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CALVES AS A POTENTIAL RESERVOIR OF *CRYPTOSPORIDIUM PARVUM* AND *GIARDIA* SP.

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Abstract: Studies on cryptosporidiosis and giardiosis were carried out between March and April 1997 on 75 calves from 9 selected farms of Wielkopolska macroregion. Faecal specimens from calves, 3–13 days old, were screened for oocysts of *C. parvum* using Ziehl-Neelsen staining and both for oocysts of *C. parvum* and cysts of *Giardia* sp. using direct immunofluorescent (MerIFluor *Cryptosporidium/Giardia*) assay. The oocysts of *C. parvum* assessed by Ziehl-Neelsen staining were revealed on 6 (67%) of 9 farms examined. The prevalence of infection ranged from 20–88%, and in some farms intensity of oocyst shedding was very high. However, in 35 calves assessed for mixed infections of *C. parvum* and *Giardia* sp., oocysts of *Cryptosporidium* were found in 18 (51%) calves and cysts of *Giardia* sp. were detected in 5 (14%) of 35 calves. Only in one calf was found coinfection with both parasites. The intensity of *Giardia* sp. infection was extremely low. Histological examination of the gut sections from immunosuppressed BALB/c mice experimentally infected with *C. parvum* isolates from calves revealed endogenous stages of *C. parvum* on the brush border of the ileum. The high prevalence and intensive shedding of cryptosporidial oocysts by calves in farms examined in this study suggests that naturally infected calves may be significant reservoirs for *C. parvum* infections in man and wild animals.

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INTRODUCTION

Numerous species of mammals have been reported to be hosts of *Cryptosporidium* spp., intestinal coccidian parasites which are a common cause of diarrheal illness in man (including those immunosuppressed or infected with AIDS) and animals [8, 12, 14, 21]. A variety of domestic animals, including calves, appear to be important zoonotic reservoirs for *C. parvum* infection in man, while wild animals may also be a source of infection [4, 5, 6, 18, 20]. Since the early 1970s, cryptosporidial infection has been recognised all over the world as a cause of diarrhoea in neonatal animals, particularly calves, [1, 13, 15, 19]. Recently, it has been estimated that over 90% of the dairy cattle herds in the United States are infected with *C. parvum* [22] and in two different parts of Europe (Poland and Portugal), about 40% of calves with diarrhoea were found to be infected [10, 16].

Because of the world-wide importance of both cryptosporidiosis and giardiosis in humans and animals, and the absence of comprehensive data on the occurrence of these parasites in calves from Poland, the present study was conducted to determine the prevalence of infections in calves from arbitrarily selected farms of Wielkopolska macroregion. Additionally, the infectivity of *C. parvum* oocysts isolated from faeces of naturally infected calves was tested by the experimental transmission to mice.

MATERIALS AND METHODS

Specimen preparation and examination of faecal samples. During February to April 1997, faecal specimens

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Table 1. Prevalence of *Cryptosporidium parvum* infection in calves on some farms from Wielkopolska macroregion.

Farm	Number of calves examined	Number of positive(%)	Number of calves shedding oocysts with intensity of:		
			(+)	(++)	(+++)
A	8	7 (88)	2	3	2
B	25	18 (72)	5	6	7
C	7	5 (71)	2	1	2
D	10	6 (60)	3	0	3
E	5	2 (40)	0	2	0
F	5	1 (20)	1	0	0
G	5	0	0	0	0
H	5	0	0	0	0
I	5	0	0	0	0
Total	75	38 (51)	13	12	14

(+) < 5 oocysts in each of 20 fields;
(++) = 5 to 10 oocysts in each of 20 fields;
(+++) > 10 in each of 20 fields, 400 × magnification.

were collected from 75 calves, aged from 3–16 days, from 9 farms in Wielkopolska region. Faecal smears prepared on glass slides were air dried, fixed in 100% methanol and stained using a modified Ziehl-Neelsen technique [9]. All slides were studied for typical acid-fast cryptosporidial oocysts at 400 × magnification. Specimens containing oocysts were categorised into three groups as follows: (+) < 5 oocysts, (++) 5 to 10 oocysts, (+++) > 10 oocysts in each of 20 microscopic fields. Thirty five samples from 5 selected farms were examined by a direct immunofluorescent technique, the MerIFluor *Cryptosporidium/Giardia* assay as described by Bajer *et al*. [3]. The oocysts of *C. parvum* and cysts of *Giardia* sp. were measured using a calibrated eyepiece graticule.

