Prevalence of *Giardia* and *Cryptosporidium* in beef cows in southern Ontario and in beef calves in southern British Columbia

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Abstract — In 1998 and 1999, fecal samples were collected from 669 beef cows on 39 farms located within 10 counties of Ontario. Overall prevalences of *Giardia, Cryptosporidium muris,* and *Cryptosporidium parvum* in cows were 8.7%, 10.6%, and 18.4%, respectively. Of the 39 farms sampled, *Giardia* was detected on 64%, *Cr. muris* on 72%, and *Cr. parvum* on 90%. *Cryptosporidium parvum* was detected in 28% of the cows in 1998 and in 5.2% in 1999. Differences between the 2 y were attributed to sampling during calving in 1998 and during gestation in 1999. In 1998, *Giardia, Cr. muris,* and *Cr. parvum* were detected in herds provided with municipal water. In 1998, 193 calves were sampled from 10 farms, representing 4 watersheds, in British Columbia. Thirty-six percent of the calves exhibited signs of diarrhea. Overall prevalences of *Giardia* and *Cryptosporidium* spp. in calves were 36% and 13%, respectively. There was evidence that calves with *Giardia* were more likely to develop scours. Restricting cattle from surface water during periods of high shedding may reduce watershed contamination.

Résumé — Prévalence de *Giardia* et *Cryptosporidium* chez des vaches de boucherie du sud de l'Ontario et chez des veaux de boucherie du sud de la Colombie-Britannique. En 1998 et 1999, des échantillons fécaux ont été prélevés chez 669 vaches de boucherie sur 39 fermes situées dans 10 comtés de l'Ontario. La prévalence globale de *Giardia*, de *Cryptosporidium muris* et de *Cryptosporidium parvum* chez les vaches étaient respectivement de 8,7 %, 10,6 % et 18,4 %. Des 39 fermes participantes, *Giardia* a été détecté chez 64 %, *Cr. muris* chez 72 % et *Cr. parvum* chez 90 % d'entre elles. *Cryptosporidium parvum* a été détecté chez 28 % des vaches en 1998 et 5,2 % en 1999. Cette différence a été attribuée au moment de l'échantillonnage : au vêlage en 1998 et en cours de gestation en 1999. En 1998, *Giardia, Cr. muris* et *Cr. parvum* ont été détectés dans des fermes alimentées par un aqueduc municipal. En 1998, 193 veaux ont été échantillonnés sur 10 fermes provenant de 4 bassins versants en Colombie-Britannique. Des signes de diarrhée étaient présents chez 36 % des veaux. Les prévalences globales de *Giardia* et de *Cryptosporidium* spp. chez les veaux étaient respectivement de 36 % et de 13 %. Il y avait des preuves que les veaux avec *Giardia* étaient plus à risques de développer de la diarrhée profuse. L'éloignement des bovins de l'eau de surface dans les périodes de fortes excrétions fécales pourrait réduire la contamination des bassins versants.

(Traduit par Docteur André Blouin)

Can Vet J 2005;46:47–55

Introduction

Giardia and *Cryptosporidium* spp. have been implicated as a cause of diarrhea in dairy calves (1,2) and are common intestinal parasites of a wide range of vertebrates (3,4). Dairy calves can excrete high numbers of cysts or oocysts for weeks, and there are indications that both diseases can potentially reduce the growth performance of ruminants (5,6). Mortality from either disease is rare, but severe cryptosporidiosis is occasionally responsible for mass fatalities in calves (7,8)

There is evidence that both parasites are zoonotic, as humans have been infected with *Giardia* spp. from

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This is Lethbridge Research Centre contribution number 38703078.

animals (9) and cryptosporidiosis in humans has been linked to infected calves (10) and household pets (11). Outbreaks of both parasites in humans have been attributed to agriculture effluent from livestock (12,13). A surface water study conducted in the vicinity of a cattle ranch in British Columbia demonstrated that numbers of *Giardia* cysts and *Cryptosporidium* oocysts in raw water from a creek passing through the ranch were higher downstream than upstream of the ranch, and that the highest concentrations of both parasites in the raw water samples were recorded coincident with peak calving time (14). Consequently, several researchers and public health officers have concluded that cattle play a major role in the contamination of surface water used for both drinking and recreational purposes (1,15,16).

In Canada, studies on the prevalence of *Giardia* and *Cryptosporidium* in cattle have focussed mainly on dairy herds (1,16). In the Fraser River valley, *Giardia* was identified on all 20 dairies examined, whereas *Cryptosporidium parvum* was found on 80% of dairy farms (1). Further examination of 505 dairy farms in Quebec indicated that 45.7% were positive for *Giardia* and 88.7% were positive for *Cryptosporidium* spp. (16). Confinement practices in dairy calf production likely increase both *Giardia* and *Cryptosporidium* infection rates, as compared with the production of beef on open range. This possibility is supported by the fact that dairy calves kept outside the barn were less likely to be infected with *Giardia* than those housed inside (16). Furthermore, reinfection of calves with *Giardia* after repeated treatment with fenbendazole illustrates that infective cysts may be readily consumed within a confined environment (17). Thus, differences in production practices may account for the one Canadian study that observed higher infection rates of *Cryptosporidium* in dairy calves (63.3%), as compared with beef calves (18.4%) (18).

Although *Giardia* and *Cryptosporidium* are prevalent in calves, it is likely that mature animals serve as reservoirs for the infection of younger animals. Periparturient increases in the excretion of *Giardia* cysts and *Cryptosporidium* oocysts have been reported in ewes (19) and we have observed a similar response in beef cows (20). However, the prevalence of *Giardia* and *Cryptosporidium* in mature beef cattle or calves has not been well documented within Canada. The objective of the present study was to determine the prevalence of *Giardia* and *Cryptosporidium* in beef cows in southern Ontario and southern British Columbia.

Materials and methods

Sample collection — southern Ontario

In 1998, 39 cow-calf farms were selected for participation in the study on the basis of their location within 10 counties distributed within the south, west, and central agricultural regions of southern Ontario with the highest beef cattle populations, and on the owners' willingness to collaborate. Three to 6 farms per county participated; on each farm, 10 cows were randomly selected from the herd for sampling. During the 1998 collection (March 31 to May 22), fecal samples were collected from 382 cows on the 39 farms: 7 in the central region, 9 in the southern region, and 23 in the western region.

Due to changes in ownership, willingness to continue the collaboration, or both, repeat samples could not be collected in 1999 from 10 of the sites sampled in 1998. Thus, during the 1999 collection (December 16, 1998 to April 21, 1999), fecal samples were collected from 287 cows on 29 of the farms. Constraints with handling systems and turnover within the cow herds precluded sampling the same individual cows in both years.

