Brief Communication:

ISOLATION OF ROTAVIRUS FROM FOALS WITH DIARRHOEA

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Summary. A rotavirus, morphologically similar to other known rotaviruses, was demonstrated in the faeces of 5 foals with diarrhoea on two properties. Four of these 5 samples produced specific intracytoplasmic fluorescence in cell culture when reacted with calf rotavirus antiserum conjugate. Sixteen affected foals from both properties were depressed, did not suckle and became recumbent. Most had a watery diarrhoea which lasted for 3 days and resulted in some dehydration and loss of body condition. Sick foals were separated from their mothers following the onset of diarrhoea and given fluid therapy and antibiotics, but despite these measures 4 of 12 affected foals on one property died. Acute and convalescent sera were collected from 6 foals, and in each case a rise in the titres of complement fixing and neutralising antibodies was demonstrated. Faecal filtrates containing foal rotaviruses were fed to gnotobiotic piglets and the effects compared with those of other rotaviruses. Viral isolates from both properties produced an asymptomatic infection in piglets. This contrasts with the other rotaviruses isolated in Australia that have been shown to cause diarrhoea in gnotobiotic piglets.

INTRODUCTION.

Diarrhoea in foals has been associated with "Foal Heat" (Johnson, Kamstra and Kohler, 1970); Salmonella spp. (Wenkoff, 1973); Corynebacterium equi (Cimprich and Rooney, 1977); Streptococcus spp., Escherichia coli and Actinobacillus equi (Catcott and Smithcors, 1972). Recently, coronaviruses and rotaviruses have been incriminated as possible causes of diarrhoea in young foals (Bass and Sharpee, 1975; Flewett, Bryden and Davies, 1975).

During an investigation of outbreaks of diarrhoea in foals on two properties in Victoria, rotaviruses were observed in the faeces of 5 sick foals. The nature of the outbreaks and the procedures used to identify the rotavirus are described in this paper.

CLINICAL OBSERVATIONS.

One outbreak of diarrhoea occurred on a well managed property where most foals are born elsewhere and brought to the stud with their mothers when the mares return for service. At the time of the outbreak there were 16 foals with their respective mares, kept in one
paddock. Twelve (75%) of the foals developed diarrhoea during a period of 6 weeks, the youngest being 3 days old and the oldest 21 days. Four of the sick foals (33·3%) subsequently died and 2 were submitted for postmortem examination.

Clinically, the affected foals appeared depressed in the initial stages, did not suck milk and became recumbent. Rectal temperature ranged between 39·5° and 41·0° and respiration was rapid and shallow (approx. 60 per min). Profuse, watery and vile-smelling diarrhoea commenced 4-12 h after the onset of depression and the sick foals became dehydrated, resulting in loss of body condition and a rough, dry coat. Pseudo-membranes were seen on the tongues of 2 foals.

Five foals with mild diarrhoea were treated by the owners with oral antibiotics and recovered within 2 to 4 days. Two untreated foals died within 24 h of the onset of diarrhoea. The remaining 5 foals were treated by one of the authors. The foals were separated from their dams and given oral and systemic antibiotics and fluids and electrolytes intravenously.

One of the treated foals, aged 15 days, that seemed to have improved 2 days after the onset of diarrhoea died overnight. The dead foal was sent to the Benalla Regional Veterinary Laboratory for postmortem examination. The second dead foal, aged 10 days, was autopsied by one of the authors. Both dead foals had lesions consistent with acute gastroenteritis. The remaining 3 foals had diarrhoea for 3 days but recovered after 6 days. Blood and faecal samples from 4 foals (3 that recovered after treatment by one of the authors and the second dead foal) were submitted for laboratory examination.

The second outbreak occurred in a smaller horse stud where 4 of 8 foals (3 to 5 weeks old) developed clinical diarrhoea similar to that described above. However, the foals were older and the disease was less severe. One faecal sample and 3 acute and convalescent sera were collected for examination on this property.

LABORATORY EXAMINATION.

Examination of faeces

Faecal samples were prepared for electron microscopy (EM) and immunofluorescence (IF) as described previously (Tzipori and Williams, 1978). Each of the 5 samples contained large quantities of virus particles with morphological characteristics typical of rotavirus. Most particles appeared to possess a double shell, with the outer shell being 68-72 nm in diameter. Four of 5 primary lamb kidney cell cultures that were inoculated with foal faecal filtrates produced specific intracytoplasmic fluorescence when reacted with calf rotavirus antiserum conjugate (Table 1).

The 4 faecal samples that were examined for virus, and the intestinal contents taken from the submitted dead foal from Property 1, were cultured by the Regional Veterinary Laboratory for bacterial pathogens. The intestinal contents of the submitted dead foal contained Salmonella bovis morbificans. No pathogenic bacteria were isolated from the other 4, nor from the faecal sample from Property 2 which was cultured at this laboratory.

Seroiogy

Seven acute and 6 convalescent sera from the 7 scouring foals on both properties were examined for complement fixing (CF) and neutralising (NT) antibodies, using a Monkey SA11 rotavirus antigen (Tzipori and Makin, 1978). The serological results are summarized in Table 1. On both properties foals had some circulating, specific antibodies against rotavirus during the acute phase, and 1 to 3 weeks later there was a rise of both CF and NT antibodies in all cases examined. The increase in CF activity appeared to be slightly greater than the increase in NT antibody.

