CORONAVIRUS INFECTION OF THE BOVINE RESPIRATORY TRACT

M.S. MCNULTY, D.G. BRYSON, G.M. ALLAN and E.F. LOGAN Veterinary Research Laboratories Stormont, Belfast BT4 3SD (Northern Ireland) (Accepted 19 March 1984)

ABSTRACT

McNulty, M.S., Bryson, D.G., Allan, G.M. and Logan, E.F., 1984. Coronavirus infection of the bovine respiratory tract. Vet. Microbiol., 9: 425-434.

Two viruses, morphologically resembling coronaviruses and antigenically indistinguishable from bovine enteric coronavirus, were isolated in bovine tracheal organ cultures from the lungs and trachea of young calves with respiratory disease. Intranasal and intratracheal inoculation of these viruses into neonatal calves resulted in a predominantly upper respiratory tract infection, which was associated with the development of mild respiratory symptoms.

INTRODUCTION

Coronaviruses are well established as enteric pathogens in young calves (Tzipori, 1981). However, there is only one report of the isolation of coronaviruses from the bovine respiratory tract (Thomas et al., 1982). This paper describes the isolation of coronaviruses from the lower respiratory tracts of calves with respiratory disease and the experimental infection of young calves with these isolates via the respiratory route.

MATERIALS AND METHODS

Antisera

A chicken antiserum to bovine enteric coronavirus, a bovine antiserum to bovine virus diarrhoea/mucosal disease (BVD) virus and rabbit antisera to bovine respiratory syncytial virus and parainfluenza virus type 3 were prepared and conjugated with fluorescein isothiocyanate as described previously (McNulty et al., 1983). A rabbit antiserum to a strain of bovine enteric coronavirus isolated in bovine tracheal-organ culture at this laboratory was prepared using the same inoculation schedule as for respiratory syncytial virus. This antiserum was used in the immunofluorescence studies of experimentally-infected calves.

Electron microscopy

Negative contrast electron microscopy was carried out on culture media harvested from tracheal-organ cultures and on samples of nasal mucus as follows. Cells in the samples were deposited by centrifugation at 1000 g for 10 min. A few drops of water were added to the cell pellet and the mixture was subjected to 2 cycles of freezing and thawing. The resulting lysate was mounted on a 400-mesh carbon-coated grid, stained with methylamine tungstate and examined in a Philips 301 electron microscope. Faeces from experimentally-infected calves were examined by method C of McNulty et al. (1979). Cell pellets from tracheal-organ cultures were fixed in 4% glutaraldehyde, post-fixed in osmium tetroxide and processed for thin section electron microscopy as previously described (McNulty et al., 1976).

Haemagglutination (HA) and haemagglutination inhibition (HI) tests

HA tests were carried out at room temperature in plastic WHO plates using 0.25 ml volumes of virus and 1% rat erythrocytes. In the HI tests, 4 HA units of virus were incubated with doubling dilutions of antiserum for 1 h at room temperature, before the addition of 1% rat erythrocytes. A local isolate of bovine enteric coronavirus which had been propagated in bovine tracheal-organ cultures was used as antigen.

Experimental infection

Two isolates of coronavirus retained from field cases (see results for details) were used to infect calves experimentally. Isolate 81-1184 was recovered from the lungs of a 12-day-old calf whilst the second isolate (82-1398) was from the trachea of a 3-4-week-old calf.

These isolates were propagated in tracheal-organ cultures prepared from bovine foetuses near term and from newborn calves as described by McNulty et al. (1983). Calves were inoculated with virus which had been passaged between 3 and 6 times in tracheal-organ culture. The medium from the organ cultures was harvested every 2–3 days and tested for HA. Those harvests with HA titres greater than 64 against rat erythrocytes were pooled and used as experimental inocula.

Calves were purchased from local dairy farms. Management was similar to that described by Bryson et al. (1978). Samples of blood and nasal mucus, and nasopharyngeal swabs were collected from each calf on arrival at the laboratory. A total of 7 calves was used, 5 were colostrum-deprived and the other 2 had received colostrum. All calves were under 7 days old when inoculated with coronavirus. Immediately before inoculation with coronavirus, and approximately every 2 days afterwards, samples of nasal mucus and nasopharyngeal swabs were taken. Nasal mucus was collected using a portable suction apparatus. Nasopharyngeal swabs, blood-, nasal mucus-, trachea- and lung-samples were taken at necropsy.

