UNIVERSITY OF CALGARY

Prevalence and Biology of *Giardia* and *Cryptosporidium* in Beef Cattle

by

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ABSTRACT

Feedlot steers demonstrated the prevalence of *Giardia lamblia* and *Cryptosporidium andersoni* decreased as the steers aged, but never reached 0%. *Giardia lamblia* cysts shed per gram of feces was intermittent throughout the study. *Cryptosporidium andersoni* oocyst shedding increased over the 257 days of the study. Feedlot steers experienced no significant differences in ADG, FE or DMI whether they were infected or non-infected, with *Giardia lamblia* or *Cryptosporidium andersoni* over the duration of the study.

Giardia lamblia cysts were excreted by twenty beef calves (100%) at some time point during this study, however only one calf (5%) excreted *Cryptosporidium parvum* on 2 separate dates. *Giardia* cysts were first detected at 3.9 $\pm$ 1.37 weeks of age. The results of this study document for the first time cumulative prevalence and infection patterns of *Giardia* and *Cryptosporidium* in beef cattle under ranch and feedlot conditions as well as their potential as environmental contaminants.

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Ig	=	immunoglobulin
ID_{50}	=	infective dose for 50% population
C	=	celcius
U.K.	=	United Kingdom
FE	=	feed efficiency
ADG	=	average daily gain
DMI	=	dry matter intake
d	=	day
PBSS	=	phosphate buffered saline solution
FITC	=	fluorescein isothiocyanate
WBC	=	white blood cells
BVD	=	bovine virus diarrhea
IBR	=	infectious bovine rhinotracheitis
SD	=	standard deviation
BIV	=	bovine immunodeficiency virus
TCR	=	T cell receptor
IEL	=	intraepithelial lymphocyte
LPL	=	lamina propria
LNL	=	lymph node
IL	=	interleukins
ELISA	=	enzyme linked immunoabsorbent assay

1. INTRODUCTION

1.1 *GIARDIA LAMBLIA*

Giardia lamblia-like parasites were first described by Leeuwenhock in 1681 finding them in his own stool, however it was more fully described morphologically by Lambl in 1859 [1, 2]. *Giardia lamblia*, however, is not a recent parasite, findings date back 2000 years to human fecal matter located in Israel and Tennessee [2]. In order to study *Giardia* it is important to understand its life cycle.

1.1.1 Life Cycle

The life cycle of the protozoan parasite *Giardia lamblia* consists of two stages: the trophozoite and the cyst [3]. The trophozoite is the feeding stage which is relatively fragile whereas the durable cyst is the most common infective stage [4]. The life cycle of *Giardia* requires no intermediate host [5]. When a cyst is ingested by a host it undergoes a process called excystation as a response to exposure to the acidic gastric pH and the pancreatic enzymes chymotrypsin and trypsin found in the duodenum [3]. The one cyst produces two trophozoites which replicate in the crypts of the duodenum and upper jejunum reproducing asexually by binary fission [3]. Encystation occurs in the ileum to some of the trophozoites possibly due to their exposure to bile salts or cholesterol starvation, creating cysts which are shed in the feces of the host [3].

1.1.2 Morphology

The *Giardia lamblia* cysts can be round or oval and measure 11 - 14 μm x 7 - 10 μm, they have four nuclei and contain axonemes and median bodies [3]. Trophozoites are 10 - 20 μm x 5 - 15 μm and have a teardrop shape [3]. Trophozoites exhibit a ventral concave sucking disk that has four pair of flagella for locomotion, two axonemes and two median bodies [3]. The sucking disk is used to attach the trophozoite to the mucosal lining of the host's intestine [3].

1.1.3 Taxonomy

Giardia belongs to the subphylum Sarcomastigophora, the superclass Mastigophora, the class Zoomastigophorea, the order Diplomonadida, and the family Hexamitide [6]. In 1951 Filice proposed that only three distinct species of *Giardia* be recognized: *Giardia duodenalis* which occurs in most mammals; *Giardia agilis* in amphibians and *Giardia muris* in rodents [7]. Today *Giardia duodenalis* may also be referred to as *Giardia intestinalis* or *Giardia lamblia*, which can be a cause for confusion [7]. Two new species have been proposed; *Giardia psittaci* found in parakeets and *Giardia ardeae* found in great herons and straw necked ibis [6]. Both of these proposed new species have different morphological characteristics from the currently recognized species [6].

1.1.4 Transmission

Giardia lamblia infections can occur with the host ingesting as few as ten viable

cysts [3]. Ingestion of cysts may occur via the fecal-oral route in a variety of ways. One such method is through direct transmission, this typically occurs between individuals such as babies and young children in day care centers, schools or residential institutions when hygiene protocols are not followed [8, 9, 11]. Direct transmission may also occur via sexual activity involving oroanal contact [8, 10].

Another common transmission method is the ingesting of cyst contaminated food which is usually traced back to a *Giardia lamblia* positive food handler who has poor hygiene or the use of *Giardia lamblia* contaminated water to wash food [6, 8, 12, 13, 14]. Contaminated ice, fruit salad and raw sliced vegetables prepared by *Giardia lamblia* positive employees have been linked to illness [12, 13, 14].

The consumption of contaminated water may also produce giardiasis [10]. This method of transmission is the most prevalent with 90 outbreaks and 23,776 cases of giardiasis reported in the United States from 1965-84, 69% of these outbreaks and 74% of the cases were related to the contamination of public water supplies [15].

Transmission of *Giardia* is enhanced by the survival of the cyst under a variety of environmental conditions. *Giardia* cysts remain viable up to one week in freezing conditions (-4°C) and two weeks when subjected to temperatures of 25°C [16]. However, when cysts were placed in water at 4°C they survived for 11 weeks, 7 weeks in soil and 1 week in cattle feces [16]. These results highlight how transmission of *Giardia* can be

enhanced when *Giardia* cysts find their way into water sources.

1.1.5 Distribution

Giardia lamblia is found worldwide, it is very common in developing countries where sanitation is poor and water treatment is not sufficient to remove or kill cysts [3]. This situation is highlighted in the prevalence of *Giardia* cysts in human feces reported at 2 - 5% in industrialized countries and 20 - 30% in developing countries [3]. Animals also carry *Giardia* worldwide. In various studies the parasite prevalence has been reported to range from 1 - 25% in domestic cats and dogs, 1 - 100% in calves and sheep, and 1 - 100% in wild mammals globally [6].

1.1.6 Clinical Symptoms

Hosts infected with *Giardia* can either display physical symptoms such as intestinal uneasiness, nausea, anorexia, watery, foul-smelling diarrhea, cramps, lethargy or be asymptomatic [1, 5, 6]. Symptoms may also be intermittent or continuous in the host with reports of some cases lasting for months or years [5].

Explanation for the various symptoms and severity of symptoms in hosts have been reported to involve factors such as the host's immune and nutritional state and possible differences among *Giardia* strains [5].

1.1.7 Pathology and Pathogenesis

Asymptomatic hosts infected with *Giardia lamblia* have been reported to show no abnormalities in duodenal and jejunal mucosa [17]. Symptomatic individuals, however, may display villous atrophy, crypt hyperplasia and epithelial cell damage [17, 18]. On rare occasions *Giardia* trophozoites can invade the mucosa of the duodenum and jejunum but usually *Giardia* is considered non-invasive by attaching itself to the surface of the mucosa [17, 18]. Under microscopic examination patches of trophozoites can be seen on villi causing a blunting of the microvillous border of epithelial cells [6, 8, 17, 21]. The specific pathogenic mechanisms are not fully understood [17]. Recently it has been suggested that the reduction in the height of the microvilli causes a loss of absorptive surface leading to the malabsorption of glucose, electrolytes and water and impairs digestion by reducing disaccharidase activity [6, 19, 20]. It is these mechanisms that are believed to cause the diarrhea experienced by symptomatic hosts [6].

Giardia infection stimulates a cellular immune response creating a multiplication of intraepithelial lymphocytes and mast cells [6]. *Giardia*-specific IgA is also produced by the host in the small intestine and milk [6]. It has been reported that *Giardia* trophozoites can be killed by IgG and IgM in the presence of complement [6]. Unfortunately exposure to *Giardia* does not seem to produce life long persistent immunity to the parasite, but there does appear to be some protective immunity developed after infection of the host [20].

1.1.8 Zoonosis

It was thought for many years that *Giardia* was host specific, but in 1978 it was shown that certain species of *Giardia* were not specific between rodents and later cysts from humans were used to infect rodents, dogs, guinea-pigs, beavers, racoons, and bighorn moufflon sheep [22, 23]. Due to the wide range of potential hosts for *Giardia* to infect it highlights the concern for environmental contamination since many warm blooded animals carry *Giardia* in watersheds where cysts could potentially find their way into water sources causing waterborne transmission [6].

1.1.9 *Giardia* and Cattle

Giardia prevalence has been reported in calves to vary from 1 to 100% [24, 25, 26]. Calves can become infected as young as 4 days of age, but 5 - 10 weeks of age is more typical, and infections can persist for greater than 8 weeks [24, 27, 28]. *Giardia* prevalence in older cattle is much lower, but periparturient rises in cyst excretion have been reported [24].

Excretion of *Giardia* cysts by calves is intermittent as reported in both natural and experimental infections, and may cause diarrhea in the host [27, 28]. Weight loss may be a result of giardiasis in calves [6].

1.1.10 Treatment/Prevention

Recently *Giardia* vaccines have been developed for both cats and dogs, these are

available in the United States with the dog vaccine also available in Canada [29, 30]. Currently there exists no registered vaccine for prevention of the disease in cattle. However, there are two chemotherapeutic agents available for treating the infection in cattle; Albendazole (20 mg/kg 1X daily for 5 days) and Fenbendazole (5 mg/kg 1X daily for 3 days) (0.8 mg/kg 1X daily for 6 days) [6]. Fenbendazole was 100% effective in eliminating cysts from calves' feces within 6 days, but reinfection was observed in some of the calves within the 28 day study period [31].

Prevention of *Giardia* infections could be enhanced by limiting the contamination of the environment (water, soil) with cysts from infected hosts [6]. In confined areas disinfection and cleaning of surfaces should occur utilizing products such as organic iodine, tincture of iodine, chlorine and bleach (hypochlorite) to reduced contact with viable cysts by potential hosts [6].

1.2 *CRYPTOSPORIDIUM ANDERSONI (MURIS) / PARVUM*

Cryptosporidium muris was first described by Ernest Edward Tyzzer in 1907 when he found these parasites in the gastric glands of mice [33, 36]. In 1912 he described a similar parasite in mice, but having smaller oocysts than the *Cryptosporidium muris* and located in the small intestine [33]. This parasite was named *Cryptosporidium parvum* and it was not until 1971 that it was reported in other hosts causing diarrhea in young beef cattle [33]. Recently *Cryptosporidium muris* found in cattle has been renamed *Cryptosporidium andersoni* based on visible morphologic differences and genotyping

[34]. Interest in *Cryptosporidium* has been on the increase since the 1993 waterborne outbreak in Milwaukee, Wisconsin involving 403,000 people [36].

