Pathogenicity of three isolates of porcine respiratory coronavirus in the USA

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The pathogenicity of three isolates of porcine respiratory coronavirus (AR310, LEPP and 1894) from the USA was assessed in specific pathogen-free pigs. Pigs inoculated with 1894 developed mild respiratory disease and pigs inoculated with AR310 and LEPP developed moderate respiratory disease from four to 10 days after they were inoculated, but all the pigs recovered fully by 14 days after inoculation. Gross and microscopic examination revealed mild (1894) to moderate (AR310 and LEPP) multifocal bronchointerstitial pneumonia from four to 10 days after inoculation. The lesions were characterised by necrotising bronchiolitis, septal infiltration with mononuclear cells, and a mixed alveolar exudate. No clinical signs or microscopic lesions were observed in control pigs that had not been inoculated.

PORCINE respiratory coronavirus (PRCV) was first isolated in 1984 from pigs in Belgium (Pensaert and others 1986) and in 1989 in the USA (Hill and others 1990). The virus is a deletion mutant of transmissible gastroenteritis coronavirus (TGEV) with tropism for the respiratory tract (Laude and others 1993), and it replicates in epithelial cells of the naso mucosa, tonsils and lungs (O'Toole and others 1989, Cox and others 1990).

European isolates of PRCV have been found to induce lesions of interstitial pneumonia without clinical respiratory disease in hysterectomy-derived, colorectum-deprived (HDCC) pigs inoculated by aerosol (Pensaert and Cox 1989) or oronasally (O'Toole and others 1989). Similarly, the AR310 isolate from the USA has been shown to induce moderate interstitial pneumonia in gnotobiotic pigs inoculated intranasally, without evidence of clinical respiratory disease (Halbur and others 1993). The ISU-1 isolate from the USA did not induce respiratory disease either in its herd of origin or in HDCC pigs inoculated oronasally (Wesley and others 1990). A French isolate of the virus induced mild respiratory disease and lesions of interstitial pneumonia in HDCC pigs (Vannier 1990) and in 25 kg specific pathogen-free (SPF) pigs inoculated intratracheally (Duret and others 1988). An isolate from Quebec, Canada, produced clinical signs of polyneuritis and dyspnoea and bronchointerstitial pneumonia in eight-week-old piglets inoculated intratracheally (Jabrane and others 1994).

The tropism of TGEV and PRCV is thought to be influenced by the spike (S) protein, and it has been suggested that the open reading frame (ORF) 3/3a gene of the two viruses may play a role in their virulence (McGoldrick and others 1999, Kim and others 2000). The AR310 and LEPP isolates from the USA have a 621 base pair (bp) deletion in the S protein (Vaughn and others 1994) which is different from the 681 bp deletion of the ISU-1/Ind-89 (Wesley and others 1991) and 1894 (Vaughn and others 1994) isolates and the 672 bp deletion of the French isolate RM1 (Rasschaert and others 1990).

This paper describes experiments to compare the pathogenicity of three isolates of PRCV from the USA (AR310, LEPP and 1894) that have been well characterised molecularly.

MATERIALS AND METHODS

Source of isolates

Isolate AR310 was isolated from a 300-sow farrow-to-feeder pig herd in Arkansas (Vaughn and others 1994) which was investigated because it had a history of endemic transmissible gastroenteritis (TGE) problems in nursery pigs. The clinical signs, microscopic enteric lesions, and repeated positive fluorescent antibody tests for TGEV on the pigs’ intestines had confirmed the diagnosis of TGE. Respiratory disease was not a major problem in the herd.

Isolates LEPP and 1894 were isolated from nasal swabs from two herds in Iowa that had antibodies to TGEV but had not shown signs of diarrhea and were therefore suspected of having a PRCV infection (Vaughn and others 1994). Isolate LEPP was from a 120-sow farrow-to-finish herd in east-central Iowa with a history of moderate endemic respiratory disease in nursery pigs. Isolate 1894 was from a 600-sow farrow-to-feeder pig herd in north-central Iowa with severe endemic respiratory disease in nursery pigs.

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Virus preparation
The isolates were cultivated by inoculating them on to a swine testis cell line (Halbur and others 1993), and they were plaque purified three times. The challenge doses were adjusted to be 107 plaque-forming units of each virus in 10 ml of inoculum.

Specific pathogen-free pigs
Thirty-two five-week-old SPF pigs were obtained from a herd which was free from TGEV, PRCV, pseudorabies virus, porcine reproductive and respiratory syndrome virus (PRRSV) and Mycoplasma hyopneumoniae.