The majority of the farms used wells as their source of water (34 sites), 2 sites were supplied with municipal water, and 3 farms used surface watering sites. During the spring sampling in 1998, the cows were maintained on pasture, whereas at the time of the 1999 collections, they were housed in confined lots and provided with preserved forage. The cows ranged from 2 to 14 y of age, but insufficient data from producers precluded calculation of a mean or median age.

Fecal samples (1/cow/y) were collected by a trained technician via rectal extraction at each farm. Approximate quantities (2 to 3 g) of fecal material were collected into preweighed, 15-mL screw-capped polypropylene tubes, then the tubes were reweighed to determine sample weight. After the addition of 5 mL of 10% (v/v) formalin, the tubes were capped and the contents were mixed thoroughly. Once a sufficient number of samples had been obtained, they were sent via courier to the University of Calgary for enumeration of *Giardia* cysts and *Cryptosporidium* oocysts.

Sample collection — southern British Columbia

During the 1998 calving season (March to May), 192 blood and fecal samples were collected from 10 different beef cattle ranches (13 to 20 animals per site) selected on the basis of their location within 4 different watersheds (Okanagan, Columbia, Thompson-Nicola, Kootenay) in the southeastern interior of British Columbia, and on the willingness of the producers to collaborate. Six of the sites were located in the Thompson-Nicola watershed, 2 in the Okanagan watershed, and 1 site in each of the Kootenay and Columbia watersheds.

Samples were collected by a team of veterinarians and animal technicians. All participants were provided with instructions to assure uniformity in data collection. Samples were collected from scouring calves (those exhibiting diarrhetic fecal production) and from non-scouring (producing feces of normal consistency) contemporaries at each site. An attempt was made at proportional representation of these groups to reflect the occurrence of scours within the entire herd at each site. For this study, diarrhetic feces was defined as material with a consistency loose enough to conform to the shape of the container into which it was collected. At the time of sampling, the age of the calf and its scouring status were recorded. The calves sampled ranged in age from 2 to 70 d (mean age 23.6 d, $s_{\bar{x}}$ 1.1).

Fecal material from each calf (estimated total weight 4 to 6 g) was deposited into 2 preweighed collection tubes, which were then weighed again to determine sample weight. Five millilitres of 10% formalin solution was added to the 1st tube (2 to 3 g fecal material). The contents of the tube were then mixed and the tube

shipped to the University of Calgary for enumeration of *Giardia* cysts and *Cryptosporidium* oocysts. The 2nd tube (remainder of fecal sample) was forwarded to a provincial laboratory in British Columbia and assessed for the presence of *Salmonella, Campylobacter jejuni, Escherichia coli* (F41, 987P, K88a, K88b or K99), rotavirus, and coronavirus.

The blood sample (10 mL) was collected from the jugular vein and serum was harvested by centrifugation at 500 × *g* for 5 min. Serum was frozen at -40°C until analyzed for calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu), iodine (I), selenium (Se), zinc (Zn), vitamin A, vitamin E, and IgG.

Animal care, sample collection and ethical review in Ontario and British Columbia were performed in accordance with the guidelines from the Canadian Council on Animal Care (21).

Sample analysis

Microscopic examination of Giardia *and* Cryptosporidium — Fecal suspensions were squeezed and rinsed through a surgical gauze sponge (Four ply, NuGauze; Johnson and Johnson, Montreal, Quebec) to yield 7 mL of filtrate. The filtrate was layered over 5 mL of 1 M sucrose (specific gravity 1.13) and centrifuged at 800 × *g* for 5 min to concentrate the cysts at the sucrose/water interface. The interface and upper layer of liquid were transferred by pipette to a clean tube and recentrifuged at 800 × *g* for 5 min. The supernatant was decanted, and the pellet was suspended in sodium phosphate buffered saline solution (PBSS) to a volume of 1 mL.

Two 150-μL samples of the concentrate were spotted onto microscope slides and allowed to air dry on a slide warmer for a minimum of 30 min. Once dry, the sample was fixed with acetone, dried, and mixed with 20 μL of either *Giardia* or *Cryptosporidium* fluorescein isothiocyanate (FITC) labelled monoclonal antibody solution (Giardi-a-glo, Crypt-o-glo; Waterborne, New Orleans, Louisiana, USA). The immunological reagents have been shown to be specific for *Giardia* spp. cysts and *Cryptosporidium* oocysts to the level of 98% (22,23) with a sensitivity of 67 cysts/oocysts per gram of feces. The sample was then incubated in a humidity chamber at 37°C for 30 min. After incubation, slides were rinsed with PBSS and the slide was allowed to air dry. Once dry, slides were mounted with glycerol (Aqua-polymount; Polysciences, Warrington, Pennsylvania, USA) and a cover slip, and the sample was examined by using an epifluorescent microscope at settings providing 200× and 400× magnification.

The number of cysts *(Giardia)* and oocysts *(Cryptosporidium)* over the spotted area were counted, which subsequently enabled the number of cysts or oocysts per gram of feces to be calculated. For samples collected from Ontario, oocysts of *Cr. muris* were differentiated from those of *Cr. parvum* based upon their size and shape (6.6 to 7.9 × 5.3 to 6.5 μm, ovoid for *Cr. muris;* 4.5 to 5.4 × 4.2 to 5.0 μm, spherical for *Cr. parvum*). This differentiation was not recorded for samples collected from calves in British Columbia. Oocysts and cysts from all samples were identified and enumerated by the same individual; previously established positive and negative control fecal samples were analyzed with each sample lot.

Bacteriology — Standard microbiologic methods were used to isolate and identify bacterial pathogens in fecal samples collected during the course of the study (24).

Isolates of *E. coli* were subcultured on E agar (25) containing glucose and citric acid as the only organic nutrients, and pilus antigens were typed by using a fluorescent antibody staining procedure (26). Colonies from E agar were suspended in PBSS and placed on microscope slides. A drop of heat-aggregated whole horse serum was added to the suspension on each slide and a smear of the bacterial preparation was created. Samples were air-dried for 30 min, fixed with acetone, and rinsed with PBSS. A solution of monoclonal antibodies for pilus antigens of F41, 987P, K88a, K88b, or K99 *E. coli* (Central Veterinary Laboratory, New Haw, Surrey, United Kingdom) was added to the samples, which were subsequently incubated in a humidity chamber at 37°C for 30 min. Slides were rinsed in PBSS; the samples were then incubated for 15 min with FITC-labeled rabbit-anti-mouse IgG, rinsed in distilled water, and air-dried. Coverslips were mounted with fluorescent mounting fluid and slides were examined at 400× magnification with an epifluorescence microscope.

Salmonella colonies were isolated by enrichment and identified by serological (*Salmonella* antisera; Difco Laboratories, Detroit, Michigan, USA) and standard biological techniques (27).