1.2.1 Life Cycle

When a *Cryptosporidium* oocyst is ingested by a host it is exposed to acid in the stomach, then bile salts and digestive enzymes in the proximal duodenum causing the oocyst to excyst, releasing sporozoites [35]. The sporozoites then infect epithelial cells of the intestinal tract causing diarrhea [35]. The sporozoites of *Cryptosporidium parvum* also migrate up the biliary tree to infect the epithelial cells of the gallbladder and bile ducts [35]. The respiratory tract may also be parasitized [36]. Sporozoites attach themselves to the epithelial cell until the microvilli surround it creating a parasitophorous vacuole [36]. Sporozoites differentiate into trophozoites where asexual reproduction occurs (schizogony) producing schizonts that then create a merozoite [36]. The merozoites exit the schizont to infect other cells and develop into a type I or type II schizont producing four merozoites [36]. It is thought that only the type II schizont merozoites undergo sexual reproduction (gametogony) producing a microgamont or macrogamont [36]. The macrogamonts may fuse and if fertilized develop into oocysts, then sporulate and exit the host in its feces or in respiratory excretions [36].

1.2.2 Morphology

Cryptosporidium parvum is the smaller of the two oocysts measuring 5.0 x 4.5 μm whereas *Cryptosporidium andersoni* measures 7.4 x 5.6 μm and both are round in

shape [36].

1.2.3 Taxonomy

Cryptosporidium belongs to the Kingdom Protozoa, Phylum Apicomplexa, Class Coccidea, Order Eucoccidiorida and Family Cryptosporidiidae [32, 36]. There have been 19 species named but Levine considers only five valid; *Cryptosporidium muris* (*Cryptosporidium andersoni*) in mammals, *Cryptosporidium meleagridis* in birds, *Cryptosporidium crotali* in reptiles, *Cryptosporidium nasorum* in fish and *Cryptosporidium parvum* in humans and other mammals [32]. *Cryptosporidium muris* in cattle has been recently renamed *Cryptosporidium andersoni* due to its morphology and location in the abomasum of cattle [34]. The material presented in this thesis will deal only with *Cryptosporidium parvum* and *Cryptosporidium andersoni*.

1.2.4 Transmission

Cryptosporidium infections occur in a similar manner to *Giardia* infections, via the fecal-oral route, with the host ingesting viable oocysts [36]. *Cryptosporidium parvum* transmission occurs through either zoonotic, environmental or non-zoonotic routes [36]. The zoonotic route of transmission can include peoples' interaction with infected companion animals resulting in the ingestion of viable oocysts [36]. Humans can also be infected through occupational exposure such as the case with veterinarians or livestock producers [36]. Occupational exposure is speculated to stimulate a high level of immunity in the host due to repeat infections [36]. Occupational transmission may also lead to

indirect zoonotic transmission where oocyst infected material is carried home secondarily infecting those at home [36].

Environmental sources of transmission in recent years have received a lot of attention due to large waterborne outbreaks [36]. Water sources are likely contaminated when fecal containing runoff from manured land finds its way into the water or by direct release of effluent into the water by drinking animals or emptying of sewage holding ponds [36, 37, 38].

Foodborne transmission is another environmental source of infection [36]. Foodborne transmission has been linked to the consumption of raw milk and sausage in the U.K., and apple cider in the U.S.A. [36]. Contamination occurs when the food product comes into contact with viable oocysts and is not further processed in a manner to kill the oocysts prior to consumption [36].

The last route of transmission causing cryptosporidiosis is non-zoonotic transmission [36]. This can be caused by person-to-person contact such as seen in daycares, senior homes or hospitals where hygiene is not adequate [36].

Transmission of *Cryptosporidium parvum* is enhanced by the low infective dose required to cause disease in the host. It has been reported that the ID_{50} for immunocompetent hosts is 132 oocysts, but infection can be caused by as few as 30

oocysts [38]. The infective dose for an immunosuppressed host has been established in mice at a single oocyst causing infection in 20% of inoculated mice [62].

A further enhancement to the transmission of *Cryptosporidium* is the viability of oocysts under a variety of environmental conditions. Oocysts have been reported to remain viable for over 12 weeks at -4^{0}C and 4^{0}C, however they degrade more quickly at 25°C and when exposed to natural microorganisms found in feces and soil [16].

1.2.5 Distribution

Cryptosporidium parvum in young calves and humans has been reported to be worldwide in distribution [36]. Infection rates among humans in developing countries (8.5%) are higher than those found among developed countries (1 - 2%) due to factors such as hygiene, water treatment and poor host immunity (malnutrition) [36]. Typically children aged 1 to 5 years and immunosuppressed individuals are most susceptible to *Cryptosporidium parvum* infections [36]. *Cryptosporidium andersoni* has been reported in older cattle in U.S.A., Canada, Mexico and Brazil [25, 36, 39, 40].

1.2.6 Clinical Symptoms

Cryptosporidium parvum infections in humans and animals typically have a 7 day incubation period after which the host exhibits secretory diarrhea, abdominal pain, nausea, fever and fatigue [1, 41, 43]. In calves, reports of high mortality have been associated with *Cryptosporidium parvum* infections [42]. *Cryptosporidium andersoni*

infected cattle are not visually any different from their non-infected pen mates, but they have been reported to have lower weight gains and reduced milk production [40, 43].

1.2.7 Pathology and Pathogenesis

Cryptosporidium parvum infections in the small intestine usually result in diarrhea due to villous atrophy, shortening of microvilli and sloughing off of enterocytes [36, 43]. It has also been proposed that *Cryptosporidium parvum* may release a toxin that causes profuse cholera-like watery diarrhea. However, it is not clear if it is the toxin or some host immune response responsible for this pathogenesis [43]. Mucosal inflammation also occurs within the host which alters intestinal water and electrolyte transport [43].

1.2.8 Zoonosis

Cryptosporidium parvum has been identified in 80 species of mammals and cross-transmission between species has been documented [36]. Human infection after exposure to horses and horse manure has been investigated in the U.K. as well as people camping on manured farm land [36]. Similar to *Giardia*, *Cryptosporidium parvum* is a concern for environmental contamination due to the wide range of potential hosts that have access to watersheds that feed potable water sources for humans [36]. *Cryptosporidium andersoni* is not known to be a zoonotic pathogen [36].

1.2.9 *Cryptosporidium* and Cattle

Cryptosporidium parvum typically infects calves at 2 weeks of age, but it has been reported in calves as young as 4 days, but rarely in calves over 1 month of age [26, 36]. Shedding of oocysts ranges between 10^5 to 10^7 per gram of feces with diarrhea lasting an average of 6 days [36]. Prevalence in dairy calves has been reported as high as 100% with beef calves reported at 20% [28, 44].

Cryptosporidium andersoni infects older cattle possibly causing impaired protein digestion associated with increased gastric pH resulting in decreased weight gains (10 - 50%), and milk production (3.2 kg/d) [45, 46]. Overall prevalence reported in dairy cattle from California was 1.74% of 8539 samples [45].

Recently *Cryptosporidium felis* has been identified in cattle on a farm in northern Poland [33]. *Cryptosporidium felis* oocysts were measured at 4.3 to 4.5 $\pm$ 0.5 µm, even smaller than those found for *Cryptosporidium parvum* [33]. This is the first report of *Cryptosporidium felis* being identified in cattle, its significance is currently unknown [33].

1.2.10 Treatment /Prevention

Presently there are no drugs registered for the prevention or treatment of *Cryptosporidium parvum* [47]. An oral vaccine for calves at birth was tested against experimental *Cryptosporidium parvum* challenge with positive results, however when

field tested the vaccine failed [47]. A recombinant protein based vaccine for *Cryptosporidium parvum* was administered to cows prior to calving; it eliminated diarrhea and reduced oocyst shedding in the calves as compared to a control group [48]. Most anticoccidial drugs are ineffective in the treatment of *Cryptosporidium parvum* however, testing of lasalocid, halofuginone, decoquinate and paromomycin under controlled conditions have shown some positive results [36, 49].

Prevention of *Cryptosporidium parvum* infections include disinfection, hygiene and adequate nutrition to reduce or prevent the spread from one animal to another [36]. These measures can include strategies such as isolation of infected calves; disinfect contaminated areas; control of mice etc. that may carry the parasite from one host to another and provide colostrum and nutritional supplements to calves [36]. In light of the limited success with experimental vaccines and drug treatments, prevention is currently the best strategy for livestock producers.

1.3 HYPOTHESES AND OBJECTIVES OF THIS STUDY

The objectives of this study were to collect information on the prevalence and biology of *Giardia* and *Cryptosporidium* in beef cattle to better understand how these parasites might effect animal productivity and the environment. These objectives were addressed in studies to determine:

Hypothesis #1 - *Cryptosporidium andersoni, Cryptosporidium parvum* and *Giardia lamblia* will have a negative effect on feedlot calves' performance.

Objectives: 1. To determine the prevalence of *Cryptosporidium andersoni, Cryptosporidium parvum* and *Giardia lamblia* in feedlot calves from weaning to slaughter.

2. To determine the effect of *Cryptosporidium andersoni, Cryptosporidium parvum* and *Giardia lamblia* infections on feedlot steers' average daily gain (ADG), dry matter intake (DMI) and feed efficiency (FE).

Hypothesis #2 - Feedlot steers infected with chronic *Cryptosporidium andersoni* are immunosuppressed by viral pathogens.

Objectives: 1. To determine which steers are chronically infected with *Cryptosporidium andersoni* from weaning to slaughter.

2. To characterize blood lymphocyte populations of chronically infected steers compared to control (uninfected) steers.

3. To determine whether or not *Cryptosporidium andersoni* infected steers are positive for immonosuppressive viral pathogens (infectious bovine rhinotracheitis, bovine virus diarrhea, bovine immunodeficiency virus and bovine leukosis virus).

Hypothesis #3 - *Cryptosporidium parvum* and *Giardia lamblia* are prevalent in beef calves.

Objective: 1. To determine the prevalence of *Cryptosporidium parvum* and *Giardia lamblia* in beef calves from birth to weaning.

Hypothesis #4 - Dams shed *Cryptosporidium andersoni* and *Giardia lamblia* during the periparturient period.

Objective: 1. To determine the prevalence of *Cryptosporidium andersoni* and *Giardia lamblia* during the periparturient period.

2.METHODS AND MATERIALS

2.1 PREVALENCE OF *GIARDIA* AND *CRYPTOSPORIDIUM ANDERSONI* AND THEIR EFFECTS ON PERFORMANCE IN FEEDLOT BEEF CATTLE

Sixty individually housed Charolais crossbred steers, weaned October 1996, weighing between 215 - 304 kg and approximately six months of age were followed for 257 d. Animal's individual pens were separated by open plank fence allowing for nose to nose contact between adjacent animals. All animals were cared for according to the Guidelines for Care and Use of Experimental Animals [52].

Animals had a 12 day period prior to initiating the study to allow animals to adapt to their environment. They were then placed on two backgrounding rations where performance data were collected with durations of 84 d and 63 d (Table 1). A 77 d finishing ration proceeded the backgrounding rations (Table 1). There was a 21 day break between the last backgrounding ration and the start of the finishing ration where no performance data were collected. The steers were fed *ad libitum*, and given access to water via automatic waterers for the duration of the study. Each week, feed weighbacks were performed and each animal was weighed. FE, ADG and DMI were calculated for each animal during each weigh period of the study, but not for the 12 d adaptation period or the 21 d interval between the second backgrounding and finishing periods.