Infection of laboratory mice with *C. parvum*. Isolates of *C. parvum* oocysts obtained from naturally infected calves were tested for their infectivity to mice. The oocysts

Table 2. *Cryptosporidium parvum* oocysts and *Giardia* sp. cysts detected by direct immunofluorescent test in calves from selected farms.

Farm	Number of calves examined	Faecal specimens (positive (%))	
		Cryptosporidium	*Giardia*
B	10	8	0
D	10	7	1*
E	5	2	2
F	5	1	0
G	5	0	2
Total	35	18 (51)	5 (14)

* coinfection of *Cryptosporidium parvum* and *Giardia* sp.

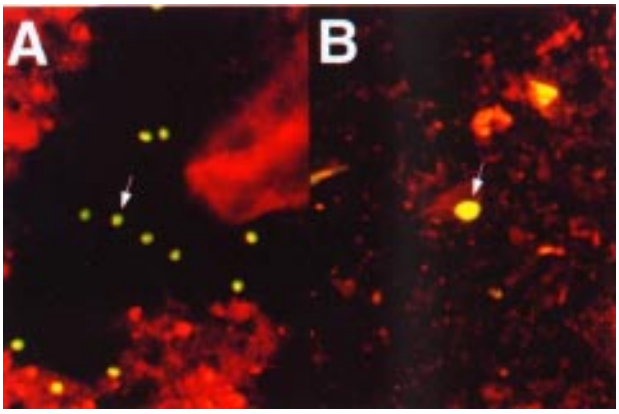

Figure 1. Oocysts of *Cryptosporidium parvum* (A) and cyst of *Giardia* sp. (B) stained with MerIFluor (*Cryptosporidium*/*Giardia*) direct fluorescence.

were isolated from faeces mixed in 2.5% w/v $K_2Cr_2O_7$, passed through wire mesh sieves and stored at 4°C for no more than 1 month prior to use. The oocysts were purified and concentrated by flotation on sucrose gradients with two specific gravities of 1.103 and 1.064 respectively [2]. The oocysts were counted in 0.02 ml volume and if necessary, the number of oocysts for inoculation was determined by adjusting the volume. Before inoculation, oocysts were incubated for 24 hrs at 37°C with antibiotics (200 µg/ml of penicilin G and 100 µg/ml of gentamycin sulfate) and subsequently washed in PBS.

Ten inbred female BALB/c mice were orally inoculated at 4 weeks of age with 10^4 oocysts each. Before inoculation, the mice were immunosuppressed using dexamethasone administered at a dose of 0.125 mg/mice/day in drinking water for 8 days. The patency of *C. parvum* infection under experimental conditions was confirmed by examination of faeces collected daily until the 7th day post inoculation (dpi). Endogenous stages of *C. parvum* were also sought in intestinal specimens of each inoculated mouse examined at necropsy on 7 dpi. Approximately 1 cm pieces of gut from the posterior jejunum and ileum were processed for histological examination after fixation in 10% buffered formal saline (pH 7.2), embedding in paraffin, sectioning at 5 µm thickness and staining with haematoxylin and eosin.

