EQUINE VETERINARY JOURNAL
Equine vet. J. (1991) **23** (6) 405-409

General Articles

The prevalence of enteric pathogens in diarrhoeic Thoroughbred foals in Britain and Ireland

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Summary

A survey of 77 normal and 326 diarrhoeic foals in Britain and Ireland from 1987 to 1989 revealed a significantly higher prevalence of Group A rotaviruses and *Aeromonas hydrophila* in diarrhoeic foals. The prevalence of cryptosporidia, potentially pathogenic *Escherichia coli*, *Yersinia enterocolitica* and *Clostridium perfringens* was similar in normal or diarrhoeic foals. Rotaviruses had a similar prevalence in all age groups of scouring foals up to three months of age, with an overall prevalence of 37 per cent among diarrhoeic foals. The number of cases of diarrhoea varied considerably from year to year, but in all three years of the survey rotavirus was a significant pathogen. A comparison of diagnostic tests for rotavirus in the faeces showed electron microscopy (EM) and polyacrylamide gel electrophoresis (PAGE) to have similar sensitivity. The Rotazyme ELISA test kit was found to have the same sensitivity as a combination of EM and PAGE. *A. hydrophila* had an overall prevalence of 9 per cent among diarrhoeic foals, although its prevalence was higher in some age groups. *A. hydrophila* has not been established previously as a significant enteric pathogen in foals. Other putative pathogens found at very low prevalence were coronavirus, the putative picobirnavirus, *Campylobacter* spp. and *Salmonella* spp. No evidence was found of synergistic effects between rotavirus, cryptosporidia and potentially pathogenic *E. coli*. Neither coccidia nor non-Group A rotaviruses were found in any of the samples examined.

Introduction

ALTHOUGH diarrhoea is common in young foals (Urquhart 1981), its infectious aetiology is not well understood (Tzipori, 1985). Different microorganisms have been implicated including rotavirus (Kanitz 1976), coronavirus (Bass and Sharpee 1975), adenovirus (Studdert and Blackney 1982), parvovirus (Baker and Ames 1987), *Escherichia coli* (expressing a variety of pilus antigens) (Tzipori *et al* 1984b), *Clostridium perfringens* type C (Dickie, Klinkerman and Petrie 1978), *Aeromonas hydrophila* (Tzipori 1985), *Clostridium difficile* (Jones *et al* 1988) *Streptococcus durans* (Tzipori, Hayes, Sims and Withers 1984a), *Bacteroides fragilis* (Myers, Shoop and Byars 1987) *Salmonella* spp. (Tzipori 1985) and *Cryptosporidium* spp. (Coleman *et al* 1989). Infection of adult horses by the coccidian, *Eimeria leuckarti*, has been reported, but has not been shown to cause disease (Barker and Remmler, 1972). Although these potential pathogens have been isolated from diarrhoeic foals, and have been shown to induce diarrhoea in some experimentally infected foals, comprehensive surveys to estimate the relative prevalence of these pathogens in diarrhoeic foals have not been conducted. Most reported surveys focus on a single pathogen, and only rotavirus has been detected consistently in diarrhoeic foals. Pathogenic enterobacteria are significant in other monogastric animals, as is cryptosporidia in ruminants. This survey concentrated on these three classes of pathogens because of their likely importance in foals, but samples also were screened for coronaviruses, the putative picobirnaviruses and for coccidia. Some aspects of the epidemiology of rotaviruses and *A. hydrophila* also were investigated.

Materials and methods

Faecal samples

Faecal samples were collected from normal and diarrhoeic foals on Thoroughbred stud farms in Britain from 1987 to 1989 and from diarrhoeic foals on Irish Thoroughbred stud farms in 1988 and 1989. In 1987, 45 normal foals on nine British stud farms were sampled weekly and samples also were collected from normal foals during outbreaks of diarrhoea from birth for up to three months. In 1989, routine samples were collected from 32 foals in British stud farms experiencing epidemics of diarrhoea. Serial daily samples were collected from these foals at intervals from birth until they were three months old. Three hundred twenty-six diarrhoeic foals on 40 study farms were sampled once when first presented for clinical examination; 86 of the foals were on Irish study farms. Episodes of 'foal heat scour' were sampled only if judged clinically serious. Samples were collected by practitioners and stored at 4°C for up to 1 week before transport.

A swab of each sample was taken into MW171 Charcoal transport medium (Transwab Company Potley, Wiltshire, UK).

