A COMPARATIVE STUDY OF *GIARDIA* AND *CRYPTOSPORIDIUM*
INFECTIONS IN FEEDLOT CATTLE IN WESTERN AUSTRALIA AND
ALBERTA, CANADA

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This thesis is presented for the degree of Doctor of Philosophy of
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I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any other educational institution.

Signed …………………………………………………………………………..

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Refereed Journal Articles:

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Abstracts:

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Alberta Agriculture and Food
Agriculture and Agri-food Canada
Dedicated to my husband and son, Philip and Mark Norregaard and my Mother and Father, Patricia and Lloyd Ralston (deceased).
Abstract

A comparative study of the parasites *Cryptosporidium andersoni* and *Giardia duodenalis* in feedlot cattle in Western Australia (n=502) and Alberta, Canada (n=852) was conducted. The objectives were to determine the prevalence, infection patterns and impact on cattle performance of these protozoan parasites. Utilizing molecular tools *G. duodenalis* was genotyped and *C. andersoni* samples were confirmed positive.

*C. parvum* was absent from all cattle sampled in Alberta, Canada and Western Australia, likely due to the advanced age of the cattle being sampled (6-36 months of age). No *C. bovis* or *C. ryanae* were observed in the study cattle.

*C. andersoni* was present in 25% of the groups of feedlot cattle sampled in Western Australia with a prevalence range of 0-26% and in all 3 of the Alberta, Canada study groups with a prevalence range of 2.9-12%. All three Alberta, Canada studies collected performance data, however, there was no significant difference between infected and non-infected steers’ ADG in the feedlot.

*G. duodenalis* was present in 83% of the groups sampled in Western Australia with prevalence ranging from 0 – 22% and all three study groups sampled in Alberta, Canada were positive with a prevalence ranging from 39 – 82%. The prevalence of *G. duodenalis* is significantly higher in the Alberta, Canada
studies as compared to the Western Australia studies, probably due to climatic factors.

Molecular characterization of a small number of the Alberta, Canada G. duodenalis positive samples (10) revealed 30% (3) genotype A, and 70% (7) genotype E. The same characterization of the Western Australia samples (10) showed 20% (2) genotype A, 40% (4) genotype E, 10% (1) genotype B, 10% (1) genotype C, 10% (1) genotype D and 10% (1) genotype B and E. Due to the unusual finding of genotypes C and D in cattle on such a small number of samples this result should be further studied to either confirm or refute the existence of genotypes C and D in cattle. Based on these results 30% of the animals from Alberta, Canada have the potential to be zoonotic (genotypes A and B) and 40% from the Western Australia studies.

The results of this study demonstrate that C. andersoni and G. duodenalis are prevalent in the study feedlot cattle in Western Australia and Alberta, Canada however the impact of these parasites was not negative on animal performance in the Alberta, Canada studies where it was measured.
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Chapter 1 Introduction
The feedlot industries in Western Australia and Alberta, Canada are highly competitive and efficient. *Cryptosporidium* spp. and *G. duodenalis* have been reported to cause weight loss and occasionally death in animals as well as having zoonotic and environmental contamination issues (Anderson, 1987; Olson *et al*., 1995; Fayer, 1997; Olson *et al*., 2004; Ryan *et al*., 2005.).

Weight loss as a result of these parasites in cattle has never been documented on a feedlot scale involving hundreds of animals under commercial feedlot management conditions. In order to address the question whether the control of these parasites would be practical and economical on a commercial basis in the feedlot industry in either country one must first study the biology, establish the prevalence and infection patterns and then assess their impact on animal performance. The comparison between Alberta, Canada and Western Australia addresses whether the impact on performance is regionalized or continental, and also if perhaps environmental and/or management differences may play a role in the prevalence of these parasites. Molecular characterization is important to confirm microscope positive samples and genotype *G. duodenalis* to assess the zoonotic risk potential that may also be a practical reason to control these parasites in feedlots in spite of their potential effect on animal performance.