Campylobacter-like colonies were cultured on Blaser's agar plates (28) at 42°C for 48 h in a microaerophilic atmosphere of 80% N_2, 10% CO_2, 5% O_2, and 5% H_2. Campylobacter-like colonies were Gram stained and examined for motility by phase-contrast microscopy. Organisms that showed corkscrew-like motility and were Gram-negative curved or spiral rods were further evaluated for their ability to hydrolyze hippurate (29).

Virology and serology — Rotavirus in fecal samples was identified by ELISA (Rotazyme II Diagnostic Kit; Abbott Laboratories, Abbott Park, Illinois, USA) and coronavirus was identified by using a standard procedure for negative staining and examination by electron microscopy (30). Radial immunodiffusion techniques (IgG SRID Kit; Veterinary Medical Research and Development, Pullman, Washington, USA) were performed to quantify serum IgG concentrations.

Serum levels of Ca, Mg, Cu, and Zn were measured by using flame atomic absorption, and P was determined by using a colorimetric kit (Diagnostic Chemicals, Charlottetown, Prince Edward Island). Selenium was determined as previously described (31). Iodine was measured by using a previously described adaptation of the Sandell-Kolthoff procedure (32) and determined by using the ferric-arsenic reduction procedure. Vitamins A and E were determined simultaneously by using a modified high-performance liquid chromatography method with dual detectors, based on the procedure of Dennison and Kirk (33).

Data analysis

Oocyst and cyst counts were log transformed and various mixed linear models (34) were used to perform an analysis of variance (ANOVA). Geometric means were

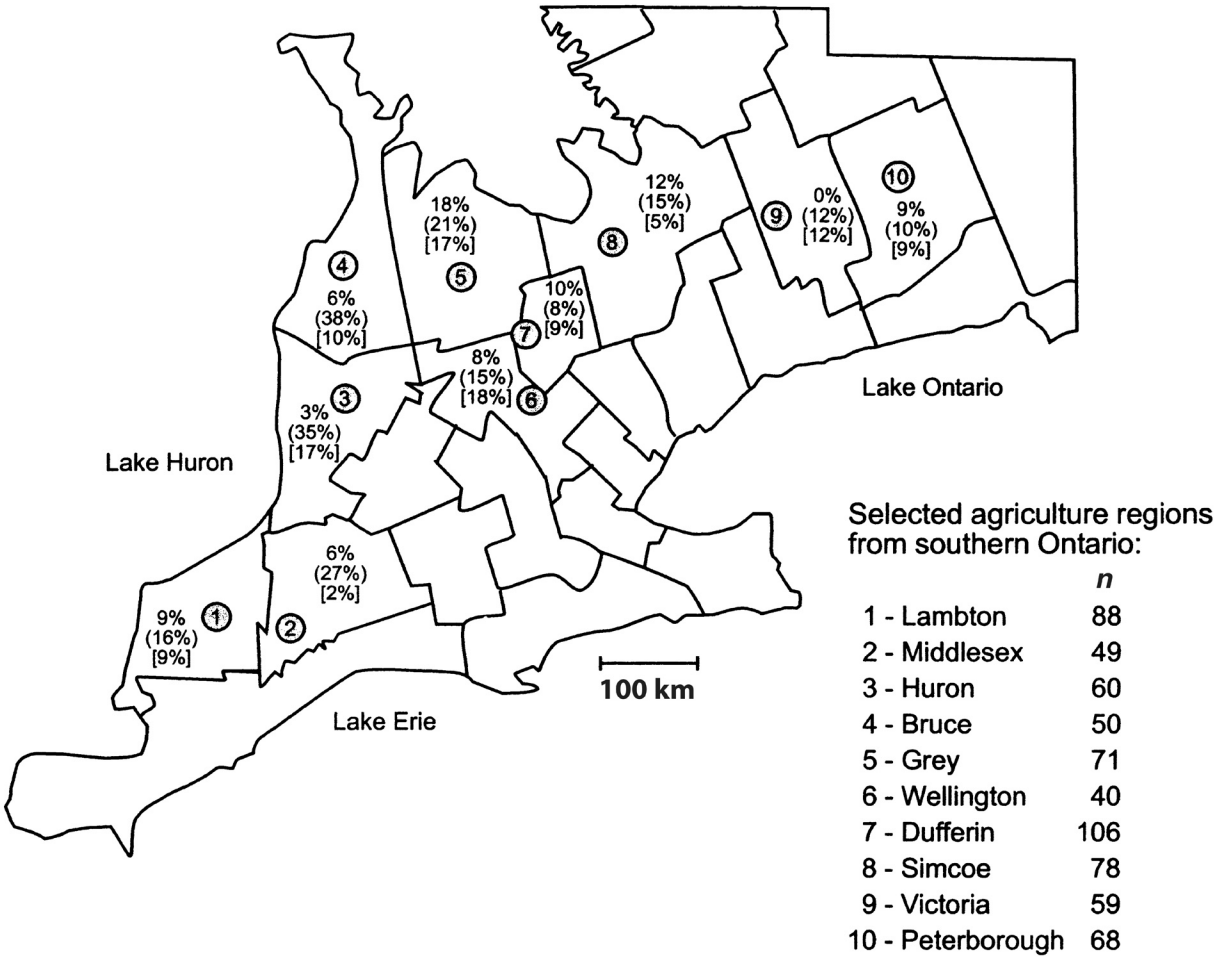

Figure 1. Prevalence of *Giardia* spp. and *Cryptosporidium* spp. infections among beef cattle in southern Ontario sampled in 1998 and 1999. Prevalences were computed as the number of samples that were positive for *Giardia* spp. or *Cryptosporidium* spp. divided by the number of samples collected within each region, multiplied by 100. Within each agricultural region, the unenclosed percentage is the prevalence of *Giardia* infection, the value in parentheses ( ) is the prevalence of *Cr. parvum,* and that in square brackets [ ] is the prevalence of *Cr. muris.* (*n*) = total number of samples collected in the region over the 2 y. Bar = 100 km.

calculated for positive animals within each year from each county and region, and multiple comparison of the means (least significant difference) was used to compare transformed cyst and oocyst counts. Categorical modeling of data (34) was used to control for clustering at the herd level and to compare the prevalence of parasitic infections in beef cows within regions and between the 2 sampling years. Prevalence was calculated as the number of infected individuals divided by the number of individuals sampled × 100. Spearman's correlation was used to compare herd level summary statistics with the prevalence of parasites in calves from British Columbia.

