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Prevalence of *Cryptosporidium* Antibodies in 10 Animal Species

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Antibodies to cryptosporidium were detected by indirect immunofluorescence in over 80% of sera from 10 animal species, including humans.

The genus *Cryptosporidium*, an enteric coccidian parasite, has been identified in a wide range of vertebrates (2). Since their discovery in 1907 (11), cryptosporidia have been reported to be associated with enteritis in a number of species, including calves (4, 5, 7, 9, 15), lambs (1, 12), turkeys (8), foals (10), humans (3, 6, 14, 17), deer (S. Tzipori, K. W. Angus, I. Campbell, and D. Sherwood, submitted for publication), and possibly others. The different cryptosporidium isolates were designated on the basis of their host origin (11, 16) and location within the gut (11) and the duration of the endogenous life cycle (16). However, we have shown that cryptosporidium isolates from calves, lambs, deer, and humans infect other species. Thus, the calf isolate induced diarrhea in lambs (S. Tzipori, K. W. Angus, E. W. Gray, I. Campbell, and F. Allan, Am. J. Vet. Res., in press) and piglets (S. Tzipori, E. McCartney, G. H. K. Lawson, A. C. Rowland, and I. Campbell, submitted for publication) and subclinical infection in laboratory animals (13). On this basis, it was argued that *Cryptosporidium*, like *Toxoplasma*, is probably a one-species genus (13).

In this communication, we describe an indirect immunofluorescence (IF) procedure for detection of antibodies to cryptosporidium and report the prevalence of antibody in a number of host species.

A 1-day-old, specific pathogen-free (SPF) lamb was inoculated with cryptosporidium originally isolated from calves with diarrhea (Tzipori et al., in press) and subsequently passaged once in SPF rats (13). The lamb was killed when moribund 48 h after the onset of anorexia and diarrhea. Four gut portions were taken from the middle and lower ileum for histology and cryostat sections. The most extensively infected site, as assessed by examination of hematoxylin-and-eosin-stained preparations, was selected for cryostat sectioning. Numerous examples of the endogenous stage of the parasite life cycle were seen. Cryostat sections from uninfected SPF lambs were used as negative controls.

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On two separate occasions, sera collected from 10 species of animals, including humans (Table 1), were diluted 1/10 in phosphate-buffered saline and tested in an indirect IF test against cryostat sections of infected and uninfected tissues. The results presented in Table 1 indicate that the majority of sera gave positive reactions with infected sections. Figure 1 shows the typical granular fluorescence coating the villi of infected gut sections.

Seven calves and eight lambs of up to 30 days of age and two newborn pigs all deprived of colostrum had no serum antibodies against cryptosporidium. Calves and lambs which were maintained under SPF conditions were still serologically negative 7 days after experimental inoculation with cryptosporidium but were positive after 14 days.

None of the eight fluorescein-conjugated antispecies antisera used for indirect procedures reacted directly with cryostat sections of infected or control gut. (Rabbit anti-sheep serum was used with sera from sheep, cattle, and deer because of their close serological relationship). It is not known whether cryptosporidial antigens are shared by other microorganisms found in the gut, but rabbit sera raised against K99 (adherence antigen of enterotoxigenic *Escherichia coli* affecting lambs) and rotavirus failed to react with the infected and control gut sections.

Further criteria of specificity were as follows. (i) The fluorescence was extracellular and had a granular character unlike that of enterotoxigenic *E. coli*. (ii) No fluorescence was detected in gut sections of age-matched uninfected SPF lambs. (iii) Negative results were obtained with mouse intestine from and an SPF colony susceptible to cryptosporidium infection. (iv) Seroconversions in calves and lambs occurred after experimental inoculations. (v) The direct IF test was negative.

This communication reports the first serological procedure for the detection of antibodies against cryptosporidium. The results show that cryptosporidia infecting different species of animals have a common antigen detectable by IF and that antibodies to cryptosporidium are very prevalent among, as well as within, the host

TABLE 1. *Prevalence of antibodies against cryptosporidium in 10 species of animals*

Species	No. of:		Positive by indirect IF	
	Sources	Samples tested	No.	%
Humans	Wide[a]	21	18	86
Dogs	Wide	20	16	80
Cats	Wide	23	20	87
Cattle	Wide	25	25	100
Sheep	3	23	23	100
Pigs	5	43	41	95
Deer	2	12	12	100
Horses	Wide	22	20	91
Mice	1[b]	11	0	
Chickens	1	25	22	88

[a] Randomized samples from blood donors at a hospital in Edinburgh.

[b] Pools (six mice per sample) from an SPF mouse colony.

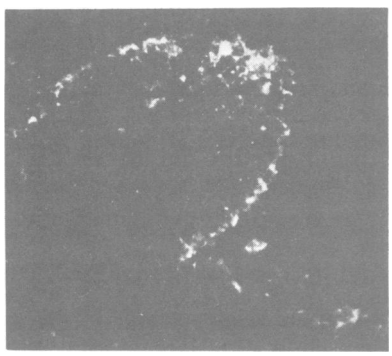

FIG. 1. *Indirect IF staining of a villus from a lamb infected with cryptosporidium.*

species. Although the number of samples tested was small, the high prevalence of positive sera suggests that the organism is indeed widespread.

Although the IF test may detect only group-specific antigen(s) and is nonquantitative, it was sufficiently sensitive to detect seroconversion in many of the samples tested. There was no difference in the quality of the fluorescence obtained with antisera from different host species.

The nature of the antigen(s) detected by IF was not examined but it is likely to be complex and to involve various stages of the parasite during its life cycle. However, since electron microscopy reveals only few oocysts adhering to the mucosa, they are unlikely to be a major source of antigen.

The lack of host specificity, the identical behavior of cryptosporidia isolated from different host species in experimental animals, and the present evidence of shared antigenicity strengthen an earlier suggestion (13) of a single-species genus.

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