Inoculation of gnotobiotic pigs

Pooled filtrates prepared from 4 faeces of foals containing virus from Property 1 were fed to 2 gnotobiotic piglets that had been obtained by caesarian section 2 days previously. No clinical diarrhoea was observed in these piglets but virus was detected by EM in their
### TABLE 1.
Details of examination of 7 foals with diarrhoea.

| Property | Age of foal (days) | Duration of diarrhoea (days) | Virus detected in faeces (EM) | Immuno-fluorescence in tissue culture | Antibody titre* | Convalescent |
|----------|--------------------|------------------------------|------------------------------|--------------------------------------|-----------------|--------------|
|          |                    |                              |                              |                                      | Acute stage     |              |
|          |                    |                              |                              |                                      | CF | NT | CF | NT |
| 1        | 3                  | 3                            | +                            | +                                    | 4  | 2  | 8  | 16 |
|          | 4                  | 3                            | +                            | +                                    | <2 | 4  | 128| 16|
|          | 8                  | 3                            | +                            | -                                    | 4  | 4  | 16 | 8 |
|          | 10                 | 4                            | +                            | +                                    | 2  | 16 | NA | NA|
| 2        | 21                 | 3                            | NA                           | NA                                   | 2  | 4  | 128| 16|
|          | 35                 | 2                            | NA                           | NA                                   | <2 | 8  | 128| 16|
|          | 21                 | 1                            | NA                           | NA                                   | 16 | 16 | 32 | 32|

*Convalescent sera were collected 7 days after recovery on Property 1, and 21 days on Property 2 and tested for complement fixing (CF) and neutralising (NT) antibodies.

†Not available.
faeces 48 h after inoculation. These piglets were subsequently challenged with 2 ml of pig rotavirus EP9 (Tzipori and Williams, 1978) 7 days after they had been fed the foal rotavirus. The piglets did not develop diarrhoea following this challenge. A sharp rise in the NT antibody levels in the sera of these pigs was observed 7 days after the challenge (Table 2). Faecal samples were collected from these pigs 48 h after the initial infection and filtrate of this material was fed to a second pair of 2-day-old gnotobiotic piglets. These piglets did not develop diarrhoea but virus was detected in the faeces of one piglet 3 days after inoculation and NT antibody in both piglets 14 days after infection (Table 2). A faecal filtrate prepared from a foal with diarrhoea on Property 2 was fed to 2 2-day-old gnotobiotic piglets and subsequently passaged in 2 more 2-day-old gnotobiotic piglets. Diarrhoea was not observed in any of these piglets and virus was only detected by EM in the faeces of 1 of the piglets. However, all 4 piglets had developed specific NT antibody titres 14 days after inoculation (Table 2). Two uninoculated control piglets remained healthy and free of specific antibody against rotavirus throughout the experiment.

**TABLE 2.**

*Details of inoculation of 2-day-old gnotobiotic pigs with rotavirus from foals on 2 properties.*

| Origin of virus | Passage no. in pairs of pigs | Virus detected in faeces (EM) | Serum neutralising antibody 14 days after inoculation |
|----------------|-------------------------------|-----------------------------|-----------------------------------------------------|
| Property 1     | 1                             | +                           | ≥ 256*                                               |
|                | 2                             | +                           | 256*                                                |
| Property 2     | 1                             | −                           | 16                                                  |
|                | 2                             | +                           | 32                                                  |

*These piglets were challenged with a pig rotavirus 7 days after they were inoculated with the horse rotavirus.

**DISCUSSION.**

The investigations reported in this paper suggest that rotavirus played a role in outbreaks of diarrhoea in foals on 2 properties. However, the disease will have to be reproduced experimentally in foals before a firm conclusion can be drawn. The isolation of *S. morbificans* from 1 of the foals that suffered a relapse and died after apparent recovery suggests that this organism could play an important secondary role in the disease. None of the other faecal samples examined contained pathogenic bacteria.

The foal rotavirus was found to infect gnotobiotic piglets but caused no diarrhoea. This is in contrast with other strains of rotavirus. In this laboratory human, calf, pig, deer and mouse rotaviruses all caused diarrhoea in gnotobiotic pigs (Tzipori, unpublished data). Although little or no virus was detected by EM on the second passage of the virus in pigs, the rise of NT antibody suggested that infection had taken place. It appears that there was a cross-protection between the pig and the foal rotavirus in pigs; it remains to be seen whether the reverse would be similar in foals.

A high titre of CF antibody was recorded in the convalescent sera compared with NT antibody. It could be that the neutralising antibody, which is more specific than the complement fixing antibody, may only be partially reactive against the monkey virus SA11 that was used as a source of antigen in these tests.
At present, neither the prevalence of this virus in the Victorian equine population nor the economic significance of the disease it produces is known. The owners of both properties investigated had not seen a disease of this type before, and it would appear likely that the causative agent had been recently introduced.

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