Upon introduction into the individual pens (0 d), fecal samples (1 - 5 g) were collected (1 collection, warm-up period). Additional samples were collected every 28 d during the first backgrounding period (84 d, 3 collections), every 21 d through the second backgrounding period (63 d, 3 collections), break period (21 d, 1 collection) and finishing period (77 d, 4 collections). Fecal samples were collected from each steer's rectum by grab sampling using a disposal latex glove. Samples were placed in weighed centrifuge tubes containing 5 mL of 5% formalin in phosphate buffered saline solution (PBSS; 0.9% NaCl, pH 7.2). The fecal sample was shaken to disperse it in the PBSS and stored at 4^{O}C until further processing (within 1 week).

Fecal analysis was performed according to previously described procedures [25, 28]. Briefly, fecal samples were filtered through surgical gauze (Nu-gauze, 4 ply, Johnson & Johnson, Montreal, Quebec), and washed with PBSS, resulting in approximately 7 mL of filtrate. The filtrate was then layered over 5 mL of 1M sucrose (sp. gr. 1.13) and centrifuged at 800 X g for 5 min in a fixed rotor centrifuge to concentrate cysts and oocysts at the sucrose layer surface. The upper filtrate layer and sucrose surface were pipetted into a clean tube and centrifuged again at 800 X g for 5 min. The resulting supernatant was decanted and the pellet suspended in PBSS to approximately 1 mL.

The suspended pellet was then applied in two 0.015 mL spots on a microscope slide (Erie Scientific Co., Portsmouth, NH.) and air dried for 30 min. on a 37 ^{O}C slide warmer. The slide was then fixed with acetone for 1 min. and left to dry. A *Giardia* - specific

fluorescein isothiocynate (FITC) - labeled monoclonal antibody solution (0.02 mL) (Giardi-a-glo, Waterborne, New Orleans, LA.) was applied to the left sample. A *Cryptosporidium* - specific FITC - labeled monoclonal antibody solution (0.02 mL) (Crypt-a-glo, Waterborne, New Orleans, LA.) was applied to the right sample. The slide was then placed in a moist container and incubated at 37^{0}C for 45 minutes. Afterwards the slide was removed from the incubator and mounted with a fluorescent antibody mounting fluid (Agua-polymount, Polysciences, Warrington, PA.) and a 22 mm^2 micro coverslip (VWR Scientific Inc., Media, PA.).

Cysts and oocysts were enumerated at 100 X and 400 X magnification respectively, dry objective, with an epifluorescence microscope (Olympus, BX60, Olympus Optical Co. Ltd., Japan). The number of cysts and oocysts per gram of feces was then calculated using a previously described formula and recorded [28]. The sensitivity of this detection method was 66 cysts or oocysts per gram of feces [28].*Cryptosporidium andersoni* oocysts were differentiated from *Cryptosporidium parvum* oocysts morphologically.

Statistical Analysis

FE, ADG and DMI values were analyzed using SAS (SAS Institute Inc.,Cary, NS.) employing the general linear model procedure. Cyst and oocyst numbers for each sampling date were log-transformed prior to analysis. Changes in cyst and oocyst counts over time were compared using ANOVA and the Student - Newman - Keuls multiple comparison of

means. Prevalence was compared to day 0 using the Fisher's Exact Test (Instat, Graphpad Inc., San Diego, Ca.). All analyses were performed using a 95% confidence interval.

Lymphocyte Profile

Blood samples were collected from 10 steers (3 positive, 7 negative for *Cryptosporidium andersoni*) on June 10, June 17, July 14, 1997 utilizing serum tubes (no anti-coagulant) and serum tubes containing EDTA (anti-coagulant). EDTA blood samples were sent to Palliser Animal Health Laboratories in Lethbridge, Alberta for differential white cell count, total white blood cell count (WBC), bovine virus diarrhea serum titre (BVD) through serum neutralization tissue culture, and infectious bovine rhinotracheitis titre (IBR) through ELISA. No anti-coagulant blood samples were sent to Dr. Reynolds' laboratory at the University of Calgary to determine lymphocyte profiles utilizing flow cytometry. Serum was extracted from the blood samples and lymphocytes were isolated utilizing bovine monoclonal antibodies as described by Pasquali et al [50]. Blood samples were also tested for bovine leukosis by ELISA and bovine immunodeficiency virus in Dr. Chris Power's laboratory utilizing polymerase chain reaction (PCR) as described in Gonzalez et al [60]. Lymphocyte profiles were compared between *Cryptosporidium andersoni* positive and negative steers for 12 monoclonal antibodies (Table 2) using Instat V2 (Instat, Graph Pad Inc., San Diego , California) Unpaired t- test. Data were reported as mean $\pm$ SD. Values of $P < 0.05$ were considered significant.

Histology

Ten of the feedlot steers were slaughtered and intestinal sections were taken from the duodenum, jejunum, ileum and abomasum to note any presence of *Cryptosporidium andersoni* (3 infected steers with *Cryptosporidium andersoni* and 7 non-infected steers). Gut segments were stapled on a cardboard sheet and placed in 10% neutral buffered formalin. These were dehydrated in ethanol, embedded in paraffin and sectioned at 6 μm. The sections were stained with hematoxylin and eosin and viewed under a light microscope.

Table 1. Ingredients and composition of diets fed to steers

Ingredients (% as-fed)	Backgrounding Diet	Finishing Diet
Barley silage	85.5	20.0
Barley, rolled	10.6	75.0
Canola meal	1.4	NA
Soybean meal	1.0	NA
Molasses	0.075	0.075
Canola oil	0.025	0.025
Calcium carbonate	4.0	4.0
Trace-mineralized salt[z]	1.0	1.0
Perma-pel[y]	0.025	0.025
Vitamin A, D, E[x]	0.015	0.015

[z] Contained 93.1% NaCl; 0.55% Mg; 0.33% Zn; 0.027% Mn; 0.03% Cu; 0.005% I; 0.007% Se.

[y] Feed pellet binder (Georgia Pacific, Bellingham, WA).

[x] Contained 10 000 IU g^{-1} Vitamin A, 1,250 IU g^{-1} Vitamin D and 10 IU g^{-1} Vitamin E.

Table 2. Monoclonal antibodies used in lymphocyte profile.

Antibody	Ig Isotype	Specificity
BAQ95A	IgG_1	CD_2
MMIA	IgG_1	CD_3
CACT138A	IgG_1	CD_4
CACT80C	IgG_1	$CD_{8\alpha}$
CACT61A	IgM	TCR_1
CACT116A	IgG_1	IL-$2R_\alpha$
PIG45A	IgG_{2b}	IgM
BIG715A	IgG_1	IgG_1
BIG312D	IgG	IgA
H58A	IgG_{2a}	MHCI
TH14B	IgG_{2a}	MHCII
BAQ15A	IgM	CD_{21}

2.2 PREVALENCE AND INFECTION PATTERN OF NATURALLY ACQUIRED GIARDIASIS AND CRYPTOSPORIDIOSIS IN BEEF CALVES AND THEIR DAMS FROM BIRTH TO WEANING

The winter and spring portions of the study were conducted on a ranch north of Calgary, Alberta, Canada. Twenty Salers cow calf pairs were selected for the study from a ranch herd based on the cow's calving dates falling between Feb 19 and March 9. The study started on Feb 19 and concluded 198 days later on September 4. During the winter and spring portions of the study (73 d) cattle were located in a large corral and fed a hay, greenfeed, barley grain based ration balanced for animal requirements and supplemented with minerals, trace minerals and vitamins according to NRC 1996 recommendations [61]. Well water was available free choice . Animals were turned out on to a fall rye pasture for grazing May 3 and transported to perennial grass summer pasture at the University of Calgary Research Facility on June 9. Cow calf pairs remained on the perennial pasture until the conclusion of the study on September 4. Animals for this study were handled according to Agriculture and Agri-Food Canada's recommended code of practice for the care and handling of farm animals - beef cattle [52].

Rectal fecal samples were collected from calves at 3 days of age and weekly until May 3. Calves were then sampled 3 times during the summer grazing period. Fecal samples were collected from the cows at the time of calving, one week later and 4 times more

throughout the grazing period. Rectal fecal samples (1-10 g) were collected using a disposable latex glove and placed in pre-weighed centrifuge tubes containing 5 ml of 5% formalin in phosphate-buffered saline solution (PBSS; 0.9% NaCl, pH 7.2). Samples were weighed and stored at 4°C until processing.

Blood samples were collected from the calves at 3 days of age utilizing serum tubes containing no anti-coagulant. Serum was extracted through centrifugation and frozen until analysis. Radial Immunodiffusion Assay (Bovine IgG_1 Test Kit, VMRD Inc. Pullman, Wash.) was used to determine IgG_1 concentration. Calves with IgG_1 concentrations between 800 and 1600 mg/dl were assumed to have partial transfer of passive immunity. Calves with concentrations > 1600 mg/dl were considered to have complete transfer of passive immunity [28].

Giardia cysts and *Cryptosporidium* oocysts were isolated and enumerated according to the methods described by O'Handley et al [28].

Statistical Analysis

Natural logarithms and geometric means of cyst counts were calculated for each sampling date and for days after cyst excretion was first detected in the calves. Natural logarithms and geometric means of cysts and oocysts excretion after calving were calculated for each sampling date for the cows. Changes in oocyst/cyst fecal counts and prevalence of

Giardia and *Cryptosporidium* throughout the study were compared using an unpaired t-test and Fisher's exact test (InStat, GraphPad Inc., San Diego, California). Data were reported as mean $\pm$ SD. Values of $P < 0.05$ were considered significant.

3. RESULTS

3.1 PREVALENCE OF *GIARDIA* AND *CRYPTOSPORIDIUM ANDERSONI* AND THEIR EFFECTS ON PERFORMANCE IN FEEDLOT BEEF CATTLE

For the purpose of this study, infected was defined as an animal that had a positive fecal sample for the particular parasite (*Giardia* or *Cryptosporidium andersoni*) on any sampling date during the course of this trial.

The prevalence of *Giardia* and *Cryptosporidium andersoni* in the 60 feedlot steers during the duration of the trial are summarized in Figures 1 and 2. There was a decrease ($P<0.05$) in the percentage of *Giardia* infected steers from d 132 to the completion of the trial (Figure 1) while *Cryptosporidium andersoni* infected steers decreased ($P<0.05$) from d 97 to the completion of the trial (Figure 2). Overall prevalence of *Giardia* and *Cryptosporidium andersoni* was 82% and 85% respectively. Combined *Giardia* and *Cryptosporidium andersoni* infections occurred at some time in 70% of the steers and infection with only *Cryptosporidium andersoni* and only *Giardia* were 15% and 10%, respectively. These parasites could not be detected in 5% of the animals for the duration of the study.

The number of *Giardia* cysts shed per gram of feces in the positive animals was intermittent throughout the trial period, while the number of *Cryptosporidium andersoni*

oocysts shed increased ($P<0.05$) after day 132 compared to d 0 (Figure 1 and 2).

Analysis of the overall performance effects of a singular *Giardia* or *Cryptosporidium andersoni* infection was not performed due to the limited numbers of animals fitting these criteria. Instead, performance parameters were compared between *Giardia* infected and non-infected animals over set periods of time. Similar comparisons were made between *Cryptosporidium andersoni* infected and non-infected animals.