1.1 *Giardia duodenalis*

*Giardia duodenalis* – like parasites were first described by Leeuwenhoek in 1681 finding them in his own stool, however, it was more fully described
morphologically by Lambl in 1859 (Flanagan, 1992; Marshall et al., 1997). The flagellate protozoan parasite, *G. duodenalis* is not a recently occurring parasite as findings date back 2000 years to human faecal matter located in Israel and Tennessee (Flanagan, 1992). Today, *G. duodenalis* is one of the most commonly identified gastrointestinal pathogens in North America and Worldwide (Wolfe, 1992; Jucket, 1996). However, it is only since the 1970’s that *G. duodenalis* has been recognized as a pathogen, prior to then it was not considered pathogenic as many infected animals and humans were asymptomatic (Wolfe, 1992).

### 1.1.1 Taxonomic Classification

*G. duodenalis* belongs to the subphylum Sarcomastigophora, the superclass Mastigophora, the class Zoomastigophorea, the order Diplomonadida, and the family Hexamitidae (Olson and Buret, 2001). The taxonomy of *G. duodenalis* has evolved over the last century with over 50 species being described in the early 1920’s and 1930’s based on host occurrence (Thompson, 2004). It was not until 1952 that Filice proposed three species based on morphological characteristics including *G. agilis* (amphibians), *G. muris* (rodents, birds, reptiles) and *G. duodenalis* (mammalian including humans) (Meyer, 1994; Thompson, 2004). *G. duodenalis* is also sometimes referred to as *G. lamblia* or *G. intestinalis* which can be a source of taxonomic confusion (Thompson et al., 2000). Subsequently two additional species have been added, *G. ardeae* (birds) and *G. psittaci* (birds) (Olson et al., 2001; Thompson, 2004). Today,
through the use of molecular techniques genetic relationships of a range of morphologically identical “strains” have been determined leading to further classification of *G. duodenalis* into genotypes and substructures or subgenotypes allowing for the recognition of the assemblages outlined in Table 1.1 (Thompson, 2004; Ortego-Pierres *et al.*, 2009). Consequently many species and genotypes of *Giardia* are now recognized and differ principally in their host (Ortego-Pierres *et al.*, 2009). Presently genotypes are described as assemblage, however a revision of the taxonomy has been proposed (Ortego-Pierres *et al.*, 2009). Currently some species and genotypes/assemblages are specific to a particular animal species or types of hosts whereas others have a large range of hosts (Ortego-Pierres *et al.*, 2009).

However, as molecular techniques are refined new genotypes and possibly species are likely to be recognized (Thompson, 2004; Ortego-Pierres *et al.*, 2009).
Table 1.1  Genotype and host range of isolates within *G. duodenalis* morphological group (Thompson, 2004)

<table>
<thead>
<tr>
<th>Genotype/Assemblage</th>
<th>Host Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoonotic/A</td>
<td>Humans, livestock, cats, dogs, beavers,</td>
</tr>
<tr>
<td></td>
<td>guinea pig, slow loris</td>
</tr>
<tr>
<td>Zoonotic/A Subgroup AI</td>
<td>Animal and human isolates</td>
</tr>
<tr>
<td>Zoonotic/A Subgroup AII</td>
<td>Human isolates</td>
</tr>
<tr>
<td>Zoonotic/B</td>
<td>Humans, slow loris, chinchillas, dogs,</td>
</tr>
<tr>
<td></td>
<td>beavers, rats, siamang</td>
</tr>
<tr>
<td>Zoonotic/B Subgroup III</td>
<td>Humans, slow loris, chinchillas, dogs,</td>
</tr>
<tr>
<td></td>
<td>beavers, rats, siamang</td>
</tr>
<tr>
<td>Zoonotic/B Subgroup IV</td>
<td>Human</td>
</tr>
<tr>
<td>Dog/ C, D</td>
<td>Dog</td>
</tr>
<tr>
<td>Livestock/ E</td>
<td>Cattle, sheep, pigs</td>
</tr>
<tr>
<td>Cat/ F</td>
<td>Cats</td>
</tr>
<tr>
<td>Rat/ G</td>
<td>Domestic Rats</td>
</tr>
<tr>
<td>Muskrats/Vole</td>
<td>Wild rodents</td>
</tr>
</tbody>
</table>