⊢	-
6	4
-	1
ρ	٩
<	C
E	-

coronaviruses
with
infections
Experimental

	זאָ צׂ איז איז מיני	5 7 V G
ສຸທິດ ຕໍ່ເ ² ຫຼື ຫຼື	2 0 H H 0	10 5, 2, 2, 2, 1,

a pii = post-initial inoculation; b Colostrum-fed; c Not done; d Infected with fungus.

Four calves were inoculated with isolate 81-1184. Two were inoculated with isolate 82-1398 and from 1 of these calves (Calf 6, Table I), the virus was re-isolated in tracheal-organ cultures and used as an inoculum for another calf. Calves were inoculated using a combined intranasal and intratracheal route, twice daily for 4 consecutive days. The morning inoculation consisted of 10 ml of virus given intratracheally and 10 ml intranasally. In the afternoon 10 ml of virus were given intranasally. In a previous study (Bryson et al., 1983), administration of the same volume of harvests from uninfected cell cultures by an identical inoculation schedule did not produce symptoms of respiratory disease or respiratory tract lesions, showing that the inoculation schedule per se is not harmful.

Virus examination

Samples of nasal mucus and cryostat sections of lung were processed for immunofluorescent staining as described by McNulty et al. (1983). Specimens for virus isolation were homogenised in Eagle's minimal essential medium containing 1000 IU of penicillin ml⁻¹, 1000 μ g of streptomycin ml⁻¹ and 100 units of mycostatin ml⁻¹ and inoculated into organ cultures of bovine trachea or into a semi-continuous line of bovine foetal lung cells (McNulty et al., 1983).

Mycoplasma and bacteria examination

Samples were examined as described by Bryson et al. (1978).

RESULTS

History of coronavirus isolates

Isolate 81-1184 was from the lungs of a 12-day old Friesian calf with a history of ill-thrift. At necropsy, there was evidence of pneumonia, pericarditis, pleurisy and septicaemia. Histological examination of the lungs revealed exudative bronchopneumonia. No epithelial syncytia or inclusion bodies were seen. Immunofluorescent examination of cryostat sections of lung with an antiserum against bovine enteric coronavirus revealed foci of cells showing cytoplasmic fluorescence (Fig. 1). Immunofluorescent staining with BVD virus antiserum also revealed the presence of BVD virus antigens in scattered cells throughout the lung parenchyma. No fluorescence was observed when antisera against respiratory syncytial virus or parainfluenza virus type 3 were used. BVD virus was isolated from lung specimens in foetal bovine lung cells. Following inoculation of bovine tracheal-organ cultures with lung material, a coronavirus (81-1184) was isolated. This material was treated with antiserum to BVD to eliminate possible contamination with this virus. Salmonella dublin was isolated from the lungs and from other organs.



Fig. 1. Focus of cells showing cytoplasmic fluorescence in lung parenchyma of 12-dayold calf with pneumonia. Direct staining with conjugated antiserum to bovine enteric coronavirus.

The second isolate (82-1398) was from the trachea of a 3-4 week old calf, one of a group of 50 Friesian calves, which had been on a calf-rearing unit for 10-14 days. The group history was of inappetance, unthriftiness and coughing. On clinical examination, most calves had respiratory rates of $30-40 \text{ min}^{-1}$; a few were moderately tachypnoeic with respiratory rates of $60-70 \text{ min}^{-1}$. Rectal temperatures were normal. Coronavirus antigen was detected by immunofluorescence in 5 of 6 samples of nasal mucus collected during the visit. Over the next few days an outbreak of diarrhoea involving about 40% of the calves occurred. The situation gradually returned to normal within a week.

