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ROTAVIRUS INFECTION IN CALVES IN BANGLADESH

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ABSTRACT

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Faecal samples from 434 calves under 1 year of age (307 diarrhoeal and 127 normal) were collected from three dairy farms and one village in selected areas of Bangladesh. The samples were tested by an enzyme-linked immunosorbent assay (ELISA) to detect the presence of rotavirus antigen. Of 402 dairy calves tested, 28 (7.0%) were positive, of which 21 (7.2%) were from diarrhoeic calves and 7 (6.3%) from non-diarrhoeic calves. Rotavirus infection varied from farm to farm (2.7–9.2%) and there was no positive response from any of the 32 village calves. Rotavirus was most commonly found in calves of 1 week of age or less (up to 22.2% in one group) but was not found in any calves later than 6 months of age. More than 80% of rotavirus-positive samples from diarrhoeic calves exhibited a titre of 128 or more (geometric mean 345 ± 4.5), whereas non-diarrhoeal calves had titres less than or equal to 128 (geometric mean = 29 ± 1.9), suggesting that rotavirus infection in calves in Bangladesh was mostly associated with diarrhoea.

Keywords: Bangladesh, calves, ELISA, prevalence, rotavirus, serology

INTRODUCTION

Rotavirus infection in calves is very common, with a worldwide distribution (McNulty, 1978; Kurstak *et al.*, 1981). The role of the virus in causing diarrhoea, especially in young subjects, is well established (Mebus *et al.*, 1969; Woode and Crouch, 1978; Castrucci *et al.*, 1988). Although rotavirus infection in diarrhoeic calves usually involves multiple enteropathogens including *Escherichia coli*, corona virus, and/or cryptosporidia (Morin *et al.*, 1978), single infections are not uncommon (Tzipori, 1981). In calves, the infection is mostly associated with diarrhoea, sometimes as the primary agent, in naturally infected and experimentally produced cases (Mebus *et al.*, 1969; Woode and Crouch, 1978) and the infection varies widely depending on various factors (McNulty, 1978; Tzipori, 1981). Calf diarrhoea (gastroenteritis syndrome) remains the most often reported clinical problem in calf management and in rural conventional cattle rearing systems in Bangladesh (Debnath *et al.*, 1990). This study was undertaken to determine the prevalence of rotavirus infection in selected dairy farms and in conventionally reared village calves in Bangladesh, and to study the prevalence of rotavirus in diarrhoeic and age matched non-diarrhoeic calves.

MATERIALS AND METHODS

Selection of calves

Farms A and B are close to each other, 1 km apart, in Dhaka district. Farm C and the selected village are 120 km away in Mymensingh district. The populations of calves under 1 year of age on the farms were 350, 160 and 75 respectively at the beginning of the study. The equivalent calf population in the village could not be ascertained but was estimated to be around 80. Calves on farms A and B were Holstein crossed (F1) with either Sindi, Sahiwal (tropical breeds) or local improved nondescript Zebu. Farm C calves were cross breeds between Sindi and Sahiwal, while most calves of the village were improved Zebu crossed with Sindi or Sahiwal. All the calves were grouped as diarrhoeic (D), having clinical diarrhoea with liquid or semi-liquid faeces, or non-diarrhoeic (N), without any abnormal fluidity of the faeces, regardless of any previous history of illness. All the calves were further categorized into four groups on the basis of age: group I, calves from birth up to 1 week of age; group II, from over 1 week to 1 month old calves; group III, calves aged over 1 month to 6 months; and group IV, calves from over 6 months to 1 year old.

Management systems

Calves were reared in individual metal calf pens until they were 1 month of age. They were then moved to pens containing 10–15 calves where they were housed up to 1 year of age. On farm C, the newborn calves were reared in groups of 3 or 4 and transferred to a common calf shed at 3 months of age where they remained up to 1 year of age with occasional moving to similar sheds. Each farm calf was bottle-fed colostrum and natural milk from their dams soon after birth, usually within 24 hours. Sick calves were usually transferred to a separate calf shed in all the dairies. The village calves were reared in the conventional way for backyard rearing systems in Bangladesh. Most farmers in the village had 2–10 cattle, the cows being used for dual purposes, i.e. draught power and milk. Their calves receive milk naturally by sucking from their respective dams. These calves usually received sufficient colostrum and milk, and occasionally roamed freely in the small grazing fields.

Sampling procedures and processing of samples

Faecal samples were collected from calves during weekly visits to each location. The diarrhoeic samples were collected first, followed by the collection of up to an equal number of normal faeces from age-matched non-diarrhoeic calves. A total of 434 samples, 307 diarrhoeic and 127 non-diarrhoeic, were collected over a 10-month period (Table I).