Examination for rotaviruses

All samples were screened for rotavirus double-stranded RNA by polyacrylamide gel electrophoresis and silver staining (Herring *et al* 1982). This method also detected possible picobirnaviruses (Gatti *et al* 1989). Also in 1988 and 1989, samples stained negatively with phosphotungstic acid were examined by electron microscopy for rotavirus particles. Some samples also were examined using the Rotazyme II enzyme-linked immunosorbent assay (ELISA) test kit (Abbott Laboratories, Chicago, Illinois).

Examination for cryptosporidia and coccidia

A faecal smear of each sample was stained with phenol auramine and examined for the presence of cryptosporidial oocysts by fluorescent microscopy. Coccidial oocysts were detected after faecal flotation on saturated sodium chloride solution.

Examination for Enterobacteriacae

A swab of each faecal sample was cultured aerobically on MacConkey and XLD agar at 37°C to isolate *Enterobacteriacae*. Salmonellae were isolated by primary culture in selenite enrichment media, then by subculture on MacConkey and XLD agar. *A. hydrophila* was isolated on sheep blood agar incorporating 10 mg/litre ampicillin. *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Aeromonas* spp. and *E. coli* were identified biochemically using the API20 system (API-bio Merieux UK, Ltd., Basingstoke, Harts, UK). *Campylobacter* spp. were isolated by microaerophilic culture on Skirrow's media at 42°C. *Clostridium* spp. were isolated by culture on neomycin blood agar under anaerobic conditions at 37°C. In all cases, only the major growth was considered positive. DNA extracted from *E. coli* isolates was hybridised to DNA probes specific for heat stable enterotoxins (STal, StTa2), verocytotoxins (VT1, VT2), heat-labile enterotoxin (LT1), enteroinvasive *E. coli*, enterohaemorrhagic *E. coli*, enteropathogenic *E. coli* adherence factor and enteropathogenic *E. coli* plasmid pLV501, as described by Fletcher *et al* (1990).

Examination for coronavirus

Samples were screened for possible equine coronaviruses by an ELISA based on polyclonal antibodies to bovine coronaviruses. Positive samples were confirmed by immunogold electron microscopy (El-Ghorr, Snodgrass and Scott, 1988).

Statistical analysis

The proportions of normal and diarrhoeic foals excreting organisms were compared by Fisher's exact test (Sokal and Rohlf 1981). The proportions of scouring foals of different ages excreting rotavirus or *A. hydrophila* were compared using an RxC G-test of independence (Sokal and Rohlf 1981). The sensitivity of the different rotavirus diagnostic methods also were compared by Fisher's exact test.

Results

The proportions of normal and diarrhoeic foals respectively excreting rotavirus, cryptosporidia, potentially pathogenic *E. coli*, *A. hydrophila*, *Yersinia enterocolitica* and *C. perfringens* in their faeces are presented in Table 1. The presence of rotaviruses was associated significantly with diarrhoea, as was *A. hydrophila*. Cryptosporidia, potentially pathogenic *E. coli*, *Y. enterocolitica* and *C. perfringens* were as frequent in faeces from normal as from diarrhoeic foals. All rotaviruses observed had group A electrophoretypes.

TABLE 1: The prevalence of possible enteric pathogens in faeces of normal and diarrhoeic foals

Potential pathogen	Foals: Normal	Foals: Diarrhoeic	Probability (P) in Fisher's exact test*
Rotavirus†	8% (6/77)	37% (122/326)	<0.001
Cryptosporidia	27& (21/77)	29% (83/285)	0.4
Escherichia coli	34% (11/32)	22% (66/304)	0.08
Aeromonas hydrophila	0% (0/32)	9% (28/304)	0.05
Yersinia enterocolitica	16% (5/32)	12% (36/304)	0.3
Clostridium perfringens	6% (2/32)	4% (12/304)	0.4

*Probability that proportions in normal and diarrhoeic foals are the same. †Rotavirus diagnosis was based on both electron microscopy and polyacrylamide gel electrophoresis

Other potential pathogens found at a very low prevalence included *Salmonella* spp. (3/304) and *Campylobacter* spp. (1/304). Coccidia were not found in any sample from 92 foals (77 diarrhoeic) tested. Presumptive equine coronaviruses were detected by ELISA in two normal yearlings and confirmed in one of them by immunogold electron microscopy (Fig 1). The particles were not recognised by a monoclonal antibody-based ELISA specific for the HE glycoprotein of bovine coronaviruses. Coronavirus-like particles were not identified in faeces from any of the 326 foals examined. Possible picobirnaviruses, characterised by two segments of double stranded RNA, were detected in six scouring foals (Fig 2).