1.1.2 Life Cycle

The life cycle of *G. duodenalis* is simple and direct, alternating between the relatively fragile motile feeding trophozoite stage and the environmentally resistant, infective cyst stage (Kirkpatrick *et al.*, 1982; Ortega and Adam,
The life cycle of *G. duodenalis* requires no intermediate host (Kirkpatrick *et al.*, 1982). Upon ingestion by the host the cyst undergoes a process called excystation, taking approximately 30 minutes, as a response to either exposure to the acidic gastric pH and the pancreatic enzymes chymotrypsin and trypsin found in the duodenum (Adam, 1991; Ortega and Adam, 1997) or bicarbonate secretion (O’Handley and Olson, 2006). Each cyst produces two trophozoites which replicate in the crypts of the duodenum and upper jejunum reproducing asexually by binary fission (Ortega and Adam, 1997). Trophozoites adhere to the intestinal epithelium using an adhesive disk on their ventral surface (Gillin *et al.*, 1996). It has been suggested that a surface membrane-associated lectin that is activated by trypsin may mediate attachment of the trophozoites to the enterocyte through phosphate residues (Jacobson and Doyle, 1996). Encystation occurs in the ileum to some of the trophozoites, possibly due to their exposure to bile salts or cholesterol starvation, creating one cyst from one trophozoite that are shed in the faeces of the host taking approximately 44 to 70 hours (Adam, 1991; Ortega and Adam, 1997).

### 1.1.3 Morphology

*G. duodenalis* cysts are either round or oval, measure 11-14 µm x 7-10 µm, have four nuclei and contain axonemes and median bodies (Ortega and Adam, 1997). Trophozoites are 10-20 µm x 5-15 µm, tear drop shaped and exhibit a ventral concave sucking disk (Ortega and Adam, 1997). They also have four
pair of flagella for movement, two axonemes and two organelles called median bodies found near the centre (the function of which is yet to be defined) (McCaffery, 1996; Ortega and Adam, 1997).

1.1.4 Transmission

*G. duodenalis* transmission can occur with the host, animal or human, ingesting as few as ten viable cysts (Ortega and Adam, 1997). This may occur via the faecal-oral route in a number of ways. Direct transmission typically occurs between individuals such as babies and young children in day care centers, schools or residential institutions when hygienic protocols are not followed (Overturf, 1994; Farthing, 1996; Juckett, 1996). Cattle may become infected through grooming and nursing.

1.1.4.1 Human Transmission

The consumption of contaminated water may lead to giardiasis (Meyer, 1985). This method of transmission is the most prevalent in humans 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 (Kent *et al.*, 1988).

Zoonotic transmission of *G. duodenalis* from food producing animals and pets also provides a less common means of infection (Ortega-Pierres *et al.*, 2009). Zoonotic transmission remains controversial, but animals have been
recognized as a potential source of infection due to the existence of zoonotic genotypes amongst the population (Ortega-Pierres et al., 2009). Zoonotic transmission can occur either from direct contact or through their contamination of water sources (Ortega-Pierres et al., 2009).

Another common human transmission method is the ingestion of food contaminated with *G. duodenalis* cysts, usually traced back to infected food handlers with poor hygienic practices or the use of contaminated water to prepare the food (Porter et al., 1990; Quick et al., 1992; Mintz et al., 1993).

1.1.4.2 Animal Transmission

Animals can become infected with *G. duodenalis* through inadvertently ingesting manure contaminated with cysts while consuming feed off the ground (Olson et al., 2004). Animals may also be infected through the consumption of contaminated water (Olson et al., 2004).