Results

Ontario

Over the 2-year study, 669 fecal samples were collected from beef cows on farms in 10 counties in southern Ontario (Figure 1). Overall prevalences of *Giardia, Cr. muris,* and *Cr. parvum* in beef cows were 8.7%, 10.6%, and 18.4%, and these organisms were detected on 25 (64%), 28 (72%), and 35 (90%) of the 39 farms sampled, respectively. The 10 counties included in the study contained approximately 47% of Ontario's beef cattle (35). *Giardia duodenalis* was identified in cows from all counties, with the exception of Victoria (Table 1). Within a county, the prevalence of *Giardia* was as high as 20%. Cyst counts varied among *Giardia*-infected cows, reaching as high as 3682 cysts/g of feces. Counts of *Cr. parvum* and *Cr. muris* oocysts in infected cows were as high as 12 323 and 175 343 per gram, respectively. In 1998, *Cr. parvum* was identified in all 10 counties (Table 1) and exhibited the highest prevalence of the parasites examined in all but 2 counties (Wellington and Dufferin). However, *Cr. parvum* was detected in only 7 of 10 counties in 1999. Prevalence across the 2 y ranged from 8.5% in Dufferin county to 38% in Bruce county. *Cryptosporidium muris* was also identified in all 10 counties, with prevalence over the 2 y ranging from 2% in Middlesex county to 18% in Wellington county.

Overall prevalence of *Giardia* in cows was higher ($P < 0.05$) in 1998 (11.8%) than in 1999 (4.5%, Table 1). In 1998, prevalence of *Giardia* was fairly consistent across regions, but in 1999, it was not detected in either the southern or the central regions. *Cryptosporidium parvum* was detected in 28% of the samples in 1998, but in only 5.2% ($P < 0.05$) of the samples in 1999. In 1998, prevalence of *Cr. muris* was higher ($P < 0.05$) in the

Table 1. Prevalence (%)[a] of *Giardia*, *Cryptosporidium parvum*, and *Cryptosporidium muris* in beef cattle in southern Ontario

		1998				1999				Both years			
Region	County	*n*[b]	*Giardia*	*Cr. parvum*	*Cr. muris*	*n*	*Giardia*	*Cr. parvum*	*Cr. muris*	*n*	*Giardia*	*Cr. parvum*	*Cr. muris*
South	Lambton	59	13.6	20.3	5.1	29	0	6.9	17.2	88	9.1	15.9	9.1
	Middlesex	29	10.3	41.4	3.5	20	0	5	0	49	6.1	26.5	2.1
	Total	88	12.5	27.3	4.6	49	0	6.1	10.2	137	8	19.7	6.6
West	Bruce	30	10	56.7	13.3	20	0	10	5	50	6	38	10
	Dufferin	56	12.5	12.5	16.1	50	8	4	2	106	10.4	8.5	9.4
	Grey	41	19.5	31.7	9.8	30	16.7	6.7	26.7	71	18.3	21.1	16.9
	Huron	30	3.3	53.5	30	30	3.3	16.7	3.3	60	3.3	35.1	16.7
	Simcoe	40	15	30	5	38	7.9	0	5.3	78	11.5	15.4	5.1
	Wellington	30	10	20	23.3	10	0	0	0	40	7.5	15	17.5
	Total	227	12.3	31.3	15.4	178	7.3	6.2	7.3	405	10.1	20.3	11.9
Central	Peterborough	38	15.8	18.4	10.5	30	0	0	6.7	68	8.8	10.3	8.8
	Victoria	29	0	20.7	13.8	30	0	3.3	10	59	0	11.9	11.9
	Total	67	9	19.4	11.9	60	0	1.7	8.4	127	4.7	11	10.2
Overall		382	11.8	28.3	12.3	287	4.5	5.2	8	669	8.7	18.4	10.5

[a]Calculated within each county as number of infected cows/number of cows sampled × 100%
[b]*n* = total number of cattle sampled in a given county

Table 2. Prevalence (%) of *Giardia* and *Cryptosporidium* spp. in beef cattle grouped by source of drinking water

	1998					1999				
Water source	Number of farms	Number of cattle	*Giardia*	*Cr. parvum*	*Cr. muris*	Number of farms	Number of cattle	*Giardia*	*Cr. parvum*	*Cr. muris*
Municipal water	2	19	31.6	31.6	10.5	1	10	0	10	0
Well water	30	294	10.2	26.2	13.6	22	217	5.1	3.2	9.7
Surface water	7	69	13	36.2	7.2	6	60	3.3	11.7	3.3
Total	39	382	11.8	28.3	12.3	29	287	4.5	5.2	8

western and central regions than in the south. Its overall prevalence that year was 12.3%. As observed with the other 2 parasites, the overall prevalence of *Cr. muris* was lower in 1999 than in 1998. Average fecal counts of *Giardia* cysts and *Cr. muris* oocysts in positive samples were higher ($P < 0.05$) in 1998 (85.9 cysts/g and 230.2 oocysts/g) than in 1999 (49.7 cysts/g and 73.5 oocysts/g). *Cryptosporidium parvum* counts did not differ ($P > 0.05$) between the 2 y.

Of the samples collected, 511 were from cows provided with well water, 129 were from cows provided with surface water, and only 29 were supplied with municipal water. *Giardia* cysts and *Cryptosporidium* oocysts were detected in cattle regardless of water source (Table 2). In fact, in 1998, prevalence of *Giardia* cysts was numerically higher in cows with access to municipal water as compared with well or surface water. However, in 1999, *Giardia* was not detected in cows with access to municipal water.

British Columbia

A total of 192 fecal samples were collected from calves representing the 4 watersheds in southeastern British Columbia (Figure 2, Table 3). Overall prevalences of *Giardia* (36%) and *Ca. jejuni* (42%) were higher ($P < 0.001$) than that of *Cryptosporidium* (13%). *Giardia* and *Ca. jejuni* were detected on all farms, except that *Ca. jejuni* was not detected at Vernon site B, whereas *Cryptosporidium* was detected on only 50% of the sites

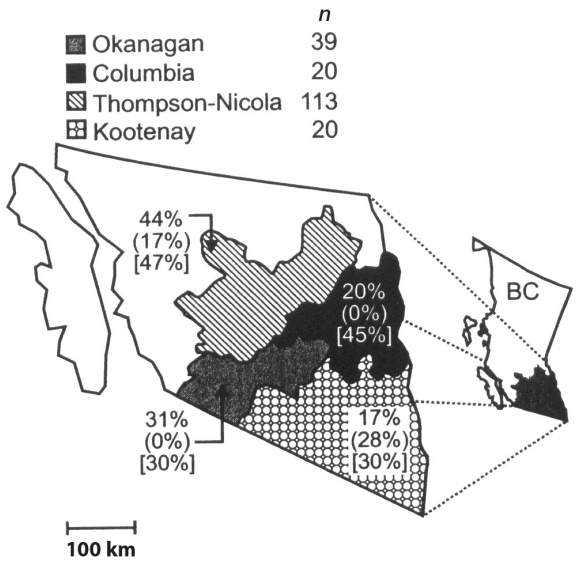

Figure 2. Prevalence of *Giardia* spp., *Cryptosporidium* spp., and *Campylobacter jejuni* infections among beef calves in southeastern British Columbia. Prevalences were computed as the number of calves infected with *Giardia* spp., *Cryptosporidium* spp. or *Ca. jejuni* divided by the number of calves sampled within each region, multiplied by 100. For each region, the unenclosed percentage is the prevalence of *Giardia* infection, the value in parentheses ( ) is the prevalence of *Cryptosporidium* infection, and that in square brackets [ ] is the prevalence of *Campylobacter* infection. (n) = total number of calves sampled in each region. Bar = 100 km.

