-old pigs.

Materials and methods
Herd survey
The sequential appearance of TGEV and M. hyopneumoniae and their corresponding antibodies was examined in one lot of feeder pigs on a farm with severe enzootic pneumonia in successive lots of pigs. Blood samples and lung tissues were collected from five eight-week-old piglets at arrival (day 0), from five pigs three weeks later (day 21), and from ten pigs at slaughter (day 168). Samples of lung tissue were homogenized and cultured by the method described by Armstrong and Friis (9) for the recovery of M. hyopneumoniae. Homogenized lung tissue samples were also inoculated on thyroid and PK cell monolayers and into chick-embryos for virus recovery. The TGEV isolates were identified by immunoelectron microscopy using the method described by Anderson and Doane (10). Mycoplasma hyopneumoniae isolates were identified by fluorescent antibody test as described by Giger et al (11). Antibodies were detected by seroneutralization for TGEV (12) and by western blot as described by Young and Ross for M. hyopneumoniae (13); specific seropositivity was scored when a stained band was visible on the 120 K protein of M. hyopneumoniae. Pulmonary lesions typical of enzootic pneumonia were scored by using the method described by Morrison et al (14). The mean pneumonia score was calculated by summing the individual scores divided by the number of lungs examined. Portions of tissue, adjacent to those which were taken for culture, were placed in 10% formal saline for histopathological examination. Sections were stained by hematoxylin and eosin and examined microscopically for lesions typical of M. hyopneumoniae (14) and TGEV infections (5).
Trial

A trial was carried out in 30 six-week-old piglets originating from a herd free of *M. hyopneumoniae*, TGEV, hemagglutinating encephalomyelitis virus, porcine cytomegalovirus, parvovirus, reovirus type 3, porcine adenovirus type 4 and swine influenza virus infections. This trial was done to evaluate the possible potentiating effect of TGEV on an experimental infection with *M. hyopneumoniae*. Piglets were assigned randomly into groups and housed separately under conventional conditions and special care was taken to avoid cross-contamination between them. Guidelines given in “Guide to the Care and Use of Experimental Animals, Volume 1” Canadian Council on Animal Care, Ottawa, Ontario were followed. Piglets were observed daily for clinical signs and were necropsied on day 14.

*Mycoplasma hyopneumoniae* strain IAF-55 isolated in our laboratory from the lungs of a pig with enzootic pneumonia was used in this trial. This strain was characterized by a fluorescent antibody test as described by Giger et al (11) and it was shown to produce lesions as described for *M. hyopneumoniae* by Ross (15). The isolate was grown on Friis broth medium (16), the culture harvested at pH 6.8 and used as such for spray administration.

Virulent Purdue strain of TGEV obtained from G. Dulac (Animal diseases Research Institute, Agriculture Canada, Nepean, Ontario) was used in this trial. TGEV was cultured on pig thyroid cell monolayers maintained in Eagle's minimum essential medium. Suspensions were used for intranasal administration at a concentration of 3000 TCID 50 per mL.

Piglets receiving *M. hyopneumoniae* alone were given 0.25 mL of suspension mixed with 0.25 mL of the culture medium. Piglets receiving a mixture of TGEV and *M. hyopneumoniae* received 0.25 mL of each suspension. The suspensions were administered intranasally using a DeVilbiss nebulizer, model 40, (DeVilbiss Ltd., Barrie, Ontario, Canada). Control pigs received 0.5 mL of an aliquot of the TGEV and *M. hyopneumoniae* growth culture media.

Results

Herd survey (Table 1)

Transmissible gastroenteritis virus was isolated from one of five lungs examined at day 0. *M. hyopneumoniae* was not recovered from these lungs. Three weeks later (day 21), TGEV isolates were obtained from three of five lungs examined and *M. hyopneumoniae* isolates were recovered from all five lungs. No other virus was recovered from these samples. Viruses or mycoplasmas were not recovered from ten lungs collected at slaughter (day 168).

Transmissible gastroenteritis virus serum antibodies were detected on days 0, 21 and 168; the mean antibody titer was higher on day 21 than on days 0 and 168 (Table 1).