Experimental design
The SPF pigs were randomly divided into four groups of eight. Pigs in three groups were inoculated intranasally with 107 plaque-forming units of isolates AR310, LEPP or 1894, and the remaining group was inoculated with an equal volume of cell culture medium. The inoculations were made by slowly dripping the material into the nares. The pigs were monitored daily for signs of enteric or respiratory disease; nasal and rectal swabs were taken after four, seven, 10 and 28 days, and on the same days two of the pigs from each group were euthanased and examined postmortem. The lungs were examined grossly and given a score to estimate the percentage of lung with pneumonia. Tissues from the respiratory and enteric systems were collected for virus isolation and samples were placed in 10 per cent neutral buffered formalin for histopathology.

Virus isolation and titration from experimental pigs
Attempts were made to isolate the viruses from the lungs, small intestines, and nasal and rectal swabs. Samples of the lungs or small intestines were homogenised in Eagle's minimal essential medium (20 per cent w/v), clarified by centrifugation at 1000 g for 10 minutes and filtered through a 0.22 μm filter. Confluent three- to five-day-old monolayers of swine testis cells in 25 cm2 flasks were inoculated with 0.2 ml of the filtrate. After incubation for one hour at 37°C, the monolayer was washed, fresh culture medium was added, and the cells were observed for a cytopathic effect. If no cytopathic effect was observed, the cells in the flask were frozen and thawed three times and the cell lysates were inoculated on to new monolayers. The samples were passed three times. The presence of TGEV was confirmed by indirect immunofluorescence, using gnotobiotic pig antisera to the Miller strain of TGEV and mouse anti-TGEV-5 monoclonal antibodies (Zhu and others 1990). The virus was titrated by the inoculation of swine testis monolayers with 10-fold dilutions of the tissue filtrates; the starting concentration was the 20 per cent w/v tissue filtrate.

Microscopic examination
Samples of lung, nasal turbinate, duodenum, jejunum, ileum and colon of all of the pigs were collected in 10 per cent neutral buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin. The lungs were inflated with formalin at the time of postmortem examination by injecting the solution into the trachea until they were fully inflated, and then the trachea was tied off and the entire lung submerged. The sections of lungs were examined blindly and assigned two scores: one for the severity of interstitial pneumonia on a scale of 0 to 6, as described by Halbur and others (1995, 1996), and the other on a scale from 0 to 3 for the severity of necrotising bronchiolitis and squamous metaplasia of the airway epithelium (0 = no lesions, 1 = mild, 2 = moderate, and 3 = severe lesions).

RESULTS

Clinical disease
The pigs inoculated with isolates AR310 and LEPP showed signs of moderate respiratory disease from four to 10 days after inoculation but had recovered by 14 days; the disease was characterised by dyspnoea, tachypnoea, mild coughing, lethargy, fever and anorexia. The pigs inoculated with isolate 1894 showed very mild signs of respiratory disease from four to seven days after inoculation. The control pigs remained normal.

Gross and microscopic lesions
Gross lesions of tan to purple-coloured consolidation, with irregular borders, were most commonly found in the middle and accessory lobes (Fig 1). The pigs inoculated with isolates AR310 and LEPP had more severe lesions than the pigs inoculated with 1894 (Table 1).

The scores of the microscopic lesions are summarised in Table 1. The pigs inoculated with AR310 and LEPP had moderate multifocal bronchointerstitial pneumonia from four to 10 days after they were inoculated. The lesions were characterised by bronchial and bronchiolar epithelial necrosis and squamous metaplasia, peribronchiolar and perivascular accumulations of mononuclear cells, septal infiltration with mononuclear cells, type II pneumocyte proliferation, and a mixed inflammatory exudate in the airways and alveolar spaces. There was also mild patchy dysplasia of the epithelium of the nasal turbinate, with a loss of cilia and mild sub-
mucosal lymphomacrophagic inflammation. No damage to the turbinates or bronchiolar epithelia was observed in the pigs inoculated with 1894, and their infected lungs had only a mild multifocal septic infiltration with mononuclear cells and a mild mixed alveolar exudate (Fig 2). No microscopic lesions were observed in the control pigs.

**Virus isolation**

Four days after they were inoculated, virus was isolated from the nasal swabs of all 24 infected pigs, seven days after seven days from six of the remaining 18, and after 10 days from only one of the remaining 12 pigs. Virus isolation from the lungs was less successful; it was isolated from the lungs of three of six infected pigs four days after they were inoculated, from one of six after seven days, and from none of six after 10 days. No virus was isolated from the lungs or nasal swabs of the control pigs, and none was isolated from the intestines of the control or infected pigs.