Transmission of *G. duodenalis* in animals can be accomplished through flies and other vectors and is enhanced through close confinement exposing animals to greater numbers of cysts, but also through the cyst’s resistance to environmental conditions extending their viability (Olson et al., 2000a; Graczyk et al., 2003). *G. duodenalis* cysts can remain viable for up to one week in freezing conditions (-4°C) and two weeks when subjected to temperatures of 25°C (Olson et al., 2000a). However, when cysts were placed
in water at 4°C they survived for 11 weeks, 7 weeks in soil and 1 week in cattle faeces (Olson et al., 2000a). Composting of manure containing cysts and oocysts demonstrated inactivation when temperatures reached > 55°C for a period of 15 days (Van Herk et al., 2004).

1.1.5 Distribution

*G. duodenalis* is ubiquitous, commonly occurring in developing countries where sanitation is poor and water treatment is not sufficient to remove or kill cysts (Ortega and Adam, 1997). Ortega and Adam (1997) reported that *G. duodenalis* is present in 2–5% of human faeces in industrialized countries and 20–30% in developing countries. In various parasite prevalence studies, *G. duodenalis* has been reported to range from 1–25% in domestic cats and dogs, 1-100% in beef and dairy calves and sheep, and 1-100% in wild mammals globally (Olson and Buret, 2001).

1.1.6 Pathophysiology and Pathogenesis

Asymptomatic hosts infected with *G. duodenalis* have been reported to show no abnormalities in the duodenal or jejunal mucosa (Wolfe, 1992). Symptomatic individuals, however, may display villous atrophy, crypt hyperplasia and epithelial cell damage resulting in malabsorption, maldigestion and hypermotility diarrhoea (Buret et al., 1992; Wolfe, 1992). *G. duodenalis* trophozoites have been reported to rarely invade the mucosa of the duodenum and jejunum, but normally they are considered non-invasive and
attach to the microvillous and basolateral membranes of the enterocyte (Buret et al. 1992; Wolfe, 1992; Farthing, 1993). When examined under the microscope, groupings of trophozoites have been observed causing blunting of the microvillous border of epithelial cells and altering their permeability resulting from a cytopathic effect caused by the infection (Buret et al., 1990b; Wolfe, 1992; Farthing, 1996; Olson and Buret, 2001; Buret et al., 2002). Corresponding with the shortening of the microvillous border there is a reduction in disaccharidase activity in the microvillous membrane (Farthing, 1993). Other pathological changes in the host observed with G. duodenalis can include accelerated gastric emptying, decreased intestinal transit time, increased contractility of smooth muscle, bacterial overgrowth leading to alterations of the intestinal architecture and bile salt deconjugation reducing lipolysis (Farthering, 1993; Halliday et al., 1995; Deselliers et al., 1997).

The mechanisms responsible for the pathophysiological changes associated with an infected host are not well understood but the severity of the disease in the host is dependant upon the strain virulence as well as the developmental, nutritional and immunological status of the host (Chin et al., 2002; Scott et al., 2002).

1.1.7 Host Response and Immunology

G. duodenalis infections in both humans and animals stimulate an immune response, which is important in eliminating the parasite, but it may also lead
to disease in the host (Heyworth, 1992; Ortega-Pierres et al., 2009). A multiplication of intraepithelial lymphocytes and mast cells producing *Giardia*-specific IgA, IgM and IgG in the small intestine and milk appear to contribute to the control and elimination of *G. duodenalis* in the host (Heyworth, 1992; Faubert, G., 1996; Olson and Buret, 2001). Unfortunately, exposure to *G. duodenalis* does not produce life long immunity to the parasite, but there does seem to be some protective immunity developed after infection of the host (Zajac, 1992).

Yanke et al. (1998) reported no IgM serum titre increase by *G. duodenalis* infected lambs pre- and post- infection. This result may be due to a difficulty in class switching of the immunoglobulins from IgM to IgG (Yanke et al., 1998). The failure of the challenged lambs to clear the *G. duodenalis* infection may be related to the insufficient humoral response shown by these lambs (Yanke et al., 1998). The development of antibodies against *G. duodenalis* is important in the protection of animals; but as demonstrated by Yanke et al. (1998) lambs are slow to develop high antibody titres which may also be the case with calves, explaining the reoccurring infections or failure to clear the infection.