There was a increase ($P<0.05$) in DMI for *Giardia* infected animals during the whole backgrounding period d 0 - d 84 and a portion of the finishing period (d 85 - d 96) (Table 3, 4, 5). A similar comparison between *Cryptosporidium andersoni* infected and non-infected steers showed a lower ($P<0.05$) ADG and DMI for non-infected steers during the first backgrounding period d 0 - d 84. During d 85 - d 96 of the finishing period *Cryptosporidium andersoni* infected steers had a lower ($P<0.05$) DMI than those non-infected steers (Table 3 and 5).

Lymphocyte Profile

Blood samples analyzed for BVD, bovine leukosis and BIV were negative for all 10 steers, two steers from the control group had positive titres for IBR (Table 6).

Results from lymphocyte profiles were expressed in two ways; as a percentage gated

and as the number of lymphocytes. There were no significant differences between the control and infected groups with the exception of T-cell receptor 1 (TCR1) expressed as a percentage where the infected group was lower ($P < 0.05$) than the control group (Table 7 and 8).

Histology

The sections from the ileum, jejunum, duodenum and abomasum in the control steers (7) were negative for cryptosporidial life cycle forms, however the abomasum sections in the *Cryptosporidium andersoni* infected steers contained cryptospridial life cycle forms including sporozoites, trophozoites, merozoites and oocysts (Figure 3).

Table 3. Performance data of *Giardia* and *Cryptosporidium* infected feedlot steers - background period 1

	Giardia				*Cryptosporidium andersoni*			
Period 1	Infected	SE	Non-infected	SE	Infected	SE	Non-infected	SE
day 0 to 28	n = 31		n = 29		n = 27		n = 33	
ADG (kg d^{-1})	0.99	0.15	1.07	0.15	0.96	0.14	1.14	0.14
DMI (kg d^{-1})	5.96	0.29	6.05	0.29	5.83	0.28	6.29	0.28
FE (kg kg^{-1})	6.35	2.07	6.82	2.08	7.08	2.02	5.76	2.01
day 28 to 56	n = 28		n = 32		n = 17		n = 43	
ADG (kg d^{-1})	0.92	0.09	0.88	0.09	0.89	0.09	0.93	0.09
DMI (kg d^{-1})	6.34	0.17	6.68	0.17	6.58	0.17	6.38	0.16
FE (kg kg^{-1})	7.19	0.98	8.42	0.99	7.76	0.99	7.71	0.97
day 56 to 84	n = 19		n = 41		n = 11		n = 49	
ADG (kg d^{-1})	1.00	0.09	1.04	0.08	1.08	0.11	0.96	0.07
DMI (kg d^{-1})	5.11	0.29	5.40	0.25	5.22	0.33	5.24	0.23
FE (kg kg^{-1})	5.38	0.77	5.54	0.66	5.06	0.87	5.82	0.60
day 0 to 84	n = 44		n = 14		n = 43		n = 15	
ADG (kg d^{-1})	0.99	0.03	0.95	0.05	1.04	0.03	0.86*	0.06
DMI (kg d^{-1})	6.11	0.09	5.58*	0.16	6.05	0.10	5.56*	0.17
FE (kg kg^{-1})	6.30	0.17	6.25	0.31	6.05	0.19	6.69	0.33

* indicates significant difference ($P<0.05$) within parasite species from infected.

Table 4. Performance data of *Giardia* and *Cryptosporidium* infected feedlot steers - background period 2

	Giardia				*Cryptosporidium andersoni*			
Period 2	Infected	SE	Non-infected	SE	Infected	SE	Non-infected	SE
day 0 to 21	n = 21		n = 39		n = 12		n = 48	
ADG (kg d^{-1})	1.25	0.13	1.44	0.14	1.36	0.13	1.30	0.06
DMI (kg d^{-1})	5.83	0.31	6.25	0.34	6.01	0.30	6.14	0.14
FE (kg kg^{-1})	7.19	1.40	4.44	1.53	6.16	1.36	4.76	0.65
day 22 to 42	n = 12		n = 48		n = 10		n = 50	
ADG (kg d^{-1})	1.10	0.21	1.13	0.11	1.12	0.17	1.10	0.08
DMI (kg d^{-1})	7.09	0.49	6.74	0.25	6.78	0.40	7.20	0.18
FE (kg kg^{-1})	6.58	2.60	6.57	1.34	6.13	2.14	7.47	0.95
day 43 to 63	n = 10		n = 50		n = 9		n = 51	
ADG (kg d^{-1})	0.80	0.19	1.02	0.10	0.89	0.16	0.95	0.08
DMI (kg d^{-1})	7.79	0.81	8.03	0.43	7.56	0.67	8.59	0.32
FE (kg kg^{-1})	10.58	3.28	9.67	1.75	9.56	2.72	11.24	1.30
day 0 to 63	n = 10		n = 50		n = 6		n = 54	
ADG (kg d^{-1})	1.08	0.05	1.19	0.07	1.15	0.06	1.11	0.03
DMI (kg d^{-1})	6.86	0.23	7.11	0.33	6.85	0.26	7.38	0.14
FE (kg kg^{-1})	6.49	0.32	6.06	0.45	6.11	0.36	6.77	0.19

Table 5. Performance data of *Giardia* and *Cryptosporidium* infected feedlot steers - finishing period 3

	Giardia				*Cryptosporidium andersoni.*			
Period 3	Infected	SE	Non-infected	SE	Infected	SE	Non-infected	SE
day 64 to 84	n = 4		n = 56		n = 8		n = 52	
ADG (kg d^{-1})	1.39	0.36	0.92	0.17	0.85	0.26	1.22	0.19
DMI (kg d^{-1})	10.86	0.80	10.08	0.39	9.67	0.56	10.88	0.42
FE (kg kg^{-1})	9.31	3.47	11.58	2.04	12.32	3.01	9.71	1.82
day 85 to 96	n = 4		n = 56		n = 8		n = 52	
ADG (kg d^{-1})	1.29	0.34	1.27	0.17	1.27	0.25	1.28	0.18
DMI (kg d^{-1})	10.48	0.87	8.37*	0.43	7.68	0.64	10.11*	0.45
FE (kg kg^{-1})	9.71	4.33	7.33	2.15	6.20	3.16	9.66	2.26
day 97 to 118	n = 0		n = 60		n = 8		n = 52	
ADG (kg d^{-1})	-	-	1.37	0.15	1.39	0.22	1.32	0.08
DMI (kg d^{-1})	-	-	9.05	0.53	8.53	0.78	10.09	0.29
FE (kg kg^{-1})	-	-	7.13	1.17	6.39	1.72	8.61	0.67
day 119 to 140	n = 3		n = 55		n = 6		n = 52	
ADG (kg d^{-1})	1.58	0.32	1.44	0.16	1.58	0.24	1.37	0.16
DMI (kg d^{-1})	8.90	1.35	8.86	0.68	8.44	1.01	9.31	0.70
FE (kg kg^{-1})	6.13	3.56	7.23	1.80	5.87	2.67	8.04	1.84
day 64 to 140	n = 16		n = 42		n = 16		n = 42	
ADG (kg d^{-1})	1.31	0.09	1.23	0.08	1.31	0.14	1.25	0.10
DMI (kg d^{-1})	9.47	0.40	9.64	0.35	9.12	0.68	10.04	0.47
FE (kg kg^{-1})	7.26	1.29	9.44	1.13	7.02	2.02	8.59	1.39
Period 1 - 3	n = 46		n = 10		n = 47		n = 9	
ADG (kg d^{-1})	1.15	0.04	1.17	0.06	1.17	0.04	1.13	0.07
DMI (kg d^{-1})	7.79	0.14	7.91	0.23	7.79	0.14	7.94	0.27
FE (kg kg^{-1})	6.95	0.27	6.85	0.43	6.81	0.26	7.14	0.50

* indicates significant difference ($P<0.05$) within parasite species from infected.

Table 6. BVD, IBR, BLV and BIV testing results for feedlot steers indicating either their negative or positive status for the particular virus.

Steer	Group	BVD Titre	IBR Titre	BLV	BIV
248	Infected	1:128 neg	neg	neg	neg
249	Infected	- neg	1:2 neg	neg	neg
356	Infected	1:64 neg	1:4 neg	neg	neg
294	Control	1:16 neg	1:29 pos	neg	neg
282	Control	1:16 neg	1:7 neg	neg	neg
246	Control	1:128 neg	1:16 pos	neg	neg
255	Control	1:64 neg	1:4 neg	neg	neg
350	Control	1:32 neg	1:6 neg	neg	neg
210	Control	1:16 neg	-	neg	neg
354	Control	1:64 neg	1:8 neg	neg	neg

Table 7. Lymphocyte profile of feedlot steers expressed as percentage.

Markers	Control % Lymphocytes	Infected % Lymphocytes
Lymphocyte %	69.5 ± 1.8	73.1 ± 2.2 (P=.25)
WBC#	14724 ± 1354.0	11367 ± 1803.5 (P=.17)
CD_2	64.4 ± 1.2	68.5 ± 2.7 (P=.13)
CD_3	81.3 ± 1.2	77.0 ± 2.7 (P=.10)
CD_4	30.0 ± .85	32.9 ± 2.5 (P=.17)
$CD_{8\alpha}$	24.4 ± .85	23.2 ± 1.9 (P=.52)
TCR_1	30.1 ± 1.3	22.8 ± 2.1 (P=.005)*
IL-$2R_\alpha$	25.8 ± 2.0	20.3 ± 2.4 (P=.12)
IgM	16.4 ± .80	18.4 ± 2.2 (P=.28)
IgG_1	12.8 ± 1.0	21.4 ± 7.0 (P=.08)
IgA	3.3 ± .81	4.3 ± 1.7 (P=.56)
MHCI	94.7 ± 3.9	99.1 ± .5 (P=.47)
MHCII	16.4 ± 3.1	22.6 ± 4.4 (P=.27)
CD_{21}	12.7 ± .88	14.5 ± 1.5 (P=.26)

* Values express the mean percentage ± SE (*) denotes values significantly differently from control values ($P < 0.05$).

Table 8. Lymphocyte profile of feedlot steers expressed as numbers of lymphocytes.

Markers	Control # Lymphocytes	Infected # Lymphocytes
Lymphocyte #	10324 ± 982.6	8330 ± 1326.2 (P=.26)
WBC#	14724 ± 1354.0	11367 ± 1803.5 (P=.17)
CD_2	6585 ± 611.7	5467 ± 751.1 (P=.30)
CD_3	8393 ± 816.7	6277 ± 944.7 (P=.14)
CD_4	3058 ± 278.9	2543 ± 317.4 (P=.29)
$CD_{8\alpha}$	2504 ± 261.8	1740 ± 343.5 (P=.12)
TCR_1	3196 ± 371.3	2064 ± 439.7 (P=.09)
IL-$2R_\alpha$	2612 ± 311.89	1783 ± 407.0 (P=.14)
IgM	1690 ± 182.4	1686 ± 400.4 (P=.99)
IgG_1	1345 ± 194.9	2042 ± 863.3 (P=.28)
IgA	300 ± 83.2	340 ± 168.4 (P=.81)
MHCI	10190 ± 976.7	8243 ± 1305.9 (P=.27)
MHCII	1640 ± 374.9	2067 ± 619.3 (P=.55)
CD_{21}	1319 ± 173.6	1281 ± 279.5 (P=.91)

* Values express the mean number ± SE (*) denotes values significantly different from control values ($P < 0.05$).

