DETECTION OF *CRYPTOSPORIDIUM* OOCYSTS AND SERUM IMMUNOGLOBULIN G (IgG) ANTIBODIES IN NATURALLY INFECTED CALVES

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ABSTRACT

Sixty three faecal as well as blood samples from a group of 15 young Friesian calves under 2 months of age at Aber Farm Bangor, U.K. were collected on monthly basis and examined for the presence of *Cryptosporidium* oocysts and serum immunoglobulin G (IgG) antibodies. Twelve (19.23%) were found positive with Cryptosporium species while in 5 (7.9%) faecal samples both *Cryptosporidium* and Eimeria were present but 46 (73.0%) samples were negative. In 9 out of 12 (75.0%) cases where *Cryptosporidium* ocysts were present, a positive IFAT was observed while in 4 out of 5 (80.0%) positives were seen in the presence of both Cryptosporium and Eimeria oocysts. In contrast only 6 out of 46 (13.1%) cases, a positive IFAT was also seen when no oocysts were recorded. Oocysts fluoresced brightly with positive serum samples and only faintly or not at all with the negative samples or the conjugate alone.

Keywords: Friesian calves, Immunoglobulin G, *Cryptosporidium* oocysts

INTRODUCTION

An investigation was carried out to observed whether meronts, merozoites, microgametocytes, microgamonts and macrogamonts possess antigenic properties or not and also whether oocysts could stimulate the production of antibodies in the colostrum of bovine milk or not (Fayer *et al.*, 1991). For this purpose immunogold stained sections of ileum from mouse, experimentally infected with *Cryptosporidium parvum*, were first stained with polyvalent antibodies in hyperimmune bovine colostrum and then stained with rabbit bovine IgA, IgM, IgG1, IgG2 and finally labelled with goat anti-rabbit gold conjugate. They found that each bovine immunoglobulin isotype in the whey recognized antigen in meronts, merozoites, microgametocytes microgamonts and macrogamonts. On the basis of these findings, they hypothesized that antigens in all stages of *Cryptosporidium parvum* provide targets of opportunity for antiparastic activity. Furthermore, hyperimmune bovine colosrum whey antobodies could help as an immunotherapeutic agent.

Cryptosporidium specific IgA, IgG and IgM were also detected from the serum, stools and duodenal fluid of Philipino children (Marc *et al.*, 1990). Lorenzo *et al.* (1993) also detected serum IgG antibodies in asymptomatic adult cattle. Using enzyme-linked immunosorbent assay, Ortega *et al.* (1993) measured the IgG levels in colostrum-deprived lambs and showed that they peaked on day 30 while IgA and IgM peaked on day 15. Furthermore, lambs which received colostrum showed anticryptosporidial IgG, IgM and IgA on day 3. On the other hand, Naciria *et al.* (1994) also observed similar results in groups of lambs which were given hyperimmune colostrum compared with colostrum-deprived lambs. They concluded that the specific *Cryptosporidium parvum* circulating antibodies have no influence on the control of cryptosporidiosis, but cause a slight decline in oocyst shedding.

The present study was therefore, planned to investigate whether IgG in the serum of naturally infected calves has a role in preventing reinfection and whether it persists during the course of infection.

MATERIALS AND METHODS

Faecal samples

Faecal samples from a group of 15 young calves under two months of age were collected at monthly intervals and examined for *Eimeria* and *Cryptosporidium* oocysts. All samples were examined by the Cross and

Moorhead (1984) staining technique to detect the presence of *Cryptosporidium* oocysts.

Serum samples

Blood samples were collected in vacutainer tubes without using anticoagulant. The sampels were transferred to the laboratory where they were kept in a refrigerator over night and the serum was separated by centrifugation at 2000 rpm for 10 minutes. Samples were stored in 5 ml plastic tubes and kept at –20°C until used.

Antigen preparation

Freshly collected faecal samples were suspended 1:1 (wt/vol) in 5% potassium dichromate solution and stored at 4°C for two months.