Table 3. Prevalence (%) of enteropathogens and scours in beef calves in southern British Columbia in 1998

Watershed	Location	Site	n[a]	Age[b]	*Giardia*	*Crypto*	*Salmonella*	*E. coli*	*Campylo*	Rotavirus	Corona	Scours
Columbia	Invermere	J	20	20.6	20	nd	nd	nd	45	5.3	21.1	50
Kootenay	Creston	F	20	22.4	16.7	27.8	nd	nd	30	7.1	14.3	10
Okanagan	Salmon Arm	A	20	20.2	40	nd	nd	nd	60	30	60	na
	Vernon	B	19	12.9	21.1	nd	nd	nd	nd	31.6	15.8	47.4
	Average		19.5	16.6	30.7	nd	nd	nd	30	30.8	38.5	—
Thompson	Kamloops	C	13	21.1	25	nd	nd	nd	38.5	nd	nd	30.8
		D	20	30.3	42.1	10.5	nd	nd	30	nd	36.8	30
		E	20	23.5	47.4	nd	nd	nd	45	7.7	53.9	5
		I	20	10.3	42.1	26.3	nd	nd	25	8.3	25	20
	Merrit	G	20	43.4	70	45	nd	5	70	20	40	80
		H	20	19.8	25	10	nd	nd	70	16.7	55	50
	Average		18.8	27.3	43.8	17.1	nd	0.9	46.9	10.5	36.8	36.2
Overall			192	23.6	35.7[c]	12.6[d]	nd	0.5[e]	41.7[c]	14.9	33.1	36

Crypto — Cryptosporidium; Campylo — Campylobacter jejuni; E. coli — Escherichia coli K88; Corona — coronavirus; nd — not detected; na — not assessed
[a]Number of calves sampled per site; not all enteropathogens were surveyed in each sample (insufficient fecal material)
[b]Mean age (days)
[cde]Within a row, values followed by different letters differ significantly ($P < 0.001$)

examined. *Salmonella* and *E. coli* K99 were not detected in any of the calves that were sampled and *E. coli* K88a was detected in only 1 calf. Coronavirus and rotavirus were present at all sites, with the exception of Kamloops. Prevalence of *G. duodenalis* was as high as 70% at site G in the Thompson watershed. Although not statistically significant, this same site also exhibited the highest prevalence of scours and the greatest prevalence of both *Cryptosporidium* and *Campylobacter*. *Giardia* cyst counts in infected calves were as high as 113 000 cysts/g of feces, whereas *Cryptosporidium* counts were as high as 132 000 oocysts/g.

Serum mineral, vitamin, and IgG levels were within normal range when averaged within a herd (Table 4; 36,37). Of note, however, is that although the association was not statistically significant, calves with scours exhibited markedly lower levels of serum Se and depressed levels of IgG than did those with normal fecal consistency (data not shown). This pattern was especially notable in the Merritt herd G, in which prevalence of scours was highest and levels of serum Se and IgG were lowest.

Prevalence of *Giardia* and *Ca. jejuni* was higher ($P < 0.001$) in 2- to 6-week-old calves than in 0- to 2-week-old calves (data not shown). In contrast, prevalence of *Cryptosporidium* was relatively constant in 0- to 6-week-old calves but notably higher ($P < 0.001$) in calves that were over 6 wk old.

Discussion

Few studies have examined the prevalence of *Giardia* and *Cryptosporidium* in beef cows; the majority of studies have focused on dairy cattle (1,2,17,23,24,38). *Giardia* was not detected when fecal samples were collected on a single occasion from 26 adult beef cows from 3 farms in Alberta (39). In another study (20), when fecal samples were collected from 20 beef cows 6 times over 25 wk, the prevalences of *Giardia* and *Cr. andersoni* among the cows ranged from 0% to 15% and 0% to 40%, respectively. Work with a single cowherd indicated that *Giardia* is more prevalent in calves than in cows (20). In our study, the prevalence of *Giardia* in beef cows on 10 different farms ranged from 0% to 20%. It has been well documented that *Giardia* cysts are shed intermittently in cattle (17). Consequently, the low prevalence of *Giardia* reported in the literature for beef cows may be associated more with the logistics of taking multiple samples from these animals under range conditions than with the actual occurrence of the infection.

As with *Giardia, Cryptosporidium* tends to be more prevalent in younger than older animals (40). When detection methods similar to ours are used, *Cryptosporidium* has generally been found to be less prevalent than *Giardia* in dairy calves (1,2). In the present study, *Cr. parvum* was more prevalent than *Giardia* in cows, and 90% of the farms had at least 1 cow that tested positive for *Cr. parvum.* Similarly, at least 1 calf was positive for a *Cryptosporidium* sp. on 89% of dairy farms ($n = 600$) examined in Quebec (16). In the present study, *Cr. muris* was also more prevalent than *Giardia* in beef cows, but, somewhat surprisingly, was not as prevalent as *Cr. parvum. Cryptosporidium muris* tends to reside in the abomasum (41) and it has been proposed that this species is far more common than *Cr. parvum* in adult cattle (42,43). The present study demonstrates that it is possible for *Cr. parvum* to be more prevalent than *Cr. muris* in beef cows. This has important implications, as clinical cryptosporidial infections in humans are caused by *Cr. parvum* (44). There has been only 1 reported case of *Cr. muris* infection in humans (44).

The decline in the prevalence of *Giardia* and *Cryptosporidum* spp. in 1999 as compared with 1998 may be the result of a number of factors. The sampling in 1999 occurred between the months of December and April, whereas the sampling in 1998 took place between March and May. Cattle were penned during the winter of 1999 but were pastured with their calves in the spring of 1998. Previous work has shown that adult cattle from herds with a high number of young calves are more likely to shed *Cr. parvum* oocysts than are those from herds that possess a lower number of juvenile animals (40,45). In 1998, newly born juvenile carriers in the herd may have served as a source of infection among adult animals.

It is also possible that a periparturient rise in the excretion of *Giardia* cysts and *Crytosporidium* oocysts during spring calving was responsible for the higher detection of these parasites in cows in 1998 compared with 1999.