Figure 1.

Mean number of *Giardia* cysts excreted by infected feedlot steers (open dot ○) and percentage of feedlot steers shedding cysts per sample date (closed dot ●). Asterix indicates significant difference ($P<0.05$) from day 0 of study.

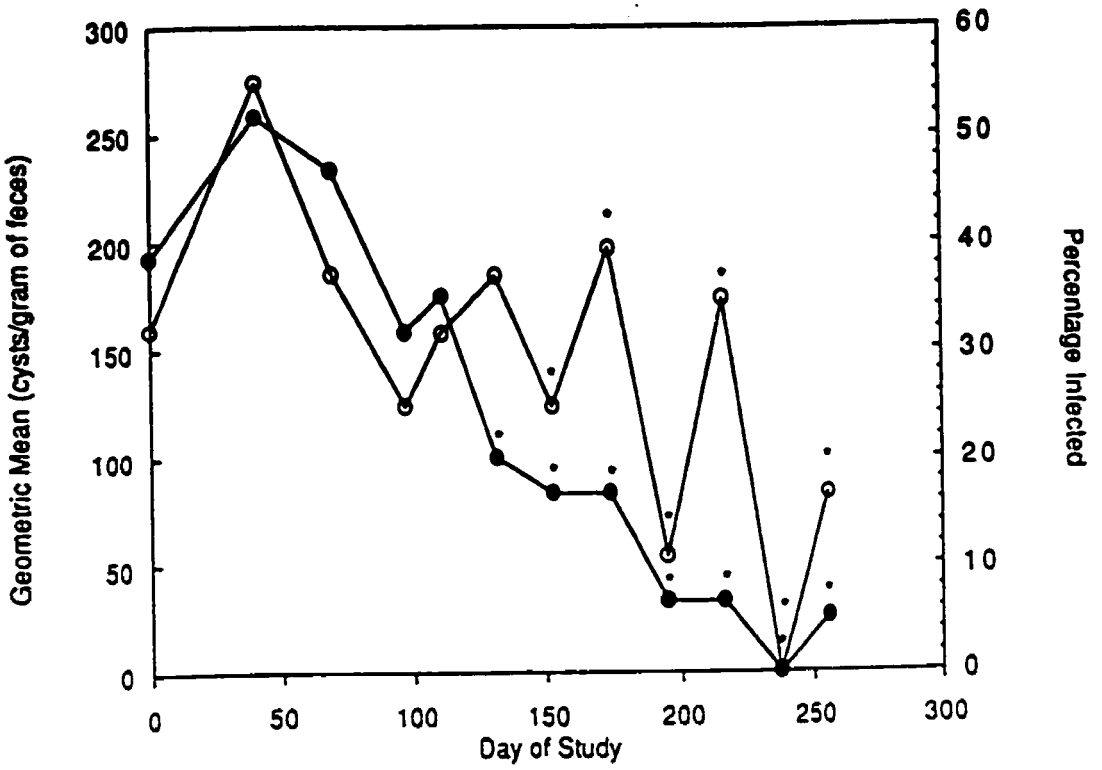

Geometric Mean (cysts/gram of feces)
Percentage Infected
Day of Study
300
250
200
150
100
50
0
60
50
40
30
20
10
0
0
50
100
150
200
250
300

Figure 2.

Mean number of *Cryptosporidium andersoni* oocysts excreted by infected feedlot steers (open dot ○) and percentage of feedlot steers shedding oocysts per sample date (closed dot ●). Asterix indicates significant difference ($P<0.05$) from day 0 of study.

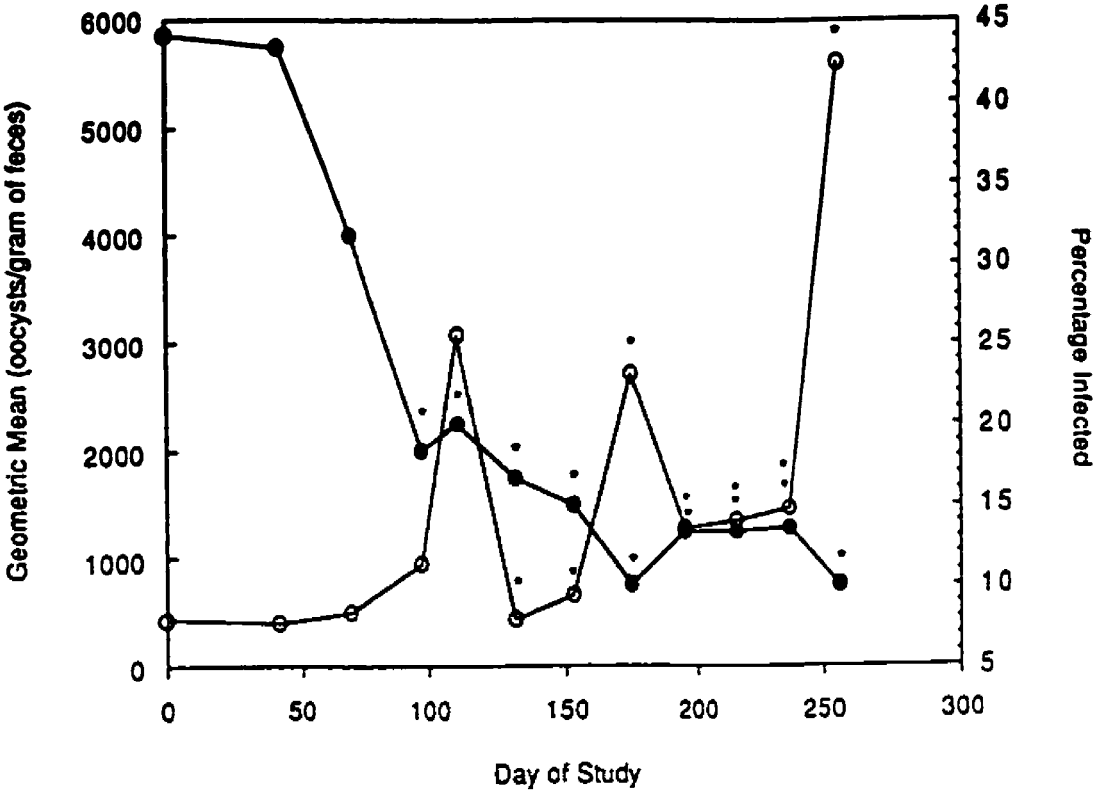
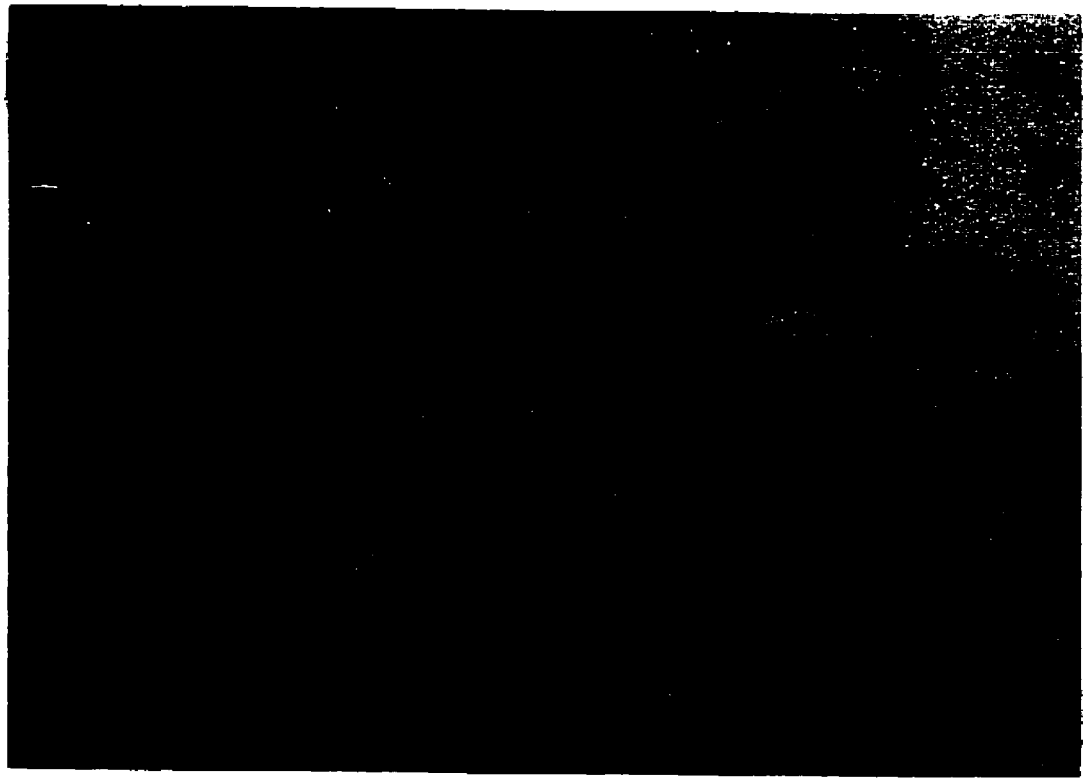

Geometric Mean (oocysts/gram of feces)
6000
5000
4000
3000
2000
1000
0
0
50
100
150
200
250
300
Day of Study
45
40
35
30
25
20
15
10
5
Percentage Infected

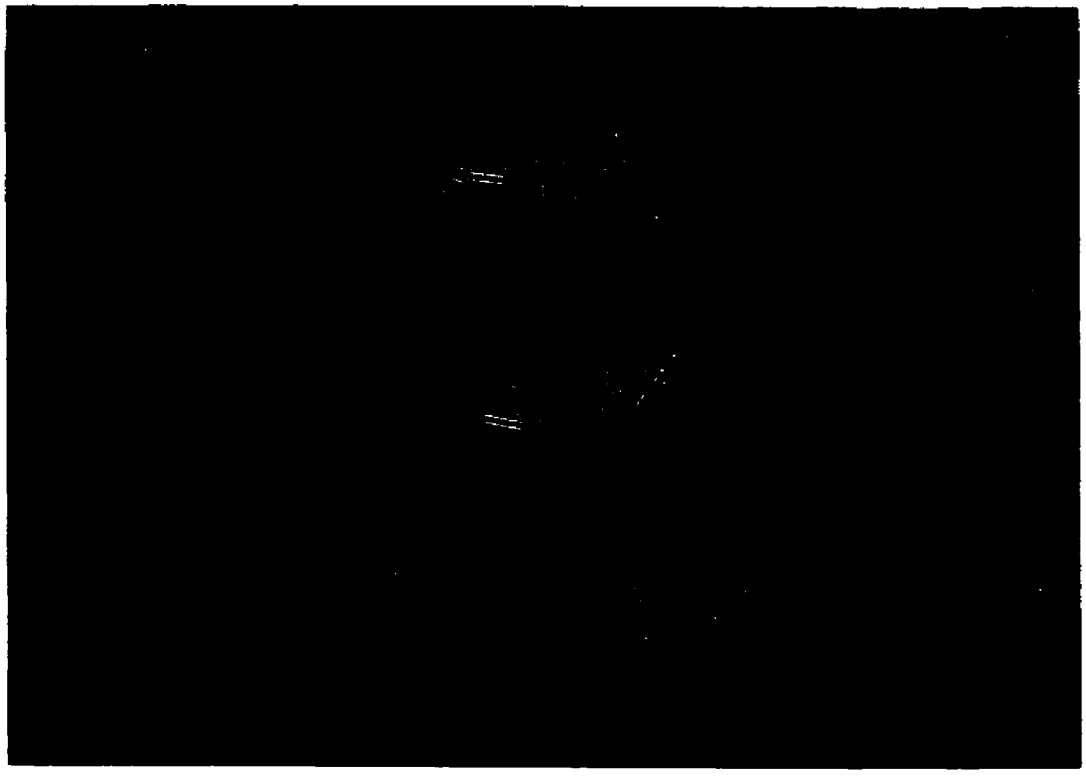
Figure 3.