Three days after the visit a calf was purchased, This calf was dull, had an induced cough and a respiratory rate of 45 min^{-1} . Harsh lung sounds were detected cranio-ventrally. There was no evidence of diarrhoea and the rectal temperature was normal. At necropsy, areas of consolidation were present at the periphery of the right apical and both cardiac lung lobes. Histologically, parenchymal collapse and small amounts of purulent exudate in some bronchioli were evident in the lungs and a mild tracheitis was also present. Coronavirus antigens were detected by immunofluorescence in a tracheal scraping, but not in nasal mucus nor cryostat sections of lung. 'A coronavirus (82-1398) was isolated in bovine tracheal organ culture from the trachea. *Mycoplasma dispar* was also isolated from the trachea. Isolates 81-1184 and 82-1398 were classified as coronaviruses for the following reasons. Coronavirus-like particles about 100 nm in diameter and surrounded by a layer of surface projections 17-22 nm in length were detected by negative contrast electron microscopy in harvests of infected tracheal-organ cultures (Fig. 2). Coronavirus-like particles were observed by thin-section electron microscopy in cytoplasmic vacuoles in epithelial cells in infected tracheal-organ cultures (Fig. 3). Cytoplasmic immuno-fluorescence was observed in cells harvésted from infected tracheal-organ cultures and stained with FITC-conjugated antisera specific to bovine enteric coronavirus (Fig. 4). No fluorescence was observed in these cells with antisera to BVD virus, respiratory syncytial virus or parainfluenza type 3 virus. Harvests from infected tracheal organ cultures agglutinated erythrocytes from rats and, to lower titres, from guinea pigs. Antiserum to bovine enteric



Fig. 2. Coronavirus-like particles in harvest of tracheal-organ culture inoculated with isolate 81-1184. Inset shows coronavirus-like particle in nasal mucus sample from calf 6 (Table I), collected 5 days post-initial inoculation.

Fig. 3. Thin-section electron microscopy of cell harvested from tracheal-organ culture infected with isolate 81-1184. Note coronavirus-like particles within cytoplasmic vacuoles and outside the plasma membrane (arrows).

Fig. 4. Cytoplasmic fluorescence in cells harvested from tracheal-organ culture infected with isolate 82-1398. Direct staining with conjugated antiserum to bovine enteric coronavirus.



coronavirus inhibited HA to the same titre as HA with homologous virus. When uninfected tracheal organ cultures were examined as described above, no virus-like particles, immunofluorescence or HA were observed.

Neither coronavirus isolate grew in primary cultures of foetal bovine kidney cells or in a diploid semi-continuous line of foetal bovine lung cells, even when 5 μ g ml⁻¹ trypsin was incorporated into the culture medium, as described by Toth (1982).

Experimental infection of calves with coronavirus isolates 81-1184 and 82-1398

The results of these experiments are summarised in Table I. Calves showed no clinical evidence of respiratory disease before inoculation with coronavirus, except for Calf 7 in which it was possible to induce a cough. No fluorescence was observed in samples of nasal mucus collected before inoculation with antisera to bovine enteric coronavirus, BVD virus, parainfluenza type 3 virus and respiratory syncytial virus.

Following inoculation with coronavirus, most calves developed a cough or a nasal discharge, but none became tachypnoeic and rectal temperatures remained normal. Four of the 5 colostrum-deprived calves developed relatively severe diarrhoea between 1-3 days post-initial inoculation (pii), which was associated with the excretion of large numbers of coronaviruslike particles in the faeces.

In the 6 calves from which samples of nasal mucus were taken ante-mortem, evidence of coronavirus replication in the nasal passages was obtained by immunofluorescent staining of cells present in the nasal mucus from 1 to 10 days pii (Table I). Particles resembling coronaviruses were detected by electron microscopy in samples of nasal mucus which contained large numbers of fluorescing cells (Fig. 2). Following necropsy, tracheal-organ cultures were established from 6 calves and coronavirus infection was demonstrated in 4 of these by immunofluorescence.

Coronavirus antigen was detected by immunofluorescence in the lungs of Calves 1 and 3. Gross lung lesions were also present in these 2 calves, but were minimal, consisting of a few scattered areas of superficial collapse in the cranial lung lobes. Gross lung lesions in the absence of coronavirus antigen were present in Calf 7, but this calf had an induced cough before inoculation.

No immunofluorescence was obtained with any samples from the calves with antisera to respiratory syncytial, parainfluenza type 3 and BVD viruses. *Mycoplasma dispar* was isolated from the lower respiratory tract of Calf 7. No mycoplasmas or pathogenic bacteria were isolated from the respiratory tracts of the remaining calves.