Table 4. Serum mineral, vitamin, and immunoglobulin (Ig) G status of calves sampled for fecal enteropathogens in southern British Columbia

				Serum levels									
Watershed	Location	Site	*n*	Ca mmol/L	P mmol/L	Mg mmol/L	Cu µmol/L	I µmol/L	Se µmol/L	Zn µmol/L	Vit A µmol/L	Vit E µmol/L	IgG g/L
Columbia	Invermere	J	20	2.71, s = 0.164	4.29, s = 1.159	0.87, s = 0.103	8.75, s = 1.613	0.38, s = 0.115	0.71, s = 0.223	16.10, s = 2.646	0.67, s = 0.204	3.79, s = 2.442	27.83, s = 5.301
Kootenay	Creston	F	20	2.39, s = 0.142	2.93, s = 0.332	0.66, s = 0.077	7.52, s = 1.165	1.57, s = 0.653	0.51, s = 0.111	14.20, s = 3.342	0.64, s = 0.192	1.54, s = 0.986	31.61, s = 7.959
Okanagan	Salmon Arm	A	20	2.63, s = 0.172	3.31, s = 0.395	0.73, s = 0.096	10.32, s = 2.156	0.81, s = 0.211	0.79, s = 0.091	17.32, s = 4.220	0.59, s = 0.136	4.21, s = 1.722	21.60, s = 3.693
	Vernon	B	19	2.79, s = 0.243	3.36, s = 0.568	0.83, s = 0.089	10.40, s = 2.770	1.25, s = 0.540	0.94, s = 0.205	17.37, s = 3.532	0.61, s = 0.195	1.50, s = 1.250	25.34, s = 6.310
	Average		19.5	2.71, s = 0.207	3.34, s = 0.482	0.78, s = 0.093	10.36, s = 2.463	1.03, s = 0.375	0.86, s = 0.148	17.34, s = 3.876	0.60, s = 0.166	2.86, s = 1.486	23.45, s = 5.002
Thompson	Kamloops	C	13	2.65, s = 0.254	3.57, s = 0.340	0.83, s = 0.111	9.99, s = 3.714	1.15, s = 1.038	0.91, s = 0.409	16.47, s = 2.753	0.80, s = 0.456	3.45, s = 2.113	24.06, s = 4.006
		D	20	2.49, s = 0.130	3.28, s = 0.736	0.76, s = 0.081	7.70, s = 2.109	1.06, s = 0.715	0.75, s = 0.101	16.96, s = 2.767	0.65, s = 0.195	1.66, s = 1.194	28.40, s = 8.192
		E	20	2.54, s = 0.210	3.33, s = 0.361	0.70, s = 0.067	7.95, s = 2.109	1.31, s = 0.612	0.87, s = 0.480	16.50, s = 3.302	0.71, s = 0.192	1.79, s = 0.772	26.62, s = 8.155
		I	20	2.40, s = 0.146	3.40, s = 0.390	0.65, s = 0.165	9.73, s = 3.218	1.28, s = 1.252	0.77, s = 0.572	15.82, s = 3.336	0.52, s = 0.170	2.38, s = 1.049	23.10, s = 4.512
	Merritt	G	20	2.50, s = 0.103	3.11, s = 0.253	0.55, s = 0.139	8.84, s = 2.408	4.76, s = 3.792	0.38, s = 0.080	13.19, s = 4.160	0.62, s = 0.171	2.73, s = 1.311	19.20, s = 3.247
		H	20	2.72, s = 0.208	3.56, s = 0.755	0.85, s = 0.112	11.74, s = 3.478	1.02, s = 0.557	0.62, s = 0.139	15.18, s = 3.119	0.74, s = 0.238	1.88, s = 1.160	26.67, s = 6.244
	Average		18.8	2.55, s = 0.175	3.37, s = 0.472	0.72, s = 0.113	9.32, s = 2.839	1.76, s = 1.328	0.72, s = 0.297	15.68, s = 5.437	0.67, s = 0.237	2.31, s = 1.267	24.68, s = 5.726
Overall average				2.58, s = 0.177	3.41, s = 0.529	0.74, s = 0.104	9.29, s = 2.474	1.46, s = 0.948	0.72, s = 0.241	15.91, s = 4.636	0.65, s = 0.215	2.49, s = 1.400	25.44, s = 5.762
Normal levels (36,37)				2.0 to 2.7	1.9 to 2.9	0.7 to 1.2	9.4 to 23.6	0.79 to 3.15	1.01 to 3.80	12.2 to 21.4	0.7 to 1.1	1.9 to 9.3	14 to 25
Deficient levels (36,37)				< 1.5	< 1.6	< 0.15	< 4.7	< 0.4	< 0.32	< 6.1	< 0.18	< 0.2	< 14

Such a scenario has been associated with the early infection of lambs (19) and calves (6) with *Giardia.* However, periparturient dairy cows were not identified as the source of infection for calves with *Cr. parvum;* rather, oocysts in the bedding and pen floor were implicated as the source of the infection (46). Others have also found *Cryptosporidium* to be more prevalent in beef calves in the spring than in the winter (2,18), a trend that agrees with our work in beef cows. Transmission between animals in the winter may be reduced due to the freezing of animal feces and a corresponding reduction in fecal-oral transmission of *Cryptosporidium* oocysts and *Giardia* cysts. *Cryptosporidium* oocysts can remain viable after freezing (47,48) and, consequently, freshly thawed feces in the spring may be a source of infection for herd members.

In the present study, provision of municipal water did not eliminate *Giardia* or *Cryptosporidium* spp. from the small number of herds that were provided with this source of water. *Giardia* and *Cryptosporidium* have been detected in dairy calves with access to both well and municipal water (1). Chlorine-based disinfectants are largely ineffective against *Cryptosporidium* (49) and their effectiveness against *Giardia* cysts declines at lower temperatures (50). Water treatment does not prevent fecal-oral contact, an event that is likely responsible for the majority of the transmission of these parasites among herd members. Consumption of water from field ponds during spring run off could also negate any benefit that water treatment has on reducing water-borne transmission of these parasites.

Most studies on the prevalence of *Giardia* and *Cryptosporidium* have been conducted on dairy calves (1,16,23,46). In the present study, the prevalence of infection with *Giardia* among range calves was lower than previously reported in dairy calves (1,16). Prevalence of *Giardia* has been shown to be greater in calves housed indoors than in those outdoors (16,23). The antihelminthic agent, fenbendazole, controls *Giardia* in dairy calves, but under confined conditions, these animals readily become reinfected if treatment is discontinued (51). In the natural environment, *Giardia* cysts are subject to desiccation and are rendered noninfective after prolonged exposure to temperatures below freezing (48). Thus, reduced confinement and the accompanying lower animal density, as well as a reduction in the survival of infective cysts in the exposed environment, may account for the reduced infection level in range calves compared with dairy calves.

