MIXED DIARRHOEAL INFECTION IN CALVES: THE RELATIVE IMPORTANCE OF 2 INTERACTING ENTEROPATHOGENS

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ABSTRACT

Some of the causes of neonatal diarrhoea in suckling calves aged up to 10 weeks were examined. The susceptibility of calves to the diarrhoea-inducing effect of calf rotavirus was found to be age dependent. Calves over 6 days old were resistant to clinical diarrhoea although they did excrete virus in their faeces and subsequently developed antibody to rotavirus. An enterotoxigenic E. coli (ETEC) inoculated orally into either gnotobiotic or suckling calves aged 1½ to 26 days did not cause diarrhoea. In contrast, diarrhoea was consistently produced in gnotobiotic and suckling calves between one and 15 days of age when they were inoculated with both calf rotavirus and ETEC. In general, diarrhoea appeared after a rotavirus incubation period of approximately 3 days and was independent of the order in which the 2 microbial agents were given.

INTRODUCTION

Diarrhoea in calves is one of the most important causes of neonatal mortality. The disease remains largely uncontrolled because of its complex aetiology. As well as different infectious agents, immunological, nutritional and environmental factors are also involved in precipitating the disease. Microorganisms most commonly encountered in field investigations of diarrhoea are enterotoxigenic E. coli (ETEC) and rotavirus (Acres et al, 1977). The role of rotavirus as a cause of diarrhoea in calves and other mammals has been extensively reviewed (Holmes, 1979).

Preliminary work has shown that calves are clinically susceptible to rotavirus infections only for the first few days of life. In the field, on the other hand, rotavirus often appears to be associated with diarrhoea in calves up to 10 weeks old. In this study a field outbreak of diarrhoea was examined to determine which other infectious or non-infectious factors act in combination with rotavirus in precipitating the disease.

MATERIALS AND METHODS

Case history - Outbreaks of diarrhoea have occurred over the last few years in a 400 suckled beef herd in Victoria, Australia. In 1978 most

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of the 400 newborn calves developed diarrhoea. Nine of the 11 faecal samples collected from scouring calves contained rotavirus (isolate C6) and 3 of them had 1 or 2, stable toxin-producing ETEC organisms (O20: K106:K99$^+$)

Experimental animals - The calves used for these experiments were either gnotobiotic (17 calves), colostrum deprived (7 calves) or suckling (16 calves).

Viruses - Calf rotavirus isolates C1, C3 and C4 (Tzipori et al, 1980) were used for experiment 1 while C6, the rotavirus isolated from the field outbreak was used for the remaining 5 experiments. A fifteen ml aliquot of faecal filtrates containing C6 was used as the standard oral inoculum for each calf. The presence of virus in faeces was determined by electron microscopy.

Bacteria - Four ml of tryptose soya broth containing 10^6 to 10^8 organisms, serotype O20:K106:K99$^+$ were used as standard ETEC oral inoculum per calf. ETEC organisms in the faeces were identified by their O antigen and were tested for K99 by the slide agglutination test.

Serology - A complement fixation (CF) test was used to estimate circulating antibody titres against rotavirus using the tissue culture adapted monkey rotavirus SA11 as antigen.

RESULTS

Experiment 1 - Seven gnotobiotic (GB) and 5 colostrum deprived (CD) calves, aged between 1 and 10 days, were inoculated with one of 4 calf rotavirus isolates. Experimental inoculation of calves with rotavirus induced diarrhoea in calves less than 7 days old. Calves 7 days old or older developed subclinical rotavirus infections only. All 12 calves developed complement fixing (CF) antibody against rotavirus.

Experiment 2 - Two CD and 4 GB calves aged between 2 hours and 26 days were inoculated with ETEC. Diarrhoea was evident only in 2 calves less than 24 hours of age. One 36 hour calf passed loose but not diarrhoeic faeces. Older calves showed no symptoms although they excreted the organism in their faeces.

Experiment 3 - Four GB calves aged between 8 and 13 days were inoculated with rotavirus and ETEC at short intervals (Table 1). Three calves

developed diarrhoea that lasted 3 to 4 days and excreted both organisms in their faeces. One calf, although dosed twice with rotavirus, failed to become infected and developed neither diarrhoea nor CF antibody against rotavirus.