No clinical sign was noted in pigs on arrival in the piggery (day 0). Diarrhea was present in pigs between days 7 and 21 and lasted for two to three days. Severe coughing was noted in the herd on day 21 but diminished later. Lesions of enzootic pneumonia were absent in the five pigs necropsied on arrival and present to various extent in four out of five lungs examined on day 21. Severe lesions were observed in each of the ten lungs examined on day 168 (average pneumonia score of 14.6 ± 5.1).

Trial

Detection of pulmonary lesions and their severity are summarized in Table 2. Pigs given *M. hyopneumoniae* alone had occasional coughing and sneezing. Pigs given a mixture of TGEV and *M. hyopneumoniae* were diarrheic and prostrate for two to three days and

### Table 1

Results of sequential microbiological and clinical assessment of a feeder pig herd

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>0</th>
<th>21</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates&lt;sup&gt;a&lt;/sup&gt; TGEV</td>
<td>1/5</td>
<td>3/5</td>
<td>0/10</td>
</tr>
<tr>
<td>M. hyopneumoniae</td>
<td>0/5</td>
<td>5/5</td>
<td>0/10</td>
</tr>
<tr>
<td>Antibodies&lt;sup&gt;b&lt;/sup&gt; TGEV</td>
<td>3/5 (23)</td>
<td>5/5 (222)</td>
<td>10/10 (69)</td>
</tr>
<tr>
<td>M. hyopneumoniae</td>
<td>0/5</td>
<td>0/5</td>
<td>10/10</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>none</td>
<td>diarrhea, severe coughing</td>
<td>sporadic coughing</td>
</tr>
<tr>
<td>Pulmonary lesions&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/5</td>
<td>4/5</td>
<td>10/10</td>
</tr>
<tr>
<td>Pneumonia score&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>7.9 ± 6.3</td>
<td>14.6 ± 5.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of pigs infected/Number of pigs examined

<sup>b</sup>Number of pigs infected/Number of pigs examined (reciprocal of geometric mean titer)

<sup>c</sup>Number of pigs with lesions/Number of pigs necropsied

<sup>d</sup>Mean ± SD
respiratory signs were not easily detectable. Pigs given both organisms had more extensive and widespread macroscopic and microscopic lesions than those given M. hyopneumoniae alone. The control group did not show clinical signs or pulmonary lesions. Antibodies to TGEV were detected two or three weeks postinfection in sera of all pigs given a mixture of TGEV and M. hyopneumoniae. Antibodies to M. hyopneumoniae were detected only in some sera of pigs infected with this microorganism; all pigs in the control group were negative.

Discussion

Previous observations led to the hypothesis that TGEV could be associated with M. hyopneumoniae in aggravating enzootic pneumonia in feeder pigs. In the present herd survey it was found that TGEV infection was present in some piglets at arrival in the piggery and was fully established three weeks later as determined by virus isolation and serology. We could not recover TGEV from lungs at slaughter although TGEV antibodies were still present. On the other hand, M. hyopneumoniae was not isolated on arrival but was recovered from each of the five lungs examined two weeks later. Antibodies to M. hyopneumoniae were neither detected on arrival nor on day 14 but were present in the ten sera collected at slaughter. No virus other than TGEV was recovered in the samples examined. These observations suggest that TGEV infection precedes active mycoplasmal infection and consequently could predispose the respiratory tract to the invasion of M. hyopneumoniae in carrier pigs.

Experimental infection showed that TGEV in association with M. hyopneumoniae increased the severity of clinical signs and the pulmonary lesions induced by the latter organism. These results add weight to the suggestion that TGEV infection, when present, should be considered a potentiating cofactor in determining the extent of development of the enzootic pneumonia process.

The diarrhea observed in this group is believed to be associated with the use of SPF piglets. Previous trials using conventional piglets receiving either TGEV alone or associated with M. hyopneumoniae did not show such gastrointestinal disturbance. Mechanisms by which infection with TGEV apparently facilitates M. hyopneumoniae infection have not been proposed but may include stress consequent to the gastrointestinal disturbance and microscopic pulmonary lesions produced by this virus. The results of this study and those of Kasza (8) support the hypothesis of a contributing role of viruses in the pathogenesis of enzootic pneumonia and should be further investigated.

References