Epidemiology of equine rotavirus infections

The ages of scouring foals that shed rotaviruses are shown in Table 2. Rotaviruses were excreted by 31 to 39 per cent of scouring foals in these groups, with the exception of the one- to two-month-olds, 57 per cent of which excreted rotavirus. These differences were not statistically significant.

The monthly prevalence of rotavirus detection in 10 stud farms over the three years of the survey is shown in Figure 3. The total number of cases varied, from six cases in 1987 to 59

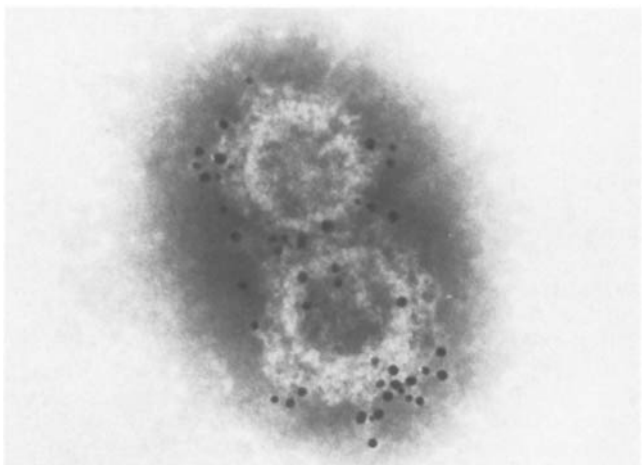

Fig 1: Coronavirus-like particles in equine faeces as seen by immunogold electron microscopy at 180,000 X using rabbit antiserum against bovine coronavirus (El-Ghorr et al *1988). 1 cm = 50 nm*

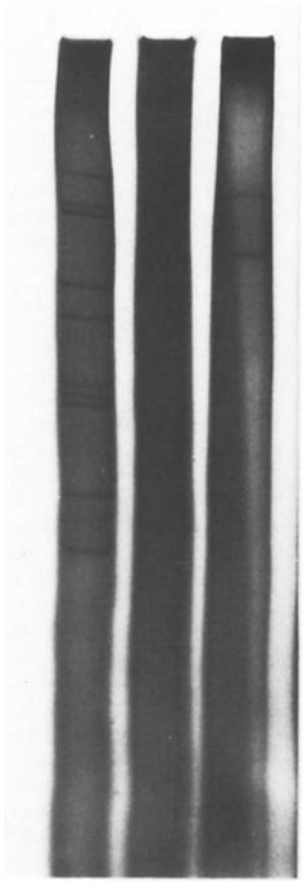

Fig 2: Putative equine picobirnavirus double-stranded RNA (two right lanes) compared with equine rotavirus double-stranded RNA (left) by polyacrylamide gel electrophoresis and silver-staining

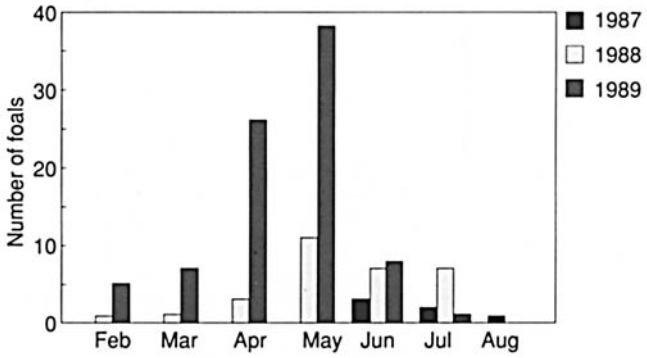

Fig 3: The monthly prevalence of rotavirus infections in foals on 10 British Thoroughbred studs from 1987 to 1989

in 1989. The distribution of cases also varied each year, with all cases in 1987 occurring from June to August, whereas in 1989 most cases occurred before June.

Comparison of rotavirus tests

A comparison of the findings with electron microscopy (EM) and polyacrylamide gel electrophoresis (PAGE) is presented in Table 3. In some cases, more than one sample from each foal was tested. Table 3 also lists the total number of samples found to be positive by either method and by both, as well as percentage figures. If both methods are assumed to be 100 per cent specific, EM had a sensitivity of 70 per cent and PAGE had a sensitivity of 78 per cent (P = 0.07).