TABLE 1 - THE RESPONSE OF GNOTOBIOTIC CALVES TO ORAL INOCULATION WITH ENTEROTOXIGENIC *E. COLI* AND ROTAVIRUS

Age at Inoculation (Days)		Clinical Diarrhoea		Shedding	
Rotavirus	*E. coli*	Incubation (Days)	Duration (Days)	Virus	*E. coli*
8	11	0.5 h	4	+	++
9	13	1	3	+	+
9	13	-	-	-	+
12	10	3	4	+	+

Experiment 4 - Four suckling calves aged between 3 and 10 days were inoculated with ETEC and 4 others aged between 6 and 10 days were inoculated with rotavirus. As with CD and GB calves, the suckling calves showed no evidence of diarrhoea to either of the two agents (Table 2). One calf inoculated with rotavirus passed solid creamy-white faeces for 1 day. The 2 calves that showed evidence of sub-clinical infection with rotavirus had lower pre-inoculation CF antibody titres (8 and < 2 respectively) than the other 2 in the group (64 and 32)

TABLE 2 - INOCULATION OF SUCKLING CALVES WITH EITHER ROTAVIRUS OR ENTEROTOXIGENIC *E. COLI*

Age at Inoculation		Clinical Diarrhoea		Shedding		Rotavirus CF antibody	
Virus	*E.coli*	Incubation (Days)	Duration (Days)	Virus	*E.coli*	Pre-inoculation	Post-inoculation
-	3	-	-	-	+	128	64
-	4	-	-	-	+	128	32
-	9	-	-	-	+	64	32
-	10	-	-	-	+	32	64
6	-	-	-	-	-	64	128
8	-	3	1*	+	-	8	64
10	-	-	-	+	-	< 2	16
10	-	-	-	-	-	32	16

*Calf passed creamy-white faeces for 1 day

Experiment 5 - Eight suckling calves aged between 5 and 15 days were inoculated with ETEC and rotavirus either simultaneously or at short intervals. (Table 3). The incubation period varied between 2 and 5 days and diarrhoea lasted 5 to 6 days in calves that were not killed earlier. There was no difference in the length of the incubation period and the severity of the diarrhoea between calves with high and low pre-inoculation CF antibody titres against rotavirus.

TABLE 3 - INOCULATIONS OF SUCKLING CALVES WITH BOTH ROTAVIRUS AND ENTEROTOXIGENIC E. COLI

Age at Inoculation		Clinical Diarrhoea		Shedding		CF Antibody	
Virus	E.coli	Incubation (Days)	Duration (Days)	Virus	E. coli	Pre- Inoculation	Post- Inoculation
5	5	3	5	+	++	512	128
7	7	3	4*	+	+	< 2	< 2
9	9	3	1*	+	+	64	8
12	12	5	5	+	+	512	64
14	14	2	1*	+	+	64	16
15	15	3	6	+	+	64	16
13	9	4	5	+	+	256	32
14	10	3	5	+	+	16	8

* Calf killed

Generally, in combined infections with ETEC and rotavirus, the disease appeared approximately 3 days after the rotavirus inoculations and was independent of which of the 2 microorganisms was given first (Tables 1 and 3). Furthermore, the disease coincided more closely with faecal excretion of rotavirus rather than of ETEC. Infections with ETEC usually became established within 24 hours of inoculation and bacterial shedding in the faeces fluctuated considerably over a much longer period than rotavirus excretion.

DISCUSSION

Calves 7 days old and older appear to be resistant to the diarrhoea-inducing effect of the 4 rotavirus isolates used. Calves older than 7 days showed evidence of sub-clinical infection followed by sero-conversion. It was shown too that the ETEC used for these experiments was capable of inducing diarrhoea in calves less than 24 hours old. Similar observations were made previously by others (Smith and Hall, 1967).

The inoculation of GB or suckling calves with both agents, simultaneously or at certain intervals, induced diarrhoea which was independent of age. The diarrhoeal illness resembled that observed in the field outbreak where the 2 microorganisms used in these experiments were isolated. This experimental work can be considered a reconstruction of the disease outbreak seen in the field. Frequently ETEC could not be detected in faeces and when present only small numbers could be isolated. As we have examined larger numbers of colonies per sample (10 colonies) than most other investigators we concluded that their presence and significance in the past may have been overlooked.

The interpretation of post-inoculation CF antibody titres was difficult as no distinction could be made between that produced by the calf or acquired from colostrum. While high level of CF antibody against rotavirus appeared to be effective in preventing infections with rotavirus alone (Table 2), in dual infections it had little effect in preventing either infection or diarrhoea. To date, microbial agents that have been associated with diarrhoea in young calves include ETEC, rotavirus, coronavirus, cryptosporidia and possibly astrovirus and Newbury agent or calicivirus. We have demonstrated that the concurrence of two of the above listed organisms can precipitate a disease in circumstances where each one acting independently may not. It is likely that there are many more combinations which would induce a disease with the severity depending partly on other non-infectious contributing factors. Only field investigations involving a sequential examination of material for viral, bacterial and protozoan enteropathogens, as well as other non-infectious factors, would lead to a correct diagnosis. It is no wonder that vaccines developed to combat only one enteric agent have been singularly ineffective in controlling diarrhoea in young domestic animals.

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