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Veterinary Parasitology, 47 (1993) 17–23
Elsevier Science Publishers B.V., Amsterdam

Diagnosis of *Cryptosporidium* on a sheep farm with neonatal diarrhea by immunofluorescence assays

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(Accepted 27 September 1992)

ABSTRACT

Xiao, L., Herd, R.P. and Rings, D.M., 1993. Diagnosis of *Cryptosporidium* on a sheep farm with neonatal diarrhea by immunofluorescence assays. *Vet. Parasitol.*, 47: 17–23.

An outbreak of diarrhea in neonatal lambs occurred on a sheep farm in northern Ohio. Diarrhea commenced as early as 1 week of age and lasted for about 3–4 days. Although 100% of the newborn lambs were affected, most had recovered by 3 weeks of age. *Cryptosporidium* infection appeared to be the cause of diarrhea. Fecal examination of nine diarrheic newborn lambs (5–10 days old), 23 older lambs (2–3 weeks old, six with diarrhea) and 23 clinically normal ewes by immunofluorescence assays revealed infection rates of 100%, 78.3% and 17.4%, respectively. Most newborn lambs had high oocyst counts. Ewes were considered to be an important source of infection for lambs.

INTRODUCTION

Cryptosporidium spp. are small coccidian parasites infecting the microvillous border of digestive and respiratory epithelial cells of vertebrates (Current and Blagburn, 1990). They have been associated with diarrheal diseases in neonatal calves and with immunosuppression in humans in numerous studies (see reviews by Angus, 1990; Current and Blagburn, 1990).

Cryptosporidium sp. was first described in sheep by Barker and Carbonell in 1974. At that time, it was thought that infection in lambs was rare and clinically inapparent. More recent studies in Europe have suggested that *Cryptosporidium* infection in lambs is probably widespread (Nagy et al., 1983; Pavlásek et al., 1986; Cvetković and Dimitrijević, 1988; Villacorta et al.,

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1991). Clinical outbreaks of diarrhea associated with cryptosporidiosis in lambs have been reported in Britain (Tzipori et al., 1981a; Angus et al., 1982), Spain (De las Heras et al., 1987) and the US (Berg et al., 1978; Anderson, 1982).

Recently, an extensive outbreak of diarrhea probably due to *Cryptosporidium* infection occurred on a sheep farm in northern Ohio. This report describes the outbreak and the results of fecal examinations. Since traditional diagnostic methods such as acid fast staining and sugar flotation have been shown to have low specificity and sensitivity (Garcia et al., 1987; Arrowood and Sterling, 1989; Rusnak et al., 1989; Current and Blagburn, 1990; Weber et al., 1991), new immunological methods were used for oocyst detection.

MATERIALS AND METHODS

Animal history

The outbreak of diarrhea started in early March 1992 in a lambing barn of a private sheep farm in northern Ohio. This barn housed about 110 ewes and 150 lambs. Half of the flock were Cheviots, the remainder were Dorset and crossbred ewes. This barn had been used for lambing in the fall of the previous year. Consecutive groups of ewes lambed in this barn, beginning in January 1992. Ewes and lambs were housed together before weaning. Problems started with suckling lambs from the second lambing period, with almost all lambs showing diarrhea. Diarrhea mostly began at 1 week of age and lasted 3–4 days. By 3 weeks of age, most lambs had recovered. Despite the high morbidity, only two deaths of diarrheic lambs occurred. The owner had tried treatments with oral neomycin, potentiated sulfas (SMZ-TMP) and spectinomycin, with no apparent efficacy. One 9-day-old lamb from the problem flock was sent to The Ohio State University Veterinary Hospital on 18 March for clinical examination and necropsy. Physical examination of the lamb suggested enteritis and necropsy revealed multifocal small intestinal cryptitis. Fecal examinations by flotation and immunofluorescent assay showed numerous *Cryptosporidium* oocysts, but no other parasitic infections.

Fecal samples

Nine, 23 and 23 rectal fecal samples were taken on 20 March from newborn lambs (5–10 days old), older lambs (2–3 weeks old) and ewes, respectively (Table 1). These sheep were all housed in the same shed. All lambs from the newborn group and six from the older lamb group had yellowish diarrhea at the time of sampling. The rest excreted firm pellets.

TABLE 1

Prevalence of *Cryptosporidium* infection in newborn lambs (5–10 days old), older lambs (2–3 weeks old) and ewes on an Ohio sheep farm with neonatal diarrhea

Group	No. examined	No. positive	Infection index[1]			
			1	2	3	4
Newborns	9	9	1	0	0	8
Older lambs	23	18	13	2	0	3
Ewes	23	4	4	0	0	0

[1]Based on the number of oocysts per ×400 field: 1, one to two oocysts; 2, three to five oocysts; 3, six or seven oocysts; 4, eight or more oocysts.

Immunofluorescence assay

Fecal samples were examined for *Cryptosporidium* oocysts by both direct and indirect immunofluorescence staining, using commercial kits from Meridian Diagnostics, Cincinnati, OH. The indirect immunofluorescence assay could detect only *Cryptosporidium* infection, while the direct immunofluorescence kit was designed for the simultaneous detection of *Cryptosporidium* and *Giardia*.