A comparison of rotavirus diagnosis by Rotazyme with combined EM and PAGE is also presented in Table 3. Again, assuming all tests to be 100 per cent specific, Rotazyme had a sensitivity of 72 per cent and combined EM and PAGE had a sensitivity of 73 per cent (P = 0.5).

Age distribution of A. hydrophila *infections*

Although there appeared to be a predominance of cases in foals aged one week to two months, the differences are not statistically significant (Table 2).

TABLE 2: Prevalence of enteric pathogens in different age groups of diarrhoeic foals

Age	Rotavirus	*Aeromonas hydrophila*
< 1 week	31% (4/13)	0% (0/11)
1 week–1 month	33% (28/85)	16% (13/79)
1 month–2 months	57% (28/49)	12% (6/49)
Over 2 months	39% (7/18)	6% (1/18)
Unknown	34% (55/161)	5% (8/147)

TABLE 3: Comparison of rotavirus diagnoses by EM and/or PAGE and/or Rotazyme

Method of diagnosis	Number of positive samples	Percentage total positive
EM only	32	22%
PAGE only	44	30%
EM and PAGE	69	48%
EM or PAGE	145	100%
Negative by either test	393	
Rotazyme only	20	27%
EM/PAGE only	21	28%
EM/PAGE and Rotazyme	34	45%
EM/PAGE or Rotazyme	75	100%
Negative by either test	71	

EM: electron microscopy; PAGE: polyacrylamide gel electrophoresis

Significance of co-infection (Table 4)

The prevalence of cryptosporidial or potentially pathogenic *E. coli* infections among rotavirus-infected, diarrhoeic foals was the same as the prevalence of these infections among all diarrhoeic foals. Similarly, the prevalence of rotavirus or potentially pathogenic *E. coli* infections among cryptosporidium-infected, diarrhoeic foals, and the prevalence of rotavirus or cryptosporidial infections among potentially pathogenic *E. coli*-infected, diarrhoeic foals, also was the same as their prevalence among all diarrhoeic foals.

TABLE 4: Prevalence of co-infection by rotavirus, cryptosporidia, and potentially pathogenic *E. coli* in diarrhoeic foals

Primary infection	Secondary infection	Prevalence of co-infection
Rotavirus	Cryptosporidia	31% (21/68)
	E. coli	19% (22/116)
Cryptosporidia	Rotavirus	33% (27/83)
	E. coli	18% (15/83)
Potentially pathogenic *E. coli*	Rotavirus	33% (22/66)
	Cryptosporidia	34% (15/44)

Discussion

This study confirms the importance of Group A rotaviruses in causing diarrhoea in young foals. Previous surveys have found rotaviruses in the faeces of diarrhoeic foals in Britain (Flewett, Bryden and Davies 1975), the United States (Kanitz 1976; Conner and Darlington 1980), Australia (Studdert, Mason and Patten 1978), New Zealand (Durham, Stevenson and Farquharson 1979), Ireland (Strickland, Lenihan, O'Connor and Condon 1982), Japan (Imagawa *et al* 1984a) and Germany (Herbst *et al* 1987). The proportion of diarrhoeic foals excreting rotavirus has varied considerably between different studies, from 11 per cent (Herbst *et al* 1987) to 62 per cent (Gillespie *et al* 1984), but most studies have implicated rotavirus in 30 to 40 per cent of cases of diarrhoea in foals, which generally supports our observations. Dwyer *et al* (1988) detected rotavirus in 3.1 per cent of 1602 samples from normal foals, and in 26.4 per cent of 269 samples from diarrhoeic foals. Our observations, therefore, confirm the highly significant association between rotavirus infection and diarrhoea, and suggest that rotaviruses are probably responsible for at least a third of all cases. Also, experimental infection of foals with equine rotaviruses induced diarrhoea (Imagawa *et al* 1984b; Higgins *et al* 1988; Browning, Chalmers, Scott and Snodgrass unpublished data). Previous surveys have not recorded the age distribution of rotaviruses. Our survey indicates that rotaviruses are significant pathogens throughout the first 3 months of a foal's life, although they may be responsible for a greater proportion of cases of diarrhoea in foals aged one to two months.