Faecal samples (ca. 10 g) were collected directly from the rectum, kept in a sterile screw-capped, labelled container, and transported to the laboratory as soon as possible in a thermostable box. Each sample was diluted 1:4 in phosphate-buffered saline (PBS, pH7.4), mixed, and centrifuged at 2500*g* for 30 min (4°C). The supernatants were separated, labelled and kept at −20°C until the samples were used for ELISA.

Enzyme linked immunosorbent assay (ELISA)

A commercially available kit (Dakopatts A/S, code K220, Denmark) was used to analyse the samples for the presence of rotavirus in the faecal materials. The test was done as described elsewhere (Ellens and de Leeuw, 1977; Ellens, 1980) and following instructions provided in the kit. Each sample was tested in duplicate. The readings were taken spectrophotometrically at 492 nm wavelength using automated equipment. Positive results were assessed by comparing the light absorbance of the test samples with that of the known standard positive and negative controls. If a test sample had an absorbance value of ≥ 6 times that of corresponding negative control, it was taken as positive. The P/N ratio was ≥ 10.

Titration of the positive samples was done with a twofold serial dilution of each sample using duplicate rows of ELISA plates and adopting the above ELISA methods. A 1:4 dilution of a positive sample was used as the initial dilution, being considered to have a titre of 4. Titre was defined as the reciprocal of the highest dilution of a sample at which it was still positive by the above ELISA interpretation.

RESULTS

The distribution of samples positive for rotavirus as between locations and ages of calves is shown in Tables I and II respectively. A higher prevalence was observed in young diarrhoeic calves than in the older groups, whereas in non-diarrhoeic samples the prevalence was higher in older calves (Table II and Figure 1). No rotavirus was detected in calves over 6 months of age.

TABLE I
The distribution of rotavirus ELISA positive samples from different sources in Bangladesh. T = total, D = diarrhoeic, N = non-diarrhoeic

Source	Number of samples								
	Tested			Positive			Percent positive		
	T	D	N	T	D	N	T	D	N
Farm A	218	165	53	20	16	4	9.17	9.70	7.55
Farm B	110	69	41	3	2	1	2.73	2.90	2.44
Farm C	74	57	17	5	3	2	6.76	5.26	11.76
All farms	402	291	111	28	21	7	6.97	7.22	6.31
Village	32	16	16	–	–	–	–	–	–

TABLE II
Rotavirus infection in different age groups of calves from dairy farms in Bangladesh. T = total, D = diarrhoeic, N = non-diarrhoeic

Age group of calves[a]	Number of samples						Percent positive		
	Tested			Positive					
	T	D(%)[b]	N	T	D	N	T	D	N
I	50	34 (68%)	16	6	6	–	12.0	17.65	–
II	220	158 (72%)	62	16	12	4	7.27	7.59	6.45
III	148	106 (72%)	42	6	3	3	4.05	2.83	7.14
IV	16	9	7	–	–	–	–	–	–

[a]As defined in the text
[b]Diarrhoeic samples as percentage of total of the respective age group

The titres in diarrhoeic calves with positive samples ranged from 16 to 4096 (median 512). The highest and the lowest titres were 128 and 16 respectively (median 32) in samples from non-diarrhoeic calves. Figure 2 shows the distribution of the titres of the positive samples from both diarrhoeic and non-diarrhoeic calves.

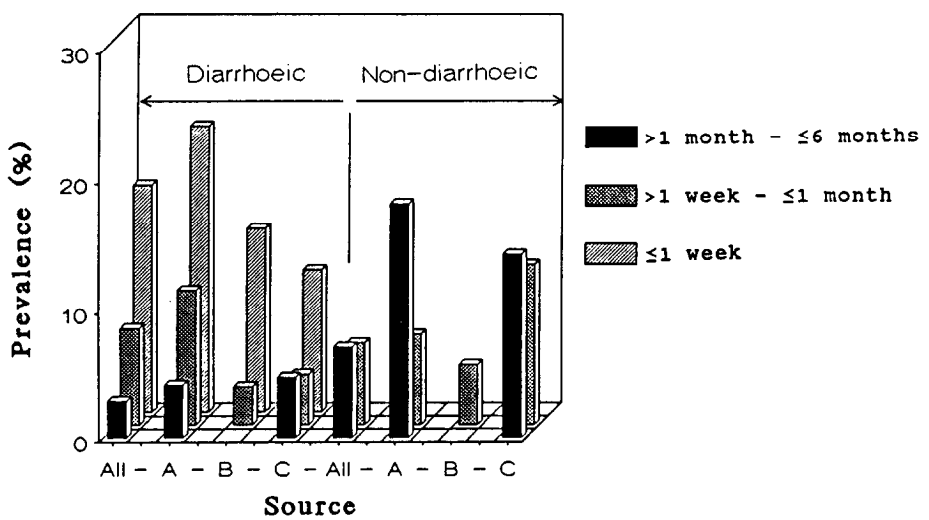

Figure 1. The distribution of age-related prevalence of rotavirus-positive samples in diarrhoeic and non-diarrhoeic calves from three selected dairy farms: A (n=218), B (n=110) and C (n=74). 'All' = mean ($\bar{x}$) response of all three farms (n=28 positive samples)