Stained fecal smears were examined at magnifications of ×200 and ×400 by epifluorescence microscopy. The entire smear (about 78.5 mm^2) was searched to verify the presence or absence of oocysts. Severity of infection was classified by the number of oocysts detected per ×400 field (0.11 mm^2), using a criteria similar to that used by Arrowood and Sterling (1989): 1 for one to two oocysts; 2 for three to five oocysts; 3 for six or seven oocysts; 4 for eight or more oocysts.

RESULTS

All newborn lambs, 78.3% of older lambs and 17.4% of ewes were found to excrete *Cryptosporidium* oocysts at the time of sampling (Table 1). Of the newborn lambs, all except one showed heavy infection (infection index 4), while only three older lambs did so. These three lambs had diarrhea at sampling. The other three diarrheic older lambs were also positive for *Cryptosporidium* infection, although the infection intensity was lower. All positive ewes had low oocyst counts (infection index 1). Oocysts were ovoid and 4–6 μm in size. Two older lambs (both with *Cryptosporidium* infection) with no diarrhea and seven ewes (one with *Cryptosporidium* infection) also had some *Giardia* cysts in their samples.

DISCUSSION

As microbiological examinations were not performed, it is difficult to make a definite diagnosis on the cause of the diarrhea. According to Mitchell and Linklater (1983), diarrhea in lambs 0–4 weeks of age is usually caused by infections of enterotoxigenic *Escherichia coli* (ETEC), *Clostridium, Salmonella*, rotavirus, or *Cryptosporidium*. Since multiple treatments with several antibiotics and sulfonamides were given during the present outbreak, it is considered less likely that bacterial infections caused the diarrhea. Since lambs usually develop an age immunity to rotavirus by 7 days (Tzipori et al., 1981c), it is unlikely that this agent is implicated in the present outbreak, where diarrhea mostly occurred after 1 week of age. Judged by the high infection rate of *Cryptosporidium* in newborn lambs and the correlation between the intensity of oocyst excretion and the occurrence of diarrhea, *Cryptosporidium* infection was probably responsible for the diarrhea outbreak. *Giardia* infection apparently did not contribute to the diarrhea, because its infection rate was low and occurred in older lambs without diarrhea.

Infection rates of sheep by *Cryptosporidium* in this outbreak were high, compared with most earlier studies in other countries (Nagy et al., 1983; Ramisse et al., 1984; Ahourai et al., 1985; Ayeni et al., 1985; Gialletti et al., 1986; Gorman et al., 1990; Villacorta et al., 1991). Prior surveys, however, were conducted mainly with healthy lambs. Studies with diarrheic lambs revealed higher infection rates (Tzipori et al., 1981a; Hiepe et al., 1985; Pavlásek et al., 1986; Cvetković and Dimitrijević, 1988). Other factors such as the type of detection method employed might also be responsible for the lower infection rates obtained elsewhere. Most other studies used acid fast staining in fecal examinations. Cozon et al. (1992) found that acid fast staining stained only 3–13% of the oocysts. Garcia et al. (1987) suggested that acid fast staining is at least ten times less sensitive than indirect immunofluorescent antibody staining. Similar results were obtained by Weber et al. (1991). Arrowood and Sterling (1989) further reported that acid fast staining had only 52% specificity. It is likely that false-negatives and false-positives were present in earlier surveys. The present report used immunofluorescence assays for the first time in a prevalence study of *Cryptosporidium* infection in domestic animals.

Despite the high *Cryptosporidium* infection rates and morbidity of lambs, mortality was relatively low on this Ohio farm. Only two lambs died and diarrhea was generally limited to 3–4 days. This is in contrast to the report by Tzipori et al. (1981a), in which 40% of diarrheic lambs died of *Cryptosporidium* infection, and diarrhea lasted 7–16 days. In that study, diarrhea occurred in artificially reared lambs deprived of colostrum, whereas in the present study it occurred in naturally reared suckling lambs. Angus et al. (1982) suggested that colostrum was protective and reduced deaths in *Cryptosporidium* infected lambs.

Few studies have been conducted to elucidate the sources of *Cryptosporidium* infection in lambs. Experimental infections with oocysts isolated from calves showed that bovine isolates of *Cryptosporidium* could infect newborn lambs and induce diarrhea (Tzipori et al., 1981b; Snodgrass et al., 1984). Based on this, Angus (1990) suggested that the close proximity of cattle, or the use of grazing land or housing recently occupied by calves, was the main source of infection for many outbreaks of lamb cryptosporidiosis.

In this study, the farm is exclusively a sheep farm and no cattle have ever been maintained on it. Cattle, therefore, were obviously not the source of infection in this outbreak. Some lactating and prepartum ewes were excreting *Cryptosporidium* oocysts, so infection may have been derived from subclinically infected ewes. As the infection in ewes was light, the contamination of the surroundings by them was probably not severe enough to induce diarrhea in lambs born during early lambings. It is possible that amplification of contamination by the infection of newborn lambs had occurred by the time diarrhea began during the second lambing period in this outbreak. A similar outbreak was also reported by Angus et al. (1982), in which diarrhea of lambs commenced midway through the lambing period. In the latter outbreak, however, no examinations for infection were conducted in ewes and ewes were not implicated as the source of infection.

There are very few surveys on the prevalence of *Cryptosporidium* infection in ewes. Although Anderson (1982) was unable to infect ewes with *Cryptosporidium* oocysts isolated from lambs, Papadopoulou et al. (1988) showed a 19.5% infection rate in adult sheep in Greece, a figure very close to the one presented here. It seems that cryptosporidiosis can be an independent ovine disease.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Jacqueline Bird for technical assistance.

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