Various cryptosporidial life cycle forms in a *Cryptosporidium andersoni* positive steer's abomasum under the light microscope. (HE stained) (A) Bar = 400 μm (B) Bar = 50 μm Arrows = cryptosporidial life cycle forms.

A



B



3.2 PREVALENCE AND INFECTION PATTERN OF NATURALLY ACQUIRED GIARDIASIS AND CRYPTOSPORIDIOSIS IN BEEF CALVES AND THEIR DAMS FROM BIRTH TO WEANING

Giardia cysts were shed by all twenty calves (100%) at some date during the duration of the study, however only one calf (5%) shed *Cryptosporidium parvum* oocysts on 2 sample dates during the trial. *Giardia* cysts were first detected at 3.9 $\pm$ 1.37 weeks of age with a range of 2 to 7 weeks of age.

The geometric mean number of *Giardia* cysts in the calf feces increased from 0 at 1 week of age to a maximum of 2230 cysts/g of feces at 5 weeks of age. The geometric mean decreased after week 5 to a low of 2 cysts/g at 25-27 weeks of age (Figure 4A). The infection rate of calves shedding *Giardia* cysts also increased from 0% at 1 week of age to a maximum at 5 weeks of age (85%) and then decreased to 21% at 25-27 weeks of age (Figure 4B).

Comparing the geometric mean from the week that the calves first shed *Giardia* cysts, the geometric mean peaked one week after initial shedding and decreased ($P<0.05$) for the rest of the trial with the exception of week 3 (Figure 5A). There was a lower ($P<0.05$) percentage of calves shedding *Giardia* cysts weeks 3-10 and 15-25 after

shedding was first detected (Figure 5B). Weeks 1, 2 and 11-13 after initial shedding were numerically lower percentages than the 100% at first detection. There was 9 calves (45%) that cleared their *Giardia* infections by week 27 of the study. A calf clearing a *Giardia* infection for the purposes of this study was defined as two or more consecutive *Giardia* negative samples.

All 20 calves had complete or partial transfer of passive immunity. The mean IgG_1 concentration in serum was 1959 $\pm$ 641 mg/dl with a minimum of 1072 mg/dl and a maximum of 4000 mg/dl for the group of calves.

The infected cows' geometric mean number of *Giardia* cysts post calving trended higher to 38.49 cysts/g of feces after 1 week and trended lower to 0 cysts/g 25-28 weeks post calving with the exception of week 20-23 (Figure 6A). These trends in geometric mean *Giardia* cyst counts, however, were not considered statistically significant when compared to the geometric mean on the day of calving. The *Giardia* infection rate of cows peaked at 15% one week post calving and decreased to 0% at 25-28 weeks post calving (Figure 6B).

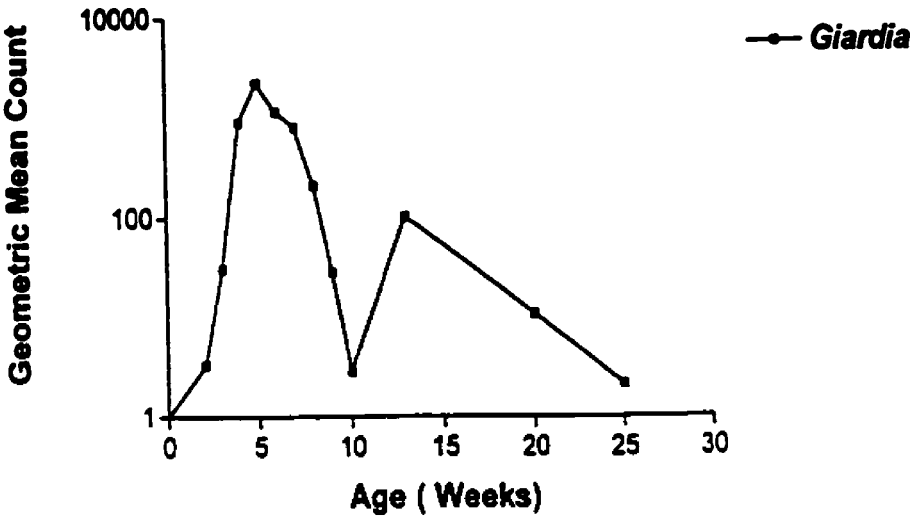
The geometric mean number of *Cryptosporidium andersoni* oocysts in the infected cow feces increased ($P<0.05$) to 37.48 oocysts /g at 1 week post calving and decreased to 0 at 13-16 weeks post calving and for the remainder of the trial (Figure 7A). The rate of infection also peaked, 40% at 1 week post calving and then decreased to 0%

from 13-16 weeks post calving to the completion of the trial (Figure 7B). There were no *Cryptosporidium parvum* oocysts detected by morphological typing in the cow's feces during the course of this study.

Figure 4.

Geometric mean numbers of *Giardia* cysts identified per gram of feces for 20 beef calves in relation to age in weeks, (A) and the percentage of beef calves shedding *Giardia* cysts in relation to age in weeks (B).

A


Geometric Mean Count
10000
100
1
0
5
10
15
20
25
30
Age (Weeks)
Giardia

B

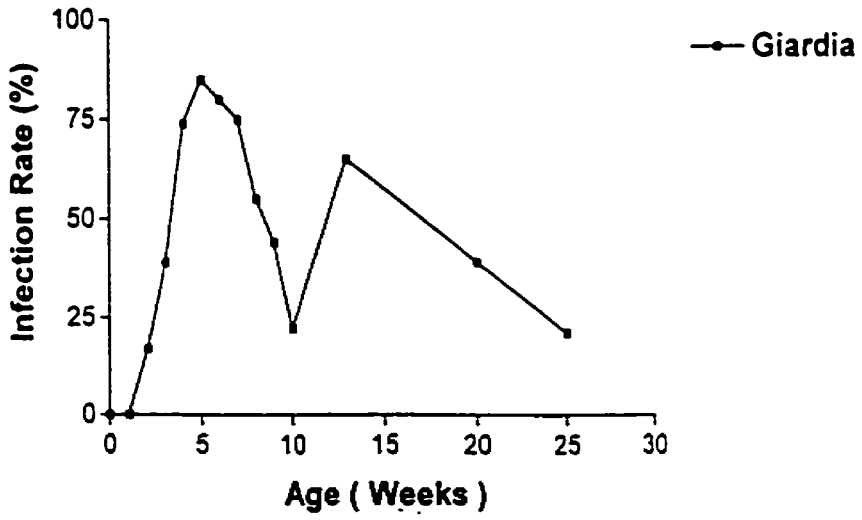
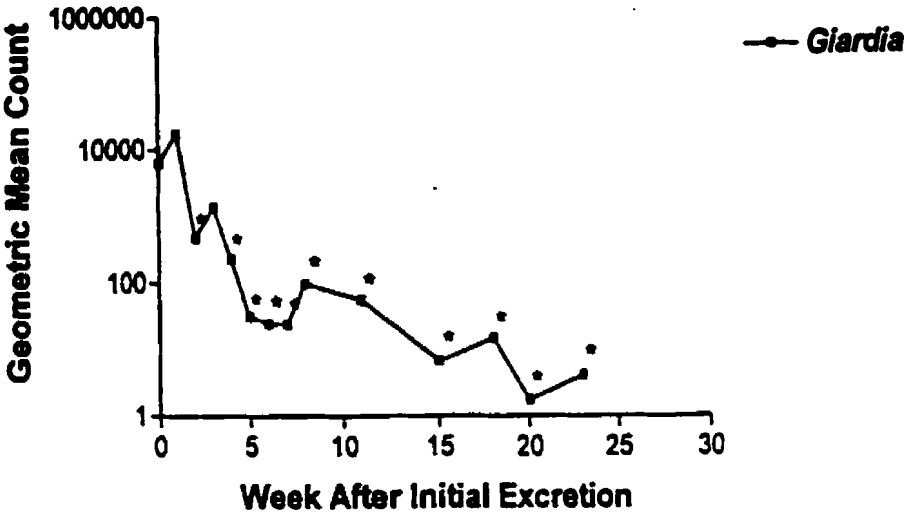

Infection Rate (%)
100
75
50
25
0
0
5
10
15
20
25
30
Age (Weeks)
Giardia

Figure 5.

Geometric mean numbers of *Giardia* cysts identified per gram of feces for 20 beef calves (A) and the percentage of beef calves shedding *Giardia* cysts (B) in relation to week after initial detection. *Significantly ($P<0.05$) different from value on week that shedding was first detected.

A


Geometric Mean Count
1000000
10000
100
1
0
5
10
15
20
25
30
Week After Initial Excretion
Giardia

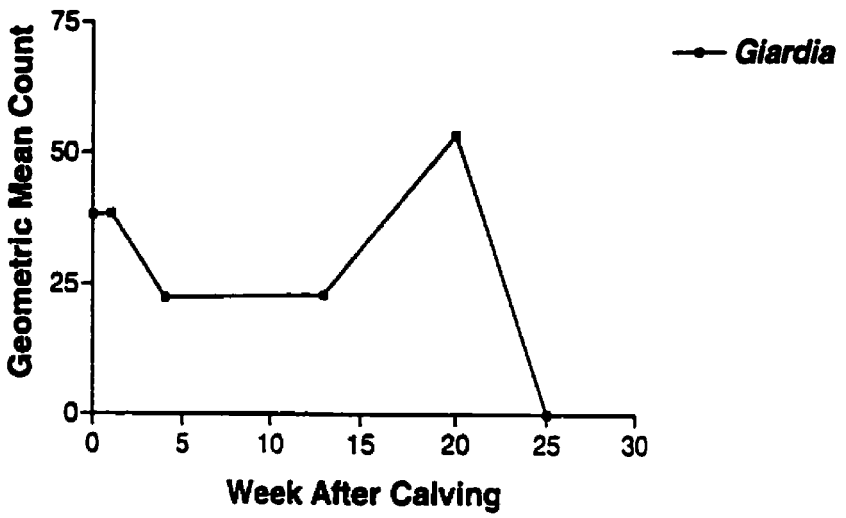
B


Infection Rate (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Week After Initial Excretion
Giardia

Figure 6.

Geometric mean numbers of *Giardia* cysts identified per gram of feces for infected beef cows in relation to week after calving, (A) and the percentage of beef cows shedding *Giardia* cysts in relation to week after calving (B).