1.1.8 **Zoonotic Potential**

*G. duodenalis* is credited as being the most common parasite to infect humans and animals worldwide (Ryan et al., 2005). The large variety of hosts for
*Giardia* increases the possible zoonotic transmission of the parasite. The recent use of molecular techniques has identified seven genotypes of *G. duodenalis* and their host range (Table 1.1) (Thompson, 2004). Only two genotypes, A and B infect humans (Thompson, 2004). Cattle are commonly infected with Assemblage E which is specific to cattle, sheep and pigs and < 10% are infected with one of the two zoonotic genotypes (Olson *et al*., 2004; Trout *et al*., 2007). In a study by Ryan *et al.* (2005) Western Australian sheep were sampled for *G. duodenalis*, and out of the 46 isolates obtained 72% were the livestock genotype, 24% were Assemblage A and 4% were an unknown genotype that closely grouped with the livestock genotype. This data suggests that the risk to public health from cattle is minimal in Western Australia if the majority of animals are infected with the livestock genotype (Caccio *et al*., 2005).

### 1.1.9 *Giardia duodenalis* and Cattle

*G. duodenalis* prevalence has been reported to vary in cattle from 1 to 100%, with it being more prevalent in young calves, typically 100% (O’Handley *et al*., 1999; Ralston *et al*., 2003; Castro-Hermida *et al*., 2006). Calves can become infected as young as 4 days of age, but more typically at 5-10 weeks of age, with infections persisting for 8 weeks or more (Xiao, 1994; Olson *et al*., 1995; O’Handley *et al*., 1999). *G. duodenalis* prevalence in older cattle is much lower, Ralston *et al.* (2002) reported a prevalence of 82% in beef feedlot cattle whereas a prevalence of only 15% was observed in mature beef.
cows with a periparturient rise in both cysts shed and prevalence of the parasite (Ralston et al., 2003). Cyst excretion is intermittent and infection in cattle may cause diarrhoea, but animals are more commonly asymptomatic (Xiao, 1994; O’Handley et al., 1999). Weight loss may be a result of giardiasis in calves (Olson and Buret, 2001), but often there is no measurable effect on feedlot animal performance (Ralston et al., 2002). G. duodenalis infections in cattle can be clinically important and may have an economic impact on animal performance as shown in a sheep model (Olson et al., 2004). Performance studies are difficult in ruminants as essentially all animals become infected and reinfected making it difficult or impossible to prevent animal infections in a contaminated environment (O’Handley and Olson, 2006).

1.1.10 Prevention and Treatment

The prevention of G. duodenalis infections in cattle is a difficult task since the cysts are ubiquitous, resist environmental decomposition and remain viable for a considerable period of time (Olson et al., 2000a). However, prevention can be enhanced by limiting the contamination of the environment (water, soil) with the cysts from infected hosts (Olson and Buret, 2001; Geurden et al., 2006a; Uehlinger et al., 2006). In confined areas, disinfection and cleaning of surfaces should occur utilizing products such as organic iodine, tincture of iodine, chlorine or bleach (hypochlorite) to reduce contact with viable cysts by potential hosts (Olson and Buret, 2001). Composting of animal
manure has been reported as an effective way of inactivating cysts when reaching internal temperatures of $55^\circ C$ for a period of 15 days (Van Herk et al., 2004). Management techniques in cattle (dairy) operations including the use of straw as bedding, litter depth, floor type, use of a maternity pen, herd size, breed and organic techniques had no effect on the risk of animals becoming infected with *G. duodenalis* (Maddox-Hytte et al., 2006).

However, the crowding of animals, periods of depopulation of animal areas and the age of animals did impact the risk of infection (Maddox-Hytte et al., 2006). Gow and Waldner (2006) reported that the age of the dam also impacts the chances of her calf becoming infected with *G. duodenalis*. Dams that were 2 years old (first calf heifers) were 2.3 times more likely to have calves shedding cysts as compared to calves of dams 4-10 years of age. Also calves 9-18 days of age and calves >18 days of age were 22.4 and 150 times (respectively) more likely to shed cysts as compared to calves $\leq 4$ days of age (Gow and Waldner, 2006). Jager et al. (2005) reported that calves receiving sufficient amounts of colostrum may acquire passive immunization, reducing the risk of *G. duodenalis* infection.