DISCUSSION

This paper reports the isolation of coronaviruses from the lungs and trachea of young calves with respiratory disease. The search for coronaviruses in the bovine respiratory tract was prompted by the known respiratory tropism of coronaviruses in other animal species and by the observation that bovine enteric coronaviruses grow in organ cultures of bovine trachea (Stott et al., 1976). In view of the antigenic similarity between the 2 respiratory coronavirus isolates described here and a bovine enteric coronavirus isolates; it is not clear whether our respiratory isolates are genuine respiratory viruses or are primarily enteric viruses which also grow in the respiratory tract, in a similar fashion to porcine transmissible gastroenteritis virus (Bohl, 1981). The occurrence of coronavirus-associated diarrhoea in most of the experimentally-infected calves tends to support the latter view.

Inoculation of young calves with the 2 coronavirus isolates produced a predominantly upper respiratory tract infection and a mild clinical disease, similar to that produced in humans by coronavirus (McIntosh, 1978). However, as different strains of other coronaviruses, e.g., avian infectious bronchitis virus, vary considerably in virulence, it is possible that bovine strains, capable of producing much more severe disease, exist.

The contribution of coronavirus to the lung lesions observed in the 2 field cases is difficult to define. In the first case, lung damage resulting from S. dublin septicaemia was present, and BVD virus was also isolated from the lungs. In the other case M. dispar was also isolated; this agent is capable of producing pneumonic lesions in young calves (Howard et al., 1976).

ACKNOWLEDEMENTS

We thank S.D. Neill and H.J. Ball for carrying out examinations for bacteria and mycoplasmas respectively, and J. Parker and J. Galway for expert help in rearing the calves.

REFERENCES

- Bohl, E.H., 1981. Transmissible gastroenteritis. In: A.D. Leman, R.D. Glock, W.L. Mengeling, R.H.C. Penny, E. Scholl and B. Straw (Editors), Diseases of Swine, 5th edn. Iowa State University Press, Ames, IA, pp. 195-208.
- Bryson, D.G., McFerran, J.B., Ball, H.J. and Neill, S.D., 1978. Observations on outbreaks of respiratory disease in housed calves (1) Epidemiological, clinical and microbiological findings. Vet. Rec., 103: 485-489.
- Bryson, D.G., McNulty, M.S., Logan, E.F. and Cush, P.F., 1983. Experimental respiratory syncytial virus pneumonia in young calves: clinical and pathological findings. Am. J. Vet. Res., 44: 1648-1655.

- Howard, C.J., Gourlay, R.N., Thomas, L.H. and Scott, E.J., 1976. Induction of pneumonia in gnotobiotic calves following inoculation of *Mycoplasma dispar* and ureaplasma (T-mycoplasmas). Res. Vet. Sci., 21: 227-231.
- McIntosh, K., 1978. Coronaviridae. In: G.D. Hsiung and R.H. Green (Editors), CRC Handbook Series in Clinical Laboratory Science. Section H: Virology and Rickettsiology, Vol. 1. CRC Press, West Palm Beach, FL, pp. 313-321.
- McNulty, M.S., Pearson, G.R., McFerran, J.B., Collins, D.S. and Allan, G.M., 1976. A reovirus-like agent (rotavirus) associated with diarrhoea in neonatal pigs. Vet. Microbiol., 1: 55-63.
- McNulty, M.S., Curran, W.L., Todd, D. and McFerran, J.B., 1979. Detection of viruses in avian faeces by direct electron microscopy. Avian Pathol., 8: 239-247.
- McNulty, M.S., Bryson, D.G. and Allan, G.M., 1983. Experimental respiratory syncytial virus pneumonia in young calves: microbiologic and immunofluorescence findings. Am. J. Vet. Res., 44: 1656-1659.
- Stott, E.J., Thomas, L.H., Bridger, J.C. and Jebbett, N.J., 1976. Replication of a bovine coronavirus in organ cultures of foetal trachea. Vet. Microbiol., 1: 65-69.
- Thomas, L.H., Gourlay, R.N., Stott, E.J., Howard, C.J. and Bridger, J.C., 1982. A search for new micro-organisms in calf pneumonia by the inoculation of gnotobiotic calves. Res. Vet. Sci., 33: 170-182.
- Toth, T.E., 1982. Trypsin enhanced replication of neonatal calf diarrhoea coronavirus in bovine embryonic lung cells. Am. J. Vet. Res., 43: 967-972.
- Tzipori, S., 1981. The aetiology and diagnosis of calf diarrhoea. Vet. Rec., 108: 510-514.