Oocysts were separated by the same method as those used for Emeria oocysts (MAFF, 1986), but a little change was made in the preparation of antigen in the case of *Cryptosporidium* oocysts. All the steps were carried at 4°C. Faeces containing oocysts were washed through mesh size 0.15 µm and the aliquot was then centrifuged at 1000 rpm for 5 minutes. This was repeated for several times until the potassium dichromate was removed. The sediment was resuspended in 20 ml distilled water and 20 ml diethyl ether and centrifuged at 1000 rpm for 5 minutes and the top three layers removed. This step was continued until sediment was again suspended in distilled water and 1 ml samples of the resulting suspension was then processed on a discontinuous Percoll (Sigma p-1644) gradient consisting of four 2.5 ml layers with densities of 1.13, 1.09, 1.05 and 1.01 g ml^{-1} and centrifuged at 700 rpm for 20 minutes. The band containing purified oocysts was then harvested and washed five times at 1000 rpm for 5 muinutes and then again resuspended in distilled water. The number of oocysts present in 1 mm3 was counted with a haemacytometer after mixing 0.2 ml with 0.8 ml of malachite green.

The slides used in the indirect fluorescence antibody test were coated with a suspension containing approximately 10,000-150000 oocysts per well and diluted in 50 µl of 0.04 M phosphate buffered saline (Lorenzo *et al.*, 1993). The oocysts in the wells were fixed in acetone for 15 minutes and then stored at –20°C until used.

Indirect fluorescence antibody test (IFAT)

A procedure was adopted as that for Eimeria species IgG anibodies (Lorenzo *et al.*, 1993). Doubling dilutions of serum from 1:64 to 1:1024 were prepared in microtitre plates. Dilutions (50µl) were transferred to appropriate slides coated with acetone-fixed oocysts of *Cryptosporidium* species. The slides were incubated at 37°C for 30 minutes in a humidity chamber. The slides were washed twice for 7 minutes in phosphate buffered saline and then rinsed in distilled water and dried. Aliquots (25µl) of fluorescein isothiocynate/ antiglobulin (IgG) conjugate (RAB/FITC, 26-686, Nordic immunological laboratories, Tilburg, Netherlands) were prepared in a similar way as described by Lorenzo *et al.* (1993) and added to the oocysts coated slides aad incubated at 37°C. Slides were washed, dried and mounted in phosphate buffered saline glycerol with a cover glass and examined with an ultra violet microscope and appropriate filters.

RESULTS AND DISCUSSION

Seropositivity of IgG antibodies in naturally infected calves

Of the 63 samples, 12 (19.23%) were found positive with *Cryptosporidium* spp. (Table 1). In 5 (7.9%) faecal samples both *Cryptosporidium* and Eimeria were present while 46 (73.0%) samples were negative. These three groups were designated C, CE and WC respectively. Titers above 64 were regarded as positive (Table 2). In 9 out of 12 (75.0%) cases where *Cryptosporidium* oocysts were present in positive IFAT was observed while in 4 out of 5 (80.0%) positive were seen in the presence of both *Eimeria* and *Cryptosporidium* oocysts. In contrast only 6 out of 46 (13.1%) cases of positive IFAT were seen when no oocysts were recorded. Figure shows IgG antibodies titers of the serum samples tested by IFAT.

Table 1: The presence or absence of coccidian oocysts and specific IgG antibodies to Cryptosporidium sp. in the sera of young calves.

Faecal examination results	No. of samples	IFAT positive	Percentage
Cryptosporidium oocysts detected	12	9	75.0
Eimeria and Cryptosporidium oocysts detected	5	4	80.0
Cryptosporidium oocysts not detected	46	6	13.1
Total	63	19	30.15

Furthermore, oocysts fluoresced brightly with positive serum samples and only faintly or not at all with the negative samples or the conjugate alone.