Prevention of *G. duodenalis* can be enhanced in cats and dogs by the use of a vaccine (GiardiaVax™) (Olson et al., 1996; 2000b). However such a vaccine is not currently available for use in food producing animals (Thompson, 2004). Uehlinger et al. (2007) reported that GiardiaVax™ was not efficacious against *G. duodenalis* in calves. These results may be due to the antigenic
differences between genetic assemblages or perhaps the calves mounted a
serological immune response after vaccination, but the vaccination may not
have stimulated immunity locally within the gut (Uehlinger et al., 2007). A
localized immune response is important in the elimination of infection
(Uehlinger et al., 2007).

Treatment of *G. duodenalis* may be necessary depending on the
circumstances. An infection can produce humoral immunity, self-limiting the
disease, but it can take >100 days for the host to produce the protective
antibodies (depending on the species) (Olson et al., 2004). There are a number
of effective chemotherapeutic agents for *G. duodenalis*, including
nitroimidazoles and benzimidazoles classifications (Thompson, 2004). In
cattle (dairy and beef) benzimidazoles (fenbendazole, albendazole) have
shown efficacy against *G. duodenalis*, however, no drug is currently
registered for treating giardiasis in ruminants (O’Handley et al., 1997;
Garossino et al., 2001; O’Handley et al., 2001; Geurden et al., 2006b;
O’Handley and Olson, 2006). It has also been reported that treatment with
fenbendazole was able to improve the mucosal microvillous structure and
function within seven days of the initiation of treatment (O’Handley et al.,
2001). Recently, Geurden et al. (2006b) has reported the effective use of
paromomycin, a broad-spectrum amino-glycoside antibiotic, which resulted in
a significant reduction of cyst excretion and a trend towards higher weight
gain and less diarrhoea in 3-5 week old dairy calves. This approach may
create concern for possible antibiotic resistance as some aminoglycosides, such as erythromycin, are important for use in human medicine.

Chemotherapeutic agents are very effective in eliminating *G. duodenalis* infections, however, re-infection of the host often occurs if the sources of environmental contamination are not eliminated which with cattle production is essentially impossible (O’Handley *et al.*, 1997; Olson *et al.*, 2004; Thompson, 2004).

### 1.2 Cryptosporidium

#### 1.2.1 Taxonomic Classification

*Cryptosporidium* spp. belongs to the Kingdom Protozoa, Phylum Apicomplexa, Class Coccidea, Order Eucoccidiorida and Family Cryptosporidiidae (Schmidt and Roberts, 1996; Fayer, 1997). This classification is a current point of debate as there is growing evidence that *Cryptosporidium* spp. is more closely related to gregarine protozoa as opposed to coccidians and therefore should be classified under this class (Barta and Thompson, 2006). There have been 19 species named but 17 are currently recognized on the basis of morphological differences, host, site of infection and genetic differences (Table 1.2) (Schmidt and Roberts, 1996; Ong *et al.*, 2002; Olson *et al.*, 2004; Fayer *et al.*, 2005). Recently, with the advancement of molecular tools, there has been three species and one genotype of *Cryptosporidium* reported that infect cattle as their primary host; *C. parvum*,
*C. andersoni*, *C. bovis* and the *Cryptosporidium* deer-like genotype (Olson *et al.*, 2004; Slapeta, 2006; Fayer *et al.*, 2005, Fayer *et al.*, 2008; Feltus *et al.*, 2008). *Cryptosporidium* deer-like genotype has now been named *C. ryanae* (Fayer *et al.*, 2008). *Cryptosporidium* appears to be species specific and it is most likely that each animal species carries its own specific genotype (Tzipori and Widmer, 2008). However, *C. parvum* is unusual as it appears to consistently infect a number of animal species (Table 1.2). Undoubtedly, refinement of the taxonomy of *Cryptosporidium* spp. will continue to occur and zoonotic genotypes will be identified. In this thesis I will only discuss those species of *Cryptosporidium* spp. that are relevant to cattle production.
Table 1.2 *Cryptosporidium* spp. and their hosts (Ong et al., 2002; Olson et al., 2004; Caccio et al., 2005; Smith et al., 2007; Fayer et al., 2005; Elwin and Chalmers, 2008; Fayer et al., 2008; Ortega-Pierres et al., 2009)

