Bovine Humoral Immune Response to *Cryptosporidium parvum†*

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Cryptosporidiosis is a diarrheal disease predominately affecting cattle and humans. Sera from experimentally infected calves and calves of various ages with no histories of exposure were evaluated for immunoglobulin G to *Cryptosporidium parvum*. An age-associated increase in immunoglobulin G was present in experimental calves and in calves with no histories of infection from 1 to 3, but not >3, months of age.

Bovine cryptosporidiosis is a zoonotic protozoal disease caused by *Cryptosporidium parvum* (3, 15). It is widespread in cattle and is frequently incriminated as a contributor to the calf diarrhea complex (1, 3, 9, 11). Clinical disease is most common in 1- to 2-week-old calves; however, cryptosporidiosis in calves over 1 month of age is uncommon (3, 11, 15). The purpose of this study was to investigate the seroepidemiologic features of cryptosporidiosis in cattle by surveying the age-associated serum immunoglobulin G response to *C. parvum* in experimentally exposed calves and in calves of various ages which had no histories of *C. parvum* exposure.

Male dairy calves that had received colostrum from their dams and were maintained on milk replacer in isolation stalls were orally infected with 10^8 *C. parvum* oocysts at 2 to 3 days of age. Oocysts used for experimental calf infections and for antigen preparation were preliminarily purified from diarrheal feces by a series of sedimentation, filtration, and centrifugation steps (5). Final purification utilized a discontinuous Percoll density gradient (17), followed by extraction in ether-water and incubation in hypochlorite (14).

Sera were obtained from experimentally infected calves at 0, 7, 14, 21, and 30 to 45 days postinfection (p.i.). Single serum samples were obtained from 279 cattle ranging from 2 weeks to >2 years of age. These cattle were obtained from sale barns, local farms, or unrelated experiments and had no histories of exposure to *C. parvum*. Enzyme-linked immunosorbent assays (ELISAs) were done by standard techniques with 2.5×10^6 sonicated oocysts per well. Each ELISA plate included a positive control consisting of serum from a cow hyperimmunized by two intramammary and three intramuscular injections of sonicated *C. parvum*, three sample controls, and a negative control. Antibody-antigen complexes were detected with horseradish peroxidase-conjugated, affinity-purified goat anti-bovine immunoglobulin G, followed by *o*-phenylenediamine–hydrogen peroxide. Reported values for each sample were the average of the A_{490} of the sample minus the average of the A_{490} of the negative control. Differences in mean A_{490} values between age groups were determined by analysis of variance and subsequent least-squares difference *t* tests and by linear correlation analysis

for age versus A_{490} value comparisons. Significance levels were *P* of <0.05.

Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 2% stacking and 15% resolving gels was used to separate 5×10^7 sonicated *C. parvum* oocysts per ml, which were denatured and reduced by boiling in SDS and beta-mercaptoethanol. Proteins from the gels were transferred to nitrocellulose, blocked with gelatin, and sequentially reacted with sample serum, affinity-purified horseradish peroxidase-labeled goat anti-bovine immunoglobulin G, and 4-chloro-1-naphthol–hydrogen peroxide.

The ELISA results for experimentally infected calves are described in Table 1, and those for calves with no history of exposure to *C. parvum* are described in Table 2. Antibody responses in experimentally infected calves were variable but generally increased with age. In calves with no history of infection, there was a significant difference in A_{490} values between the 1-month-old group and all other groups. Additionally, antibody levels and age had a significant positive correlation from 1 to 3 months of age ($r = 0.613$); however, after this time, there was considerable variation in A_{490} values and poor correlation between age and A_{490} values (r, 0.454 to 0.258).

Immunoblot profiles of sera from experimentally infected calves were similar but varied in the intensity and time of appearance of antigen bands (Fig. 1). By 14 to 21 days, samples from each calf had reactivity to antigen bands at 140 to 90, 55, 38, and 11 to 14 kDa. By 21 days p.i., additional antigens were detected for some calves at 38 and 22 kDa, whereas for other calves the antigen banding pattern began to decrease in intensity. The most consistent features of immunoblots of samples from calves with no history of infection were indistinct narrow bands at >120 kDa, occasional faint bands at 55, 38, and 14 kDa, and rare faint bands at 22 kDa.

The results of this study are similar to those of previous studies which demonstrated that antibodies to *C. parvum* antigens are stimulated by experimental infection and that this response is reflected in generally increasing ELISA values and reactivity to specific antigens on immunoblots (4, 7, 10, 12, 18, 19). However, similar or higher ELISA values in the absence of strong or consistent reactivity on immunoblots to *C. parvum* antigens also occurred for calves of various ages with no histories of infection. There was a correlation between ages and ELISA values for 1 to 3 month

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† Publication no. 92-610J of the Kansas Agricultural Experiment Station.