A


Geometric Mean Count
75
50
25
0
0
5
10
15
20
25
30
Week After Calving
Giardia

B

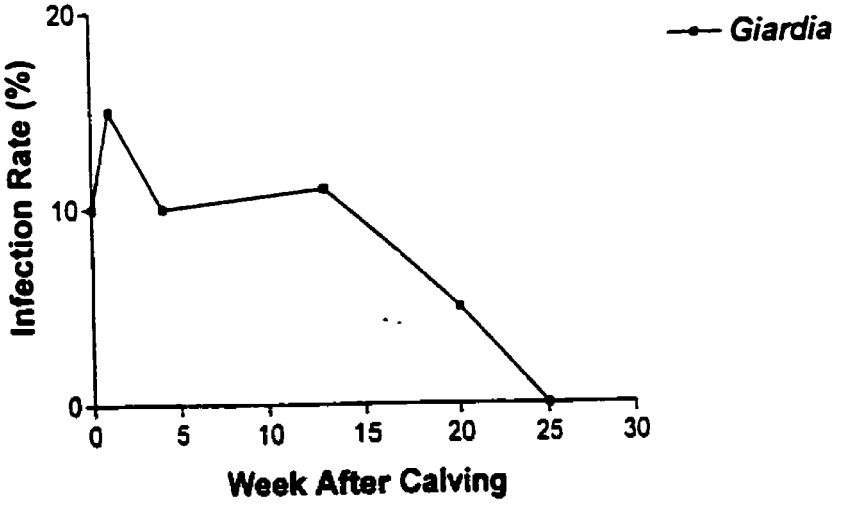
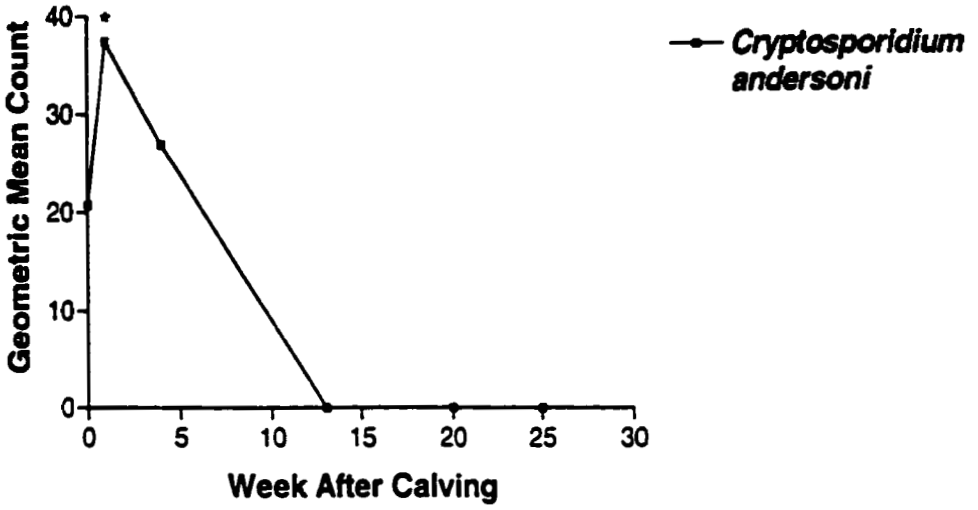

Infection Rate (%)
20
10
0
0
5
10
15
20
25
30
Week After Calving
Giardia

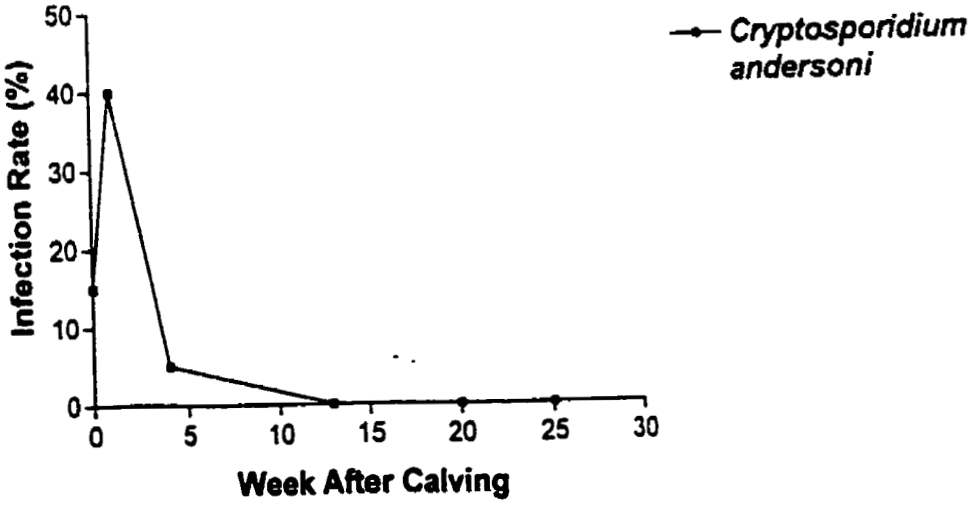
Figure 7.

Geometric mean numbers of *Cryptosporidium andersoni* oocysts identified per gram of feces for 20 beef cows (A) and the percentage of beef cows shedding *Cryptosporidium andersoni* oocysts (B) in relation to week after calving.

A


Geometric Mean Count
40
30
20
10
0
*
0
5
10
15
20
25
30
Week After Calving
Cryptosporidium andersoni

B


Infection Rate (%)
50
40
30
20
10
0
0
5
10
15
20
25
30
Week After Calving
Cryptosporidium andersoni

4. DISCUSSION

4.1 PREVALENCE OF *GIARDIA* AND *CRYPTOSPORIDIUM ANDERSONI* AND THEIR EFFECTS ON PERFORMANCE IN FEEDLOT BEEF CATTLE

Overall prevalence of *Giardia lamblia* in the 60 feedlot steers during the 257 d course of this trial was 82%. In a study by Olson et al. (1997) beef cattle older than six months of age had a reported *Giardia* prevalence of 11 %, which is considerably lower than that which was found in the present study [44]. Buret et al. (1990) observed a 10.4% prevalence of *Giardia* in adult cattle, but stated that this was likely an underestimate of the true prevalence of infection due to the intermittent shedding of cysts and only one fecal sample was collected from each animal [53]. Fecal samples were collected only once from cattle in both of these previous studies, whereas animals in this study had 12 samples collected. The 12 individual samples collected per animal over a 257 d period give a more accurate picture of actual prevalence of *Giardia* in cattle over six months of age. Stress of weaning and introducing animals to the feedlot, may have led to temporary relaxation of immunity causing a high prevalence of *Giardia* and *Cryptosporidium* during the first backgrounding stage. Xiao and Herd et al. (1994) found a periparturient rise in *Cryptosporidium parvum* oocysts and *Giardia* cyst excretion in ewes caused by relaxation of immunity [54].

Significant reductions in *Giardia* prevalence occurred from day 132 until the

conclusion of this study. Xiao (1994) and Olson et al. (1997) reported that *Giardia* infections are more prevalent among calves as compared to older cattle [25, 27]. In our study the cattle were six months of age at day 0 as compared to the younger cattle (< 20 weeks) examined by Xiao (1994). Despite this difference in age we also saw a significant reduction in the prevalence of *Giardia* as the animals matured [27]. The number of *Giardia* cysts excreted fluctuated throughout the duration of the study giving an intermittent cyst shedding pattern. During infections, up to 10^5 *Giardia* cysts were shed per gram of feces. This suggests that infected animals may continue to shed large numbers of cysts into maturity. *Giardia* infected steers had a significantly higher DMI compared to non-infected steers during the first backgrounding period d 0 - d 84 and the finishing period interval of d 85 - d 96. Steers may have increased DMI to compensate for malabsorption caused by the *Giardia* infection. Olson et al. (1995) reported a significant reduction in rate of weight gain and impairment in FE in market lambs infected with *Giardia* compared to uninfected lambs attributed to malabsorption and maldigestion of carbohydrates, fats and vitamins [24]. These reductions were not found in the present study. Further study is required to determine if the age of animals, level of infection, environmental challenge or ration type are responsible for the difference that we saw between the two studies. In a subsequent study done by Olson et al (1996) it was reported that kittens infected with *Giardia* had a higher DMI than those not infected [30]. These results are consistent with our observations during the first backgrounding period and interval d 85 - d 96 of the finishing period.

Cryptosporidium andersoni prevalence in the feedlot steers over the 257 d study was 85%. Anderson (1991) reported the prevalence of *Cryptosporidium andersoni* in cattle as high as 4.7% in the United States [55]. In a pen of feedlot cattle prevalence was 11.8% as reported in California by Anderson (1991) [55]. Since prevalence of *Cryptosporidium andersoni* in the steers used in our study was 85% over the 257 d period, this is significantly higher than the *Cryptosporidium andersoni* levels reported previously. This may be attributed to the extended period of our study, the high number of samplings, and the sensitivity of the analysis procedure utilizing a *Cryptosporidium* specific FITC monoclonal antibody. There was a significant reduction in prevalence of *Cryptosporidium andersoni* from d 97 throughout the entire study but it never reached 0%. Anderson reported that dairy cows that were sampled more than once, continued to shed *Cryptosporidium andersoni* oocysts, a result that was not confirmed in younger feedlot steers as we saw a reduction in prevalence [45]. Certain feedlot cattle may develop protective immunity and clear the infection, similar to what has been reported for *Cryptosporidium parvum* by O'Handley et al. [28].

Cryptosporidium andersoni oocyst shedding of infected steers increased significantly from d 132 throughout the duration of the study. The peak that we see on day 257 is due to only very few animals shedding high numbers of *Cryptosporidium andersoni* oocysts (up to 10^5 oocysts were shed per gram of feces).

ADG and DMI were significantly lower in non-infected steers compared to

Cryptosporidium andersoni infected steers during period 1 (84 d). Impaired performance in cattle infected with *Cryptosporidium andersoni* has been reported. Esteban et al. (1995) reported a 3.2 kg/d reduction in milk production by dairy cows shedding *Cryptosporidium andersoni* oocysts compared to non-infected cows [46]. Anderson (1990) describes cattle with *Cryptosporidium andersoni* as having hypertrophy of gastric mocosa, hyperplasia of mucus neck cells, thinning of lining epithelium and dilation of gland lumens [45]. Anderson (1990) suggests that these pathological changes may impair protein digestion [45]. Plasma pepsinogen concentrations of *Cryptosporidium andersoni* infected steers were on average twice that found for non-infected steers and weight gains of some of these infected steers were 10 - 50% below normal [45]. The maldigestion of protein may be due to its reliance on pepsin, which is derived from pepsinogen and activated at a relatively acid pH, which was raised in infected animals whereby impairing the process [45]. In the present study ADG was not reduced by *Cryptosporidium andersoni* infections.