Prevalence of scours was highest among calves for site G at Merritt in the Thompson watershed. There were individual calves in that herd with serum Se and IgG levels that were below normal (data not shown), a factor that contributed to this site's low herd average of these parameters (Table 4). Prevalences of *Giardia* and *Campylobacter* were also highest in the site G herd, a finding that supports the notion that *Giardia* could play a role in calf scours (17). Low serum IgG concentrations at 24 h of age have been closely correlated with the prevalence of scours in calves from birth to 9 or 16 wk of age (24,52) and, in those studies, numerous enteropathogens were isolated from calf feces. The present study supports the hypothesis that passive immunity does

not affect the level of infection of *Cryptosporidium* (52,53).

The overall prevalence of *Giardia* and *Cryptosporidium* among groups of cattle penned or pastured together is heavily influenced by the time of year at which samples are collected. Prevalence of these pathogens in cows is highest during the calving season and management strategies targeted at reducing environmental contamination are likely to be particularly critical at this point in the cow-calf cycle. Although the prevalance of *Giardia* and *Cryptosporidium* was lower in range calves than in dairy calves, there was evidence that calves exhibiting scours also were likely to harbor *Giardia.* Low Se levels suggests that management strategies that enhance the nutritional status of calves may also reduce the prevalence of these enteropathogens.

The public health significance of *G. duodenalis* and *Cr. parvum* isolated from cattle has been investigated with the use of polymerase chain reaction-based molecular tools (54–56). In the present study, these organisms could not be genotyped, because the formalin fixation precluded isolation of DNA from the cysts and oocysts. In North America and Australia, most human cases of cryptosporidiosis appear to be of human origin (*Cryptosporidium hominis* or *Cr. parvum* genotype 1), which are morphologically indistinguishable from the common mammalian species, *Cr. parvum* genotype 2 (54,57). In the United States, cattle have not been identified as the source of any waterborne outbreak of *Cr. parvum* in humans and in Canada, only one outbreak (in Cranbrook, British Columbia) has been attributed to the bovine *Cr. parvum* genotype (54). Because the specific *Cr. parvum* genotype could not be identified in this study, it is difficult to assess the zoonotic significance of the high prevalence of this parasite in beef cows. *Giardia* is also very common in calves and cows, but recent molecular analysis of cysts from a variety of animals showed that more than 95% of the samples contained cysts that were of the nonzoonotic livestock genotype (55,56).

Acknowledgments

The authors thank C. Cockwill, N. Guselle, J. Greenfield, G. Gourlay, and B. Puls for their assistance with these studies. They also thank all of the producers in Ontario and British Columbia who provided us with access to their cattle. CVJ

References

1. Olson ME, Guselle NJ, O'Handley RM, et al. *Giardia* and *Cryptosporidium* in dairy calves in British Columbia. Can Vet J 1997;38:703–706.
2. Xiao LH, Herd RP, Rings DM. Concurrent infections of *Giardia* and *Cryptosporidium* on two Ohio farms with calf diarrhea. Vet Parasitol 1993;51:41–48.
3. O'Donoghue PJ. *Cryptosporidium* and Cryptosporidiosis in man and animals. Int J Parasitol 1995;25:139–155.
4. Erlandsen SL. Biotic transmission — Is giardiasis a zoonosis? In: Thompson RCA, Reynoldson JA, Lymbery AJ, eds. *Giardia:* From Molecules to Disease. Cambridge, UK: Cambridge Univ Pr, 1994:83–97.
5. Olson ME, McAllister TA, Deselliers L, et al. Effects of giardiasis on production in a domestic ruminant (lamb) model. Am J Vet Res 1995;56:1470–1474.
6. Ralston BJ, Cockwill CL, Guselle NJ, Van Herk FH, McAllister TA, Olson ME. Prevalence of *Giardia* and *Cryptosporidium andersoni* and their effects on performance in feedlot beef cattle. Can J Anim Sci 2003;83:153–159.
7. Fayer R, Speer C, Dubey J. The general biology of *Cryptosporidium*. In: Fayer R, ed. *Cryptosporidium* and Cryptosporidiosis. Boca Raton: CRC Pr, 1997:1–41.
8. Olson ME, Ralston BJ, O'Handley R, Guselle NJ, Applebee AJ. What is the clinical and zoonotic significance of Cryptosporidiosis in domestic animals and wildlife. In: Thompson RCA, Armson A, Ryan UM, eds. *Cryptosporidium:* From Molecules to Disease. Elsevier Sci, 2004 (In press).
9. Majewska, AC. Successful experimental infections of a human volunteer and Mongolian gerbils with *Giardia* of animal origin. Trans R Soc Trop Med Hyg 1993;88:360–362.
10. Reif, JS, Wimmer L, Smith JA, Dargatz DA, Cheney JM. Human cryptosporidiosis associated with an epizootic in calves. Am J Public Health 1989;79:1528–1530.
11. Egger M, Nguyen XM, Schaad UB, Krech T. Intestinal cryptosporidiosis acquired from a cat. Infection 1990;18:177–188.
12. Gradus MS. Water quality and waterborne protozoa. Clin Microbiol News 1989;11:121–125.
13. LeChevallier MW, Norton WD, Lee RG. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. Appl Environ Microbiol 1991;57:2610–2616.
14. Ong C, Moorehead W, Ross A, Isaac-Renton J. Studies of *Giardia* spp. and *Cryptosporidium* spp. in two adjacent watersheds. Appl Environ Microbiol 1996;62:2798–2805.
15. Garber LP, Salman MD, Hurd HS, Keefe T, Schlater JL. Potential risk factors for *Cryptosporidium* infection in dairy calves. J Am Vet Med Assoc 1994;205:86–91.
16. Ruest N, Faubert GM, Couture Y. Prevalence and geographical distribution of *Giardia* spp. and *Cryptosporidium* spp. in dairy farms in Quebec. Can Vet J 1998;39:697–700.
17. O'Handley RM, Olson ME, McAllister TA, et al. Efficacy of fenbendazole for treatment of giardiasis in calves. Am J Vet Res 1997;58:384–388.
18. Mann ED, Sekla LH, Nayer GPS, Koschik C. Infection with *Cryptosporidium* spp. in humans and cattle in Manitoba. Can J Vet Res 1986;50:174–178.
19. Xiao L, Herd RP, McClure KE. Periparturient rise in the excretion of *Giardia* sp. cysts and *Cryptosporidium parvum* oocysts as a source of infection for lambs. J Parasitol 1994;80:55–59.
20. Ralston BJ, McAllister TA, Olson ME. Prevalence and infection pattern of naturally acquired giardiasis and cryptosporidiosis in range beef calves and their dams. Vet Parasitol 2003;114: 113–122.
21. Olfert EE, Cross BM, McWilliams AA, eds. Guide to the Care and Use of Experimental Animals. 2nd ed. vol. 1. Ottawa, Ontario: Can Council Anim Care, 1994.
22. Olson ME, Thorlakson CL, Deselliers L, Morck DW, McAllister TA. *Giardia* and *Cryptosporidium* in Canadian farm animals. Vet Parasitol 1997;68:375–381.
23. Xiao L, Herd RP. Infection patterns of *Cryptosporidium* and *Giardia* in calves. Vet Parasitol 1994;55:257–262.
24. McDonough SP, Stull CL, Osburn BI. Enteric pathogens in intensively reared veal calves. Am J Vet Res 1994;55:1516–1520.
25. Francis DH, Remmers GA, DeZeeuw PS. Production of K88, K99 and 987P antigens by *Escherichia coli* culture on synthetic and complex media. J Clin Microbiol 1982;15:181–183.
26. Mullaney CD, Francis DH, Willgohs JA. Comparison of seroaggultination, ELISA and indirect fluorescent antibody staining detection of K99, K88 and 987P pilus antigens of *Escherichia coli.* J Vet Diagn Invest 1991;3:115–119.
27. Jang SS, Biberstein EL, Hirsh DC. A Diagnostic Manual of Veterinary and Clinical Bacteriology and Mycology. Davis, California: Univ California Pr, 1992:51–60.
28. Atlas, RM. Handbook of Microbiological Media, 2nd ed. Boca Raton: CRC Pr 1996:243–244.
29. Skirrow MB, Benjamin J. Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J Hyg Camb 1980;85:427–442.
30. Isenberg H. Direct specimen testing: viral and chlamydial infections. In: Isenberg H, ed. Essential Procedures for Clinical Microbiology. Washington, DC: ASM Pr, 1998:533–550.
31. Horwitz W, ed. Official Methods of Analysis, procedure 25. 126. Washington DC: Assoc Analytical Chemists, 1980:1018 pp.