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. galli</em></td>
<td>Finches, chickens</td>
</tr>
<tr>
<td><em>C. canis</em></td>
<td>Dogs, Humans</td>
</tr>
<tr>
<td><em>C. hominis</em></td>
<td>Humans, monkeys</td>
</tr>
<tr>
<td><em>C. molnari</em></td>
<td>Marine fish</td>
</tr>
<tr>
<td><em>C. felis</em></td>
<td>Cats, Humans</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
<td>Cattle, Humans and other mammals</td>
</tr>
<tr>
<td><em>C. muris</em></td>
<td>Rodents, Humans</td>
</tr>
<tr>
<td><em>C. wrairi</em></td>
<td>Guinea pig</td>
</tr>
<tr>
<td><em>C. andersoni</em></td>
<td>Cattle, Humans and other livestock</td>
</tr>
<tr>
<td><em>C. meleagridis</em></td>
<td>Turkeys and humans</td>
</tr>
<tr>
<td><em>C. baileyi</em></td>
<td>Poultry</td>
</tr>
<tr>
<td><em>C. serpentis</em></td>
<td>Reptiles</td>
</tr>
<tr>
<td><em>C. saurophilum</em></td>
<td>Lizards</td>
</tr>
<tr>
<td><em>C. suis</em></td>
<td>Pigs, Humans</td>
</tr>
<tr>
<td><em>C. bovis</em></td>
<td>Cattle, Sheep</td>
</tr>
<tr>
<td><em>C. scophthalmi</em></td>
<td>Fish</td>
</tr>
<tr>
<td><em>C. ryanae</em></td>
<td>Cattle, Sheep</td>
</tr>
</tbody>
</table>
1.2.2 Life Cycle

1.2.2.1 *C. andersoni*

Upon ingestion by cattle, *C. andersoni* oocysts are exposed to stomach acid, followed by bile salts and digestive enzymes in the proximal duodenum causing the oocysts to excyst, releasing sporozoites (Chen and LaRusso, 2000). Sporozoites then infect the superficial cells of the mucosa in the abomasum (Olson et al., 2004). Sporozoites differentiate into trophozoites that initiate asexual reproduction (schizogony) producing schizonts that produce numerous merozoites (Fayer, 1997). The merozoites exit the schizont to infect other cells and develop into a Type I or Type II schizont producing four merozoites (Fayer, 1997). Type I schizonts contain six to eight nuclei which become incorporated into six to eight merozoites when the schizont is mature (Fayer and Ungar, 1986). The merozoite may invade a new host cell where it develops into another Type I or Type II schizont (Fayer and Ungar, 1986). It is thought that only the Type II schizont merozoites undergo sexual reproduction (gametogony) producing either a microgamont or macrogamont (Fayer, 1997). The macrogamonts may fuse and if fertilized develop into oocysts which sporulate and leave the host in the faeces or occasionally in respiratory excretions (Fayer, 1997). The entire lifecycle is completed within 72 hours post-infection (Hijjawi et al., 2002).
1.2.2.2 *C. parvum*

The life cycle of *C. parvum* is identical to *C. andersoni* with the exception that the sporozoites infect the cells of the mucosa in the intestine and not the abomasum (Olson *et al*., 2004). *C. ryanae* and *C. bovis* are closely related to *C. parvum* making it likely that they both infect the mucosa in the intestine.

1.2.2.3 *C. bovis* and *C. ryanae*

The life cycles of *C. bovis* and *C. ryanae* are identical to that described above for *C. andersoni* with the exception that the sporozoites infect the cells of the mucosa in the intestine and not the abomasums (Fayer *et al*., 2005; 2008).