**DISCUSSION**

This is the first report of the reproduction of respiratory disease in SPF pigs infected with single isolates of PRCV derived from the USA. Other studies with Canadian (Jabrane and others 1994) and French (Vannier 1990) isolates have obtained evidence of clinical disease and interstitial pneumonia. Together, these findings confirm that some isolates of PRCV, such as AR310 and LEPP, should be considered to be primary respiratory pathogens in young growing pigs. The experimentally induced lesions appear to be severe enough at least to predispose pigs to secondary bacterial or mycoplasmal infections or to play a role in combined viral infections with, for example, PRCV and PRRSV (Van Reeth and others 1996, Hayes and others 1998), or PRCV and swine influenza virus (Van Reeth and others 1996).

Two of the isolates from the USA, AR310 and LEPP, appear to be more virulent than those described in Europe (Pensaert and others 1986, O'Toole and others 1989, Pensaert and Cox 1989, Vannier 1990), possibly as a result of differences in the sites of replication of the European and American isolates. European isolates apparently replicate primarily in alveolar epithelial cells (Cox and others 1990), whereas the American isolates appear to replicate predominantly in bronchial and bronchiolar epithelial cells (Sirinarumit and others 1996).

There were differences in the pathogenicity of the three isolates of PRCV in the five-week-old SPF pigs; isolates AR310 and LEPP induced moderate disease and moderate broncho-interstitial pneumonia, whereas 1894 induced very mild disease and only mild multifocal interstitial pneumonia. Isolates AR310 and LEPP have a 621 bp deletion in the S protein (Vaughn and others 1994), whereas the less virulent strain 1894 has a 681 bp deletion (Vaughn and others 1994), as has ISU-1/1nd-89 (Wesley and others 1991); another less virulent European isolate, RM4, has a 672 bp deletion (Rasschaert and others 1990). The virulent strains examined to date have an intact ORF3/3a gene, whereas the non-virulent strains have an altered ORF3/3a gene which may make the gene or its product non-functional (Vaughn and others 1995). Isolates AR310 and LEPP have an intact ORF3/3a gene, whereas 1894 has a 23-nucleotide deletion (Vaughn and others 1995). These results support the suggestion by Vaughn and others (1995), McGoldrick and others (1999) and Kim and others (2000) that the genetic differences between the isolates may contribute to the observed differences in their pathogenicity.

The virus isolation results indicate that PRCV persists for only a short period in the lungs but can be isolated more readily and for a longer period from nasal swabs. It was not possible to isolate the virus from the small intestine of the experimentally inoculated pigs, suggesting that it may replicate to only a limited extent in the gut. In the authors' experience, the best specimens to take from field cases are nasal swabs from acutely affected pigs.

The results of this work suggest that there are differences in pathogenicity between these three isolates of PRCV from the USA. Two of them should be considered as primary pathogens in growing pigs, but all three isolates induced enough damage to the lungs of the pigs to predispose them to more severe disease if they became infected with other viruses or bacteria.

![FIG 2: Microscopic sections of lungs from (a) a normal control pig (x 80), (b) a pig four days after it was inoculated with the low virulent isolate 1894 (x 80), (c) a pig four days after it was inoculated with the moderately virulent isolate LEPP showing moderate multifocal septal infiltration (x 80), and (d) mild necrotising bronchiolitis (x 200). Haematoxylin and eosin](image)
Effects of journey and lairage time on steers transported to slaughter in Chile

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Steers representative of the most common type, weight and conformation slaughtered in Chile were transported for either three or 16 hours and held in lairage for three, six, 12 or 24 hours. Measurements of liveweight, carcase weight, and the postmortem pH and colour of muscle were made to assess the economic and welfare effects of the different transport and lairage times. Compared with the short journey, the longer journey was associated with a mean (se) reduction in liveweight of 8·5 (2·8) kg, and there was a further decrease of 0·42 (0·18) kg for every hour that the animals were kept in lairage after 16 hours of transport, an increase in final muscle pH, a decrease in muscle luminosity and an increase in the proportion of carcases downgraded because they were classified as ‘dark cutting’. The carcase weights also tended to be lower after the longer journey and after longer periods in lairage.

IN Chile, cattle generally undergo a long road journey to slaughter because, while animal production takes place all over the country, most of the slaughterhouses are situated in the capital, Santiago, where the majority of the population live (Gallo and others 1995, Matic 1997). National regulations limit the journey time to 24 hours without a break (Anon 1993a), but this limit is frequently exceeded (Gallo and others 1995), so that animals are often deprived of food and water for long periods. According to Chilean national regulations (Anon 1994a), after the animals arrive at the slaughterhouse

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