32. Dunn JT, Crutchfield HE, Gutekunst R, Dunn AD. Two simple methods for measuring iodine in urine. Thyroid 1993;3:119–123.
33. Dennison DB, Kirk JR. Quantitative analysis of vitamin A in cereal products by high-performance liquid chromatography. J Food Prot 1979;42:1376–1384.
34. Statistical Analysis Systems. SAS/STAT User's Guide, Version 8. Cary, North Carolina: SAS Institute.1999.
35. Ontario Ministry of Agriculture and Food (homepage on the internet). Guelph, Queen's Printer for Ontario; c2004 [last updated June 15, 2004, cited July 5, 2004]. Statistical Tables, Livestock by Country, Cattle 1998 and Cattle 1999 [about 2 screens]. Available from http://www.gov.on.ca/OMAFRA/english/stats/livestock/index.html
36. Puls R. Mineral Levels in Animal Health: Diagnostic Data, 2nd ed. Clearbrook British Columbia: Sherpa Int, 1994.
37. Puls R. Vitamin Levels in Animal Health: Diagnostic Data and Bibliographies. Clearbrook, British Columbia: Sherpa Int, 1994.
38. Fuente R, Luzon M, Ruiz-Santa-Quiteria JA, et al. *Cryptosporidium* and concurrent infections with other major enteropathogens in 1- to 30-day-old diarrheic calves in central Spain. Vet Parasitol 1999;80:179–185.
39. Buret A, denHollander N, Wallis PM, Befus D, Olson ME. Zoonotic potential of giardiasis in domestic ruminants. J Infect Dis 1990; 162:231–237.
40. Atwill ER, Johnson EM, das Gracas M, Pereira C. Association of herd composition, stocking rate and duration of calving season with fecal shedding of *Cryptosporidium parvum* oocysts in beef herds. J Am Vet Med Assoc 1999;215:1833–1838.
41. Anderson BC. Abomasal cryptosporidiosis in cattle. Vet Pathol 1987;24:235–239.
42. Anderson BC. Prevalence of *Cryptosporidium muris*-like oocysts among cattle populations of the United States: preliminary report. J Protozool 1991;38:148–152.
43. Bukhari Z, Smith HV. Detection of *Cryptosporidium muris* oocysts in the faeces of adult dairy cattle in Scotland. Vet Rec 1996; 138:207–208.
44. Casemore DP, Wright SE, Coop RL. Cryptosporidiosis — human and animal epidemiology. In: Fayer R, ed. *Cryptosporidium* and Cryptosporidiosis. Boca Raton: CRC Pr 1997:65–92.
45. Atwill ER, Johnson EM, Klingborg DJ. Age, geographic, and temporal distribution of fecal shedding of *Cryptosporidium parvum* oocysts in cow-calf herds. Am J Vet Res 1999;60:420–425.
46. Atwill ER, Harp JA, Jones T, Jardon PW, Checel S, Zylstra M. Evaluation of periparturient dairy cows and contact surfaces as a reservoir of *Cryptosporidium parvum* for calfhood infection. Am J Vet Res 1998;59:1116–1121.
47. Fayer R, Nerad T. Effects of low temperature on viability of *Cryptosporidium parvum* oocysts. Appl Environ Microbiol 1996;62:1431–1433.
48. Olson ME, Goh J, Phillips M, Guselle N, McAllister TA. *Giardia* cyst and *Cryptosporidium* oocyst survival in water, soil and cattle feces. J Environ Qual 1999;28:1991–1996.
49. Rose JB, John JT, LeChevallier M. Waterborne Cryptosporidiosis: incidence, outbreaks and treatment strategies. In: Fayer R, ed. *Cryptosporidium* and Cryptosporidiosis. Boca Raton: CRC Pr 1997:93–109.
50. Jarroll EL, Bingham AK, Meyer EA. *Giardia* cyst destruction: effectiveness of six small-quantity water disinfection methods. Am J Trop Med Hyg 1980;20:8–11.
51. O'Handley RM, Cockwill C, Jelinski M, McAllister TA, Olson ME. Effects of repeat fenbendazole treatment in dairy calves with giardiosis on cyst excretion, clinical signs and production. Vet Parasitol 1999;89:209–218.
52. Quigley JD, Martin KR, Bemis DA, et al. Effects of housing and colostrum feeding on the prevalence of selected infectious organisms in feces of Jersey calves. J Dairy Sci 1994;7:3124–3131.
53. Harp JA, Woodmansee DB, Moon HW. Effects of colostral antibody on susceptibility of calves to *Cryptosporidium parvum* infection. Am J Vet Res 1989;50:2117–2219.
54. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium:* transmission, detection and identification. Int J Parasitol 2000;30:1305–1322.
55. O'Handley RM, Olson ME, Fraser D, Adams P, Thompson RCA. Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. Vet Parasitol 2000;90:193–200.
56. Appelbee AJ, Frederick LM, Heitman TL, Olson ME. Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada. Vet Parasitol 2003;112:289–294.
57. Morgan-Ryan UM, Fall A, Ward LA, et al. *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens.* J Eukaryot Microbiol 2002;49:433–440.