Lymphocyte Profile

The *Cryptosporidium andersoni* infected steers in the lymphocyte portion of the study were not immunocompromised due to BVD, bovine leukosis, IBR, or BIV as they all tested negative. The significantly lower level of TCR1 in the infected group as compared to the control group when expressed as a percentage of lymphocytes is an

interesting finding. Pasquali et al, studying the lymphocyte dynamic patterns in cattle during a primary infection with *Cryptosporidium parvum* collected lymphocytes from the intraepithelial (IEL), lamina propria (LPL) and draining lymph node (LNL) in 9 day old calves during a primary *Cryptosporidium parvum* infection and from non-infected calves as a control [50]. Infected calves exhibited an increase ($P < 0.05$) in the percentage of CD_2, CD_3, CD_4 and CD_8 T cells in the IEL as compared to the control calves [50]. IL2R percentage numerically increased and IgG cells numerically decreased in the IEL of infected calves compared to controls [50]. In the LPL percentages of CD_3, CD_8 and IL2R T cells increased significantly in the infected calves as compared to the control calves [50]. No significant differences were seen in the LNL [50]. Abrahamsen also studied lymphocyte populations in calves with *Cryptosporidium parvum* and reported a significant increase in CD_4 and CD_8 T cells in the IEL, LPL and Peyer's patch of the ileum demonstrating that *Cryptosporidium parvum* stimulates a strong cell - mediated response in calves [51]. These reported increases in CD_4 and CD_8 in infected calves versus control calves are not consistent with my findings. I could find no references in the literature to lower TCR1 levels in infected animals versus controls, only that their function remains largely unknown [51]. In all of the literature that I read the studies were done on *Cryptosporidium parvum* infected calves, perhaps *Cryptosporidium andersoni* infection in mature cattle does not stimulate a strong cellular or humoral response in the host as reflected by the chronicity of the infection.

4.2 PREVALENCE AND INFECTION PATTERN OF NATURALLY ACQUIRED GIARDIASIS AND CRYPTOSPORIDIOSIS IN BEEF CALVES AND THEIR DAMS FROM BIRTH TO WEANING

Beef calves' cumulative infection rates for *Giardia* and *Cryptosporidium parvum* were 100% and 5% respectively in this study. Cumulative infection rate studies on beef calves have not been previously reported to the author's knowledge, however, a point prevalence study reported *Cryptosporidium parvum* oocyst shedding at 10.3% for beef calves < 1 month, 12.9% at 1 to 2 months, 5.9% at 2 to 3 months, 4.9% at 3 to 4 months and 0.6% over 4 months of age [56]. Overall point prevalence of *Cryptosporidium parvum* oocyst shedding for the 915 beef calves in the reported study was 5.6% [56], similar to the 5% cumulative prevalence we found in our study. Point prevalence levels for *Giardia* cyst shedding in beef calves have been reported at 31% [44]. This level is substantially lower than our 100% cumulative infection rate, possibly due to the underestimating of infection levels inherent to the utilization of a point prevalence study due to the intermittent cyst excretion characteristic of *Giardia* [28]. This study followed the calves for 198 days after birth, with 14 samplings.

Cumulative infection rates in dairy calves for *Giardia* and *Cryptosporidium parvum* previously have been reported to be 100% [28]. These results are consistent with our finding for *Giardia*, but are much higher than our *Cryptosporidium parvum* infection rates. These results suggest that the lower stocking densities of beef calves in comparison

to dairy calves may have reduced the transmission of *Cryptosporidium parvum* among calves [56]. *Giardia* transmission between calves may not have been reduced by the lower stocking density because of the continual high numbers of cysts excreted by the calves, creating a higher level of contamination, making the calves more likely to come into contact with the cysts. Perhaps in the case of *Giardia* compared to *Cryptosporidium parvum* even less stocking density is required to reduce the prevalence within the calves. Periparturient cows also excrete low levels of *Giardia* cysts which may add to the source of contamination. In contrast none of the cows excreted *Cryptosporidium parvum* oocysts in the present study. Beef calves also readily have access to colostrum that may assist in their immune defenses against *Cryptosporidium parvum*, whereas dairy calves usually have limited access to colostrum and cytotoxic anti-cryptosporidium antibodies which are also present in cow's milk [56]. It has been reported that passive lacteal immunity and passive colostral immunity were not protective against *Cryptosporidium parvum* orally inoculated neonatal mice or calves [57, 58].

Occasionally beef calves in particular herds may experience high prevalence (100%) of *Cryptosporidium parvum* resulting in death losses of 30-35% of calves (100+ head herds) (Ralston et al, unpublished data). To the authors' knowledge occurrences appear to be rare and certainly do not constitute the norm in the Alberta Cattle Industry.

In this study the mean age that *Giardia* cysts were first detected in the beef calves was 3.9 $\pm$ 1.37 weeks of age with a range of 2 to 7 weeks of age. Previous studies have

reported *Giardia* infections being most prevalent in calves 3 to 10 weeks of age, which is consistent with our findings [27].

Giardia cyst counts in calves one week of age were 0 and reached a peak at 5 weeks of age, decreasing until the conclusion of the study. Infection rate also followed the same trend peaking at 85% at 5 weeks of age, with 21% of the calves still shedding low levels of cysts at 25 - 27 weeks of age. These results differ from those reported in a previous dairy calf study where cysts excreted peaked at 2 weeks of age and infection rate peaked at 6 weeks of age (100%) [26]. The delayed intensity of cyst shedding for beef calves compared to dairy calves may be due to immune status of the dairy calves [59]. Some beef calves continued to shed cysts up until the conclusion of the study suggesting that giardiasis may be chronic in some animals, similar to what was reported in dairy calves [28]. The reported age at peak infection of dairy calves is very close to what we observed for beef calves [26].

The dams of calves involved in this study showed a small numerical periparturient rise in *Giardia* cyst shedding and geometric mean rising from 38.25 at calving to 38.49 one week post calving and then decreased to 0 at weeks 25 - 28 with the exception of week 20-23 (53.59). The percent of cows infected with *Giardia* followed a similar pattern with 10% infected at calving, peaking at 15% 1 week post calving and then decreasing to 0% infected at week 25 - 28. This increase may have been more dramatic if cyst and oocyst levels were enumerated from the cows prior to calving. However, cows were

selected from a herd based on the date they calved so pre-calving sampling was not feasible. Previously reported studies in sheep have observed a significant rise in *Giardia* cyst excretion 2 weeks prior to lambing with peaks at 0 and 4 weeks postpartum prior to returning to low levels at weeks 6 and 8 [27, 54]. Our study did show an increase 1 week post calving and then a decrease in cyst excretion. The periparturient rise is explained in the literature as the relaxation of immunity due to hormonal alterations during late pregnancy and early lactation causing the excretion of *Giardia* cysts in higher numbers [27].

The cows did experience a significant periparturient rise in the *Cryptosporidium andersoni* excreted one week post calving (37.48) as compared to the levels at calving (20.76). There was a numerically higher infection rate at 1 week post calving (40%) as compared to calving (15%). Both excretion of oocysts and percent of animals infected reached 0 at weeks 13 - 16 and maintained that level until the completion of the study. It has been previously reported that ewes increase *Cryptosporidium parvum* oocyst shedding at parturition, but the periparturient rise in *Cryptosporidium andersoni* has not been well documented prior to this study [54].

5. CONCLUSION

Based on the findings of these studies *Giardia* and *Cryptosporidium andersoni* play an important role in cattle and their impact on the environment. Calves in the cow

calf study were chronically infected with *Giardia* throughout the study to weaning. In the feedlot study steers came into the study after weaning infected with *Giardia,* levels decreased throughout the trial until the time of slaughter, but they never reached zero. These results highlight the fact that we can expect cattle herds under the age of two to be chronic shedders of *Giardia* cysts and efforts should be made to avoid the contact of their manure with water sources to reduce further infection of other hosts. However, considering that wildlife and humans may also carry *Giardia*, all potential sources of contamination would have to be addressed in order to effectively reduce the risk of water-borne infection. In the author's opinion it is not effective to lay blame on one potential source of contamination without addressing all of the potential sources in an integrated management system.

Cryptosporidium parvum had a very low prevalence in the calves in the cow calf study and was non-existent in the feedlot study highlighting the minimal contamination risk that range cattle over 2 months of age would pose to watersheds based on these studies results. However, this may not be true for all herds.

The impact of *Giardia* and *Cryptosporidium andersoni* infections on animal performance in the feedlot requires more study utilizing larger numbers of animals to determine if treatment is economical from a production aspect.

5.1 PREVALENCE OF *GIARDIA* AND *CRYPTOSPORIDIUM ANDERSONI* AND THEIR EFFECTS ON PERFORMANCE IN FEEDLOT BEEF CATTLE

This trial shows the prevalence of *Giardia* and *Cryptosporidium andersoni* decreased as the steers aged, but never reached 0%. *Giardia* cysts shed per gram of feces was intermittent throughout the trial, highlighting the importance of longitudinal studies to determine the prevalence within a population. *Cryptosporidium andersoni* oocyst shedding actually increased with time. Feedlot steers experienced no significant differences in ADG, FE or DMI whether they were infected or non-infected, with *Giardia* or *Cryptosporidium andersoni* over the duration of the study. Significant differences were noted in DMI and ADG at various stages of the trial, however it appears that the animals experienced compensatory gain so no long term performance effects were experienced. Animals coming off of range and entering the trial at weaning time were already naturally infected with *Giardia* and *Cryptosporidium andersoni.* In light of the zoonoses of these parasites and the potential for water contamination, perhaps control measures on range, especially those located on water bodies, should be investigated. The impact of *Giardia* and *Cryptosporidium* on animal performance requires more study utilizing larger numbers of animals to determine if treatment is economical from a production aspect.

5.2 PREVALENCE AND INFECTION PATTERN OF NATURALLY ACQUIRED GIARDIASIS AND CRYPTOSPORIDIOSIS IN BEEF CALVES AND THEIR DAMS FROM BIRTH TO WEANING

The prevalence and infection pattern of naturally acquired giardiasis and cryptosporidiosis in twenty beef calves and their dams from birth to weaning were determined in this study. *Giardia lamblia* cysts were shed by all twenty calves (100%) at some date during the duration of the study, however only one calf (5%) shed *Cryptosporidium parvum* on 2 sample dates during the trial. *Giardia* cysts were first detected at 3.9 $\pm$ 1.37 weeks of age with a range of 2 to 7 weeks of age. The geometric mean number of *Giardia* cysts in the calf feces increased from none at 1 week of age to a maximum of 2230 cysts/g of feces at 5 weeks of age and then decreased to 2 cysts/g at 25-27 weeks of age. Infection rate of calves shedding *Giardia* cysts followed a similar pattern peaking at 85% at 5 weeks of age and then decreasing to 21% at 25-27 weeks of age. Comparing the geometric mean from the week that the calves first shed *Giardia* cysts, the geometric mean peaked one week after initial shedding and decreased ($P<0.05$) for the remainder of the trial with the exception of week 3. There was a lower ($P<0.05$) percentage of calves shedding *Giardia* cysts weeks 3-10 and 15-25 compared to when shedding was first detected. Weeks 1, 2 and 11-13 after initial shedding were numerically lower than week 0 (100%). All calves had complete or partial transfer of passive immunity as measured by IgG_1 levels. The rate of *Giardia* infection (15%) numerically increased at 1 week post calving compared to values at calving (10%). The rate of

infection (40%) numerically increased and the geometric mean number of *Cryptosporidium andersoni* oocysts in the cow feces (37.48 oocysts/g) increased ($P<0.05$) at 1 week post calving and decreased to 0 at 13-16 weeks post calving. The results of this study document for the first time cumulative prevalence and infection patterns of *Giardia* and *Cryptosporidium* in beef cattle under ranch conditions.

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