1.2.3 Morphology

1.2.3.1 *C. andersoni*

*C. andersoni* oocysts are ellipsoidal in shape and measure approximately 7.4 x 5.5 μm with a range of 6.0–8.1 by 5.0-6.5 μm (Fayer, 1997; Lindsay *et al*., 2000). The colorless oocyst wall is <1 μm thick, lacks a micropyle and has a suture at one end (Lindsay *et al*., 2000).
Figure 1.1 *C. andersoni* oocysts in the abomasum of a feedlot steer

1.2.3.2 *C. parvum*

*C. parvum* is ellipsoidal in shape, similar to *C. andersoni*, but is considerably smaller measuring 5.0 x 4.5 µm (Fayer, 1997).

1.2.3.3 *C. bovis*

*C. bovis* oocysts are morphologically indistinguishable from *C. parvum*, oocysts are shed in faeces fully sporulated, contain four sporozoites, but lack sporocysts (Fayer *et al.*, 2005). Oocysts measure 4.76-5.35 µm by 4.17-4.76 µm with a mean size of 4.89 x 4.63 µm (Fayer *et al.*, 2005).
1.2.3.4 *C. ryanae*

*C. ryanae* oocysts are 2.92 – 4.41 µm x 2.94 – 3.68 µm with a mean size of 3.73 x 3.16 µm (Fayer *et al.*, 2008). Fayer *et al* (2008) reported one or two sporozoites per oocyst were visible in different focal planes but the total number in each oocyst could not be clearly visualized.

1.2.4 Transmission

*Cryptosporidium* spp. infections in animals and humans occur in a similar manner to *G. duodenalis* infections, via the faecal-oral route with the host ingesting as few as 10 viable oocysts (Fayer, 1997). *C. parvum* transmission can occur through zoonotic, environmental or non-zoonotic routes (Fayer, 1997).

1.2.4.1 Human Transmission

The zoonotic route of transmission can include peoples’ interaction with infected animals, such as occupational exposure in the case of veterinarians, livestock producers or pet owners, resulting in the ingestion of viable oocysts (Fayer, 1997). Occupational exposure is speculated to stimulate a high level of immunity in the host due to repeat infections and may also be responsible for indirect zoonotic transmission when oocyst infected material is carried home secondarily infecting those at home (Fayer, 1997).
Environmental sources of transmission in recent years have received a lot of attention due to large water-borne outbreaks (Gajadhar and Allen, 2004). The current system of large volumes of water processed in modern treatment plants and the distribution systems increase the risk of large-scale water-borne disease outbreaks (Gajadhar and Allen, 2004). Accidental contamination of water supplies with human sewage, due to system failure or human error, is responsible for the outbreaks in Milwaukee, U.S.A. and North Battleford, Canada (Gajadhar and Allen, 2004). Effluents from farms are often blamed for these outbreaks, but it has been shown that they are seldom the source of infection (Gajadhar and Allen, 2004). Surface water may also become contaminated when running water containing faeces enters the water course or when faeces are deposited directly by grazing animals or the emptying of sewage lagoons (Wallis et al., 1996; Fayer, 1997; Graczyk et al., 2000).

Foodborne transmission is another environmental source of infection that has been linked to the consumption of raw milk, frozen tripe and sausage in the U.K., apple cider, chicken salad, fruit and vegetables in the U.S.A. (Fayer, 1997; Millar et al., 2002; Gajadhar and Allen, 2004). However, a recent study by Moriarty et al. (2005) found no contamination of 288 beef carcasses in a slaughter facility, despite 21 animals testing positive by faecal analysis post slaughter for *C. andersoni* (54.5%) and *C. parvum* (45.5%). Generally, contamination occurs when a food product comes into contact with viable
oocysts and is not further processed in a manner to kill the oocysts prior to consumption (Fayer, 1997).

The last route of transmission causing cryptosporidiosis is non-zoonotic transmission caused by person-to