RESULTS

Prevalence and intensity of infections with *C. parvum* and *Giardia* sp. in calves. In the Wielkopolska macroregion oocysts of *C. parvum* were found on 6 (67%) of the 9 farms (Tab. 1). The prevalence of infections ranged from 20–88%, and intensity of infections were heavier on farms A, B and C. Using the MerIFluor test, oocysts of *C. parvum* were detected in 18/35 (51%) calves and cysts of *Giardia* sp. in 5/35 (14%) (Tab. 2). *C. parvum* oocysts were round, measuring approximately 4–6 µm, while *Giardia* sp. cysts were oval, measuring approximately 11–15 µm. Only one calf from farm D had a

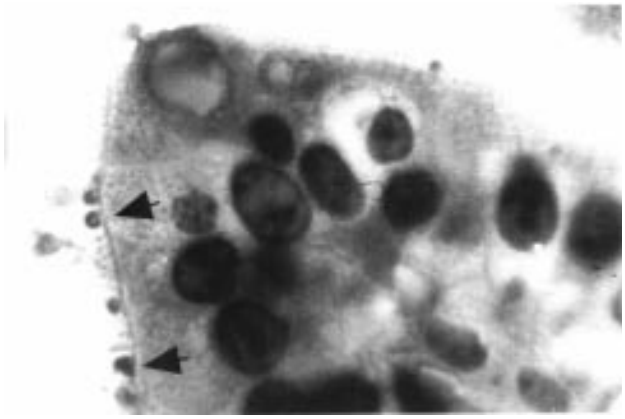

Figure 2. Mouse ileum section showing endogenous stages of *Cryptosporidium parvum* on the brush border, magnification × 1000. Parasites are arrowed.

mixed infection of *C. parvum* and *Giardia* sp. Figure 1 shows oocysts of *C. parvum* and cyst of *Giardia* sp. stained with MerIFluor (*Cryptosporidium/Giardia*) direct fluorescence.

Infection of BALB/c mice with *C. parvum* isolated from calves. Oocysts of *C. parvum* were detected in the faeces of all experimentally infected mice from 2–7 dpi. Histological examination of the gut sections on 7 dpi revealed endogenous stages of *C. parvum* in all inoculated mice (Fig. 2).

DISCUSSION

The detection of both cryptosporidial oocysts and *Giardia* sp. cysts using direct immunofluorescent (MerIFluor *Cryptosporidium/Giardia*) test creates very sensitive and specific diagnosis of infection with these parasites.

The high prevalence and intensity of *C. parvum* infections in calves on some farms in the Wielkopolska macroregion indicates that they are common parasites in this region. In contrast, the low prevalence and intensity of *Giardia* sp. infection on farms indicates they are rare in this region. Calves infection with *C. parvum* in some studied farms creates a condition for zoonotic transmission and environmental spreading of oocysts through direct or soil-water routes.

Experimental infections showed that *C. parvum* isolates from naturally infected calves were infective to immunosuppressed laboratory mice. According to Current and Blagburn [6], adult laboratory mice cannot be readily infected with *C. parvum*, whereas neonates (1-day old mice) orally inoculated with *Cryptosporidium* oocysts of calf origin were heavily infected [16]. It seems more likely that *C. parvum* from calves may be infective to wild rodents and could contribute to zoonotic reservoirs of infection.

However, controlling the spread of *C. parvum* or *Giardia* sp. requires the reduction or elimination of parasites from the environment, but this seems extremely difficult because oocysts and cysts are very resistant to various commonly used disinfectants [8]. Under favourable conditions they remain infectious for a relatively long time. Thus, not only immunosuppressed persons [10] but also immunocompetent farmers, veterinary, medical and laboratory workers are at high risk of infections associated with ingesting of cysts or oocysts from the environment contaminated with faeces of infected calves. Infections with both parasites in immunocompetent persons could be serious, but are generally self-limited and leave the host solidly immune to reinfection.

Very high prevalence and intensive shedding of cryptosporidial oocysts by calves on some farms presented in this study may indicate that naturally infected calves pose an essential source of *C. parvum* infections enabling wide environmental spreading of oocysts through direct or soil- water routes into wild animals and humans.

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