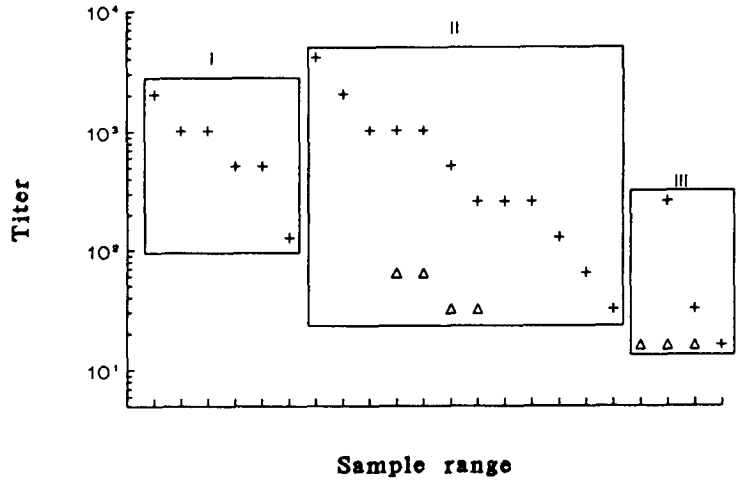

Figure 2. Rotavirus antigen titres in faecal samples from diarrhoeic (+) and non-diarrhoeic (△) calves. Age groups shown as I, II and III represent calves of not more than 1 week, from over 1 week to 1 month, and from over 1 month to 6 months of age respectively

DISCUSSION

Little was known about the status and viral aetiology of calf diarrhoea in Bangladesh under field conditions, although rotavirus-associated human infantile diarrhoea is prevalent (Sack *et al.*, 1980; Huq *et al.*, 1982). A 7.22% infection of diarrhoeic calves as detected in the present study appears low in comparison with findings elsewhere (de Leeuw *et al.*, 1980; Moerman *et al.*, 1982; Bellinzoni *et al.*, 1987). Rotavirus infection may vary widely from place to place or even from farm to farm (Tzipori, 1981). Our findings, however, show similarity with limited observations (Debnath *et al.*, 1987) in the area of Dhaka in diarrhoeic calves up to 1 month old. The variation in the rate of infection on different farms ($p=0.09$) needs more investigation before reaching any firm conclusions. Hygiene measurement is one of the important factors, along with other interacting variables (Tzipori, 1981). Farm B, which had the lowest prevalence (2.73%) of the virus differed significantly ($p=0.05$) from farm A and was noted to have a better cleaning and disinfecting procedure than the other two farms. A higher infection rate (Table II) in the first week of life suggests widespread rotavirus in this group of younger calves. Other studies (Acres and Babiuk, 1978; de Leeuw *et al.*, 1980) showed similar results. In farm C, infection in the non-diarrhoeic calves was relatively high (Table I). Subclinical infection is not uncommon on premises where clinical infection occurs in calves (de Leeuw *et al.*, 1980; Snodgrass and Sherwood, 1983). The overall infection rate in non-diarrhoeic calves in this study corresponds with some other reports (Bellinzoni *et al.*, 1987).

As numerous factors (Tzipori, 1981) interplay in precipitating clinical diarrhoea, it is difficult to make absolute conclusions based on the limited information we have. However, rotavirus infection has been shown to be more important than other agents in diarrhoea in young calves of around 1 week of age (4–14 days) (de Leeuw *et al.*, 1980). Although our findings did not reveal a significant difference in rotavirus

infection between diarrhoeic and non-diarrhoeic calves, rotavirus was mostly associated ($p = 0.1$) with young diarrhoeic calves.

The age-related ($r = 0.90$) prevalence in diarrhoeic calves (Figure 1) tends to agree with the report by Tzipori (1981). The absence of detectable rotavirus antigen in non-diarrhoeic calves up to 1 week old and the high prevalence rate in diarrhoeic calves in the same age group (Table II) emphasizes the association between rotavirus and diarrhoea in such calves.

The higher viral antigen titres (Figure 2) in samples from diarrhoeic calves, for which the geometric mean (GM) of titre was 345 ± 4.5 as against those from non-diarrhoeic calves (GM = 29 ± 1.9) confirms an association of the virus with diarrhoea in these young dairy calves.

The small numbers of conventionally reared village calves makes it difficult to draw firm conclusions from the absence of rotavirus in these animals. The study, however, suggests that the rotavirus infection in young calves also exists in different locations of the country outside the Dhaka region and is the first report of the existence of the virus in high concentration in association with diarrhoea in young dairy calves in that country.

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