TABLE 1. Sequential ELISA A_{490} values for seven experimentally infected calves at day 0 (preinoculation) and at days 7, 14, 21, and 30 to 45 p.i.

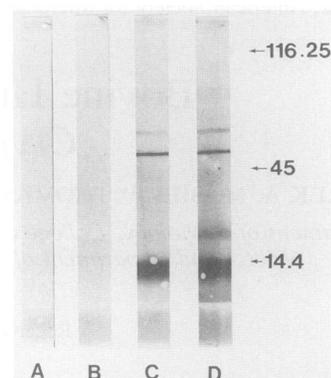| Calf no.	A_{490} at day p.i.:				
	0	7	14	21	30–45
1	1.999	0.225	0.681	0.807	ND[a]
2	0.931	0.605	0.650	0.765	ND
3	0.753	0.909	0.963	0.864	ND
4	1.147	0.789	1.113	1.841	ND
5	0.747	1.031	1.081	1.334	2.849
6	0.390	0.396	0.590	0.836	0.491
7	0.175	0.275	0.575	0.613	0.839

[a] ND, not done.

FIG. 1. Sequential immunoblot profiles for calf no. 1 at day 0 (lane A) and at 7 (lane B), 14 (lane C), and 21 (lane D) days p.i. Locations of molecular weight markers (in thousands) are indicated on the right.

old calves, but this trend did not hold for older calves and was not associated with any consistent immunoblot pattern.

Several factors may contribute to this lack of correlation between ELISA values and reactivity to specific *C. parvum* antigens. The complex composition of *C. parvum* makes it probable that multiple antigenic carbohydrates and glyco-proteins are associated with the organism (7, 13). Many of these antigens may be detected in ELISAs but may not be recognized when denatured samples are utilized for immunoblot analysis. Possible cross-reactivity with contaminating antigens in the ELISA or a low concentration of anti-*C. parvum* antibodies in all samples is an additional factor to consider. Poor correlation between antibody level and specific antigen reactivity was also demonstrated in a similar study using human sera (16). Similarly, uninfected persons sometimes had antibody titers which were comparable to titers in infected persons (2).

A variety of *C. parvum* antigens which could contribute to the ELISA response have been detected (6, 10, 13, 16, 18). A 22-kDa antigen in the current study reacted strongly with sera from all experimentally infected calves but weakly with sera from only a few calves with no history of exposure. In another study, a 20-kDa antigen was recognized by bovine sera at 3 and 16 weeks p.i. but not by sera at 20 and 28 weeks p.i. (10). Similarly, reactivity to a 20-kDa antigen occurred in calves by 9 to 14 days p.i. (18). In humans, a 23-kDa antigen was recognized by sera from 45% of ELISA-positive individuals who had no previous histories of infection and by 93% of sera from individuals with histories of infection (16). The broad 14- to 11-kDa antigen recognized in the current study by sera from all experimentally infected calves and a

few calves with no history of exposure is most likely the same as the 11-kDa antigen described as the sole reactive antigen at 14 days p.i. in another calf study (18). Additional bands at 55 and 38 kDa were recognized by sera from all experimental calves but rarely by sera from calves with no history of exposure.

Cryptosporidium parvum oocysts can be detected in a high proportion of cattle, with incidence rates from 38.7 to 40.7% (1, 11). Importantly, oocysts were detected in 60% of 8- to 14-day-old calves but were not found in calves >21 days old (11). A serologic study estimated that 58% of cattle from farms with confirmed cryptosporidiosis and 40.9% of cattle selected at random had antibodies to *C. parvum* (8). It is likely that many of the 269 cattle with no history of exposure had been previously exposed to *C. parvum*; yet, neither the ELISA nor immunoblots had any consistent recognizable variation which could be used as a predictor of the exposure status of the animal.

This survey demonstrated an age-associated increase in serum immunoglobulin G to *C. parvum* in experimentally infected calves and young calves with no histories of infection. In cattle from 3 to 24 months of age, however, there were no significant relative age-associated changes in ELISA or immunoblot reactivity. Any association between this serologic trend and the age-associated incidence of cryptosporidiosis in cattle is yet to be investigated.

TABLE 2. Mean ELISA A_{490} values for calves of various ages with no history of exposure to *C. parvum*

Age (months)	No. of samples	Mean ELISA A_{490} value	SEM
1	38	1.181	0.106
2	12	1.662	0.184
3	13	2.013	0.128
4	44	1.942	0.059
5	17	1.727	0.137
6	11	1.831	0.175
8	22	2.062	0.063
10	22	1.643	0.085
11	35	1.954	0.071
14	9	2.090	0.066
18	44	1.921	0.054
24	12	2.263	0.091

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