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CORONAVIRUS REPLICATION IN THE INTESTINAL AND RESPIRATORY TRACTS DURING INFECTION OF CALVES

D.J. REYNOLDS


Abstract

Coronaviruses have been isolated from cases of neonatal calf diarrhoea in many parts of the world and were revealed in the respiratory tract recently, during a study of calf pneumonia. In experiments on gnotobiotic calves an enteric coronavirus replicated in the nasal epithelium and a respiratory isolate was shed in faeces. The enteric coronavirus prevented subsequent infection with either homologous enteric or the respiratory isolate. A dual tropism for this agent was emphasized by the detection of coronavirus antigen in intestinal and respiratory epithelium of naturally-infected calves.

Many of the bovine coronavirus (BCV) strains identified in cases of neonatal calf diarrhoea have been related to the virus described originally by Stair et al. (1972). However in a search for new microorganisms in outbreaks of calf pneumonia (Thomas et al., 1982) the presence of a coronavirus was demonstrated in material from the respiratory tract. Coronavirus replication sites in the intestinal and respiratory tracts were investigated to study the relationship between these viruses.

Materials and Results

A double-sandwich enzyme-linked immunoabsorbent assay (ELISA) was developed with hyperimmune serum prepared in a gnotobiotic calf by immunization with BCV. Coronavirus in faeces could be detected by this test and confirmed by specific blocking with a second immune serum. Virus antigen in epithelial cells from nasal swabs was demonstrated by immunofluorescence staining.

BCV excretion occurred after oral inoculation with the strain described by Bridger et al. (1978) and could be found in faeces for four consecutive days, after a 1-2 days incubation period. Six animals were monitored for virus excretion from the respiratory tract, and was found in each case, from 3 to 8 days after inoculation. Figure 1 shows the bright, cytoplasmic fluorescence associated with coronavirus antigen in columnar, ciliated epithelial cells. When the respiratory isolate of BCV (Thomas et al., 1982) was administered by intra-nasal and

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>Inoculum</th>
<th>Origin</th>
<th>BCV excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Faeces</td>
</tr>
<tr>
<td>1</td>
<td>a</td>
<td>Enteric</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>Enteric</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
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<tr>
<td></td>
<td>b</td>
<td>Respiratory</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>Enteric</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>Respiratory</td>
<td>-</td>
</tr>
</tbody>
</table>

i : Bridger et al. (1978).  
ii : Thomas et al. (1982).
intra-tracheal inoculation, a similar pattern resulted with virus excretion in faeces and nasal swabs. Table 1 summarises these results and shows that when a calf was challenged with the respiratory virus, after recovery from infection with enteric coronavirus, there was no detectable virus excretion from either faeces or respiratory tract.

Tissues from calves with naturally-occurring coronavirus infection have been examined. In two calves with severe diarrhoea, BCV antigen was demonstrated by immunofluorescence in distal parts of the small intestine, caecum and colon. In addition epithelial cells of nasal mucosa and trachea were infected.

**Conclusions**

The ability of bovine coronavirus to replicate in intestinal and respiratory epithelium cells has been demonstrated and was not dependent on the original source of virus. ELISA on faeces or immunofluorescence on nasal swabs were both satisfactory means of virus detection. Two isolates from differing sources were, however, closely related, in that the enteric strain induced protection from infection with the respiratory virus. Natural infection of gut and respiratory tract was found during investigation of coronavirus replication sites; the relevance of this finding for pathogenesis and epidemiological study is being investigated.

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**References**


**Question**

*From Dr. Bourne to Dr. Reynolds*

How long does protective immunity persist following experimental infection?

**Answer**

Experimental reinfection has been attempted only at up to 3 weeks after first inoculation.

**Question**

*From Dr. La Bonnardière to Dr. Reynolds*

Have you studied alveolar macrophages infection?

**Answer**

Frozen sections of the complete lung were tested by immunofluorescence. Coronavirus antigen has not been seen so far.

**Question**

*From Dr. Viso to Dr. Reynolds*

Did you check other parts of the respiratory tract?

**Answer**

Yes, coronavirus-infected cells were found in the nasal epithelium. Also the upper and middle trachea.

**Question**

*From Dr. Pearson to Dr. Reynolds*

You showed immunofluorescence against coronavirus antigen in the colonic crypts. Do you see any pathological changes in the crypts associated with the infection?

**Answer**

There were microscopic necrotic lesions in the colonic crypts.