There were comparatively few cases of diarrhoea in foals aged less than one week or more than 2 months. As most foals remained on the studfarms from birth until 3 months of age this distribution is unlikely to be due to sampling bias. Foals aged less than 1 week probably gained passive protection against enteric infection from the higher concentrations of antibody in colostrum and milk bathing the gut. Foals older than 2 months as well as being more tolerant of upper intestinal insult due to development of colonic function, might have developed active immunity as a result of sub-clinical infections.

The variation in the total number of rotavirus cases each year also was reflected in the month of peak prevalence; the greater the number of annual cases, the earlier the month of peak prevalence. This probably reflects the relative concurrence of high levels of exposure in a population of susceptible animals. When environmental contamination occurs early in the season, a greater exposure to rotavirus is present when the maximum number of susceptible foals are on the studfarm (April/May). Two studies (Conner and Darlington 1980; Traub-Dargatz *et al* 1988) noted an association between the introduction of foals to studfarms and epidemics of rotavirus-associated diarrhoea, but it was not established whether the epidemic resulted from introduction of a new strain or multiplication and dissemination of an endemic strain. The considerable movement of stock between studs and the observation that many stud farms are affected concurrently in years of higher prevalence, suggests that epidemics are probably caused by increased levels of contamination rather than introduction of new strains. The association with introduction might be related more to the stress of transport and its effect on general levels of immunity, and also reduced ingestion of the mare's milk during and/or after transport resulting in lowered passive protection. Although the ability of milk antibody to protect foals from rotavirus infections has not been established, vaccination of the dam has been used successfully to protect calves from rotavirus diarrhoea by increasing the amount of antirotavirus antibody in the milk (Snodgrass 1986).

No significant difference was found between the sensitivity of EM and PAGE for rotavirus diagnosis. Similarly, no significant difference could be found between the sensitivity of diagnosis by both EM and PAGE and the Rotazyme ELISA kit, assuming the ELISA to be 100 per cent specific. However, it is apparent from these results that any survey based on only one of these diagnostic methods will seriously underestimate the true prevalence of rotavirus infections. The lack of an absolute standard against which to compare these assays hampers interpretation the estimates of sensitivity. Because the structural morphology and the segmented double stranded RNA genome of rotaviruses are characteristic, it is reasonable to assume that EM and PAGE approach 100 per cent specificity, but it is not possible to assume this unreservedly for an ELISA test. All three assays, however, are probably sufficiently accurate as a herd test.

Even though rotaviruses have been shown to be a significant cause of diarrhoea in very young foals, diarrhoea in the foal often goes undiagnosed. Three agents warrant further consideration. *A. hydrophila* has been isolated from diarrhoeic foals (Tzipori 1985), but has not been shown to be a significant pathogen in foals prior to this study. It may have been responsible for up to 9 per cent of all cases, and further focussed study of its epidemiology appears worthwhile, particularly of the trend observed for higher prevalence in foals aged 1 week to 2 months in which it might have been responsible for as many as 16 per cent of all diarrhoea cases. The evaluation of enterotoxin production by *A. hydrophila* from diarrhoeic foals may be of value in future investigations because enterotoxigenic *A. hydrophila* are a common cause of prolonged diarrhoea in human infants (Burke *et al* 1983). Second, cryptosporidia, although not shown to be significantly more prevalent in diarrhoeic foals, still warrants attention because of its high prevalence and its potential activity as a co-pathogen in mixed infections (Tzipori 1985). We found no association between diarrhoea and the relative number of cryptosporidial oocysts in faecal smears, and no evidence of an increased prevalence of cryptosporidia in co-infections with other pathogens. Nevertheless, cryptosporidia might contribute to the severity of the diarrhoea.

The role of pathogenic *E. coli* in diarrhoea in foals remains unclear. The prevalence of *E. coli* detected by the virulence probes was similar in normal and diarrhoeic foals, and co-infection with these *E. coli* and either rotavirus or cryptosporidia did not appear significant. Application of *in vitro* methods such as gut-organ culture (Batt, Embaye, Hunt and Hart 1989) offers an approach to detection of *E. coli* strains with pathogenic mechanisms other than those for which probes are currently available.

Acknowledgments

This study was supported by the Horserace Betting Levy Board. We thank practitioners in Britain and Ireland who supplied faecal samples and S. Wright who performed the examinations for cryptosporidia and coccidia.

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Received for publication: 17.12.90
Accepted: 4.4.91