In the present survey, seropositivity by IFAT was seen in 30.15% of the samples taken. This prevalence is

very similar to that found in calves with confirmed cryptosporidiosis by Villacorta *et al.* (1989). They recorded seropositives by IFAT of 44.18% at the time of acute infection and also four months later. Lorenzo *et al.* (1993) detected a higher percentage (63.35%) of seropositives by IFAT from asymptomatic adult cattle.

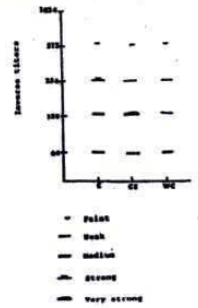

Fig. 1: Results of IFAT for serum IgG antibodies to Cryptosporidium performed on serum samples from naturally infected calves. Titers of 128 or above were considered as positive. C, represents that Cryptosporidium detected, Ce, Cryptosporidium and Emeria detected and WC no coccidia (Both Eimeria and Cryptosporidium) detected.

We have observed a seroprevalence of 13.1% (6/46) where no *Cryptosporidium* spp. oocysts were recorded in the faeces. The presence of IgG antibodies to *Cryptosporidium* does not necessarily correlate with the presence of acute or active infection. Since oocytst are excreted for a relatively short period of time, the presence of IgG antibodies in the absence of oocysts may indicate a previous infection some months or years (Ungar *et al.*, 1988). Ungar *et al.* (1989) showed that IgG antibodies to *Cryptosporidium* in man may persist for at least one to two years after infection. Tzipori and Campbell (1981) and Koch *et al.* (1985) also demonstrated that in excess of 50% of persons with no history of *Cryptosporidium* infection show anti *Cryptosporidium* IgG.

Campbell and Current (1983) reported no cross-reactivity with other coccidia (Toxoplasma, Sarcocystis and Isospora) in their IFAT procedure, Ungar *et al.* (1986) proved that there is no cross reactivity with other intestinal protozoan parasites for IgG in ELISA procedures, except in one or two cases of infection with the human coccidian *Isospora belli*. Villacorta *et al.* (1990) could not find any cross reactions with *Toxoplasma gondii* or Sarcocystis species. On the other hand, Ortega *et al.* (1991) could not find any cross reactions between *Cryptosporidium* and Eimeria spp. in sheep. Furthermore, their titers were similar to our four cases of Eimeria and *Cryptosporidium* positive for oocysts. Moreover, a strong and bright fluorescence was observed in oocysts where the cases were positive with *Cryptosporidium* spp. and while only a weak or faint reaction seen in the cases which were negative. Similar results were presented by Casemore (1987), who demonstrated that oocysts fluoresced brightly with positive samples but only faintly or not at all with negative samples or with conjugate alone.

Table 2: Titers of the specific IgG antibodies to Cryptosporidium sp. in sera of calves with and without presence of oocysts in faeces.

Months	1				2				3				4				5			
Serum dilution	64	128	256	512	64	128	256	512	64	128	256	512	64	128	256	512	64	128	256	512
Calf No.																				
18	+	+	+	-	-	-	-	-	-	-	-	-		N. Ob				N. Ob		
19	-	-	-	-	-	-	-	-	+	+	+	-		N. Ob				N. Ob		
20	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-		N. Ob		
21	-	-	-	-	-	-	-	-	+	++	+	-		N. Ob				N. Ob		
22	-	-	-	-	+	++	++	+	-	-	-	-	-	-	-	-		N. Ob		
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		N. Ob		
24	-	-	-	-	+	++	+	-	-	-	-	-		N. Ob				N. Ob		
25	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-		N. Ob		
26	-	-	-	-	+	+	+	+	-	-	-	-	+	++	+	-		N. Ob		
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		N. Ob		
28	+	+	++	+	-	-	-	-	-	-	-	-	-	-	-	-	+	++	++	+
29	-	-	-	-	-	-	-	-	+	+	++	+	-	-	-	-	+	+	+	+
30	-	-	-	-	+	+	++	+	+	++	+	+	-	-	-	-	-	-	-	-
31	+	++	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-

+ = weak or detectable fluorescence, ++ = Bright fluorescence; - = Negative; N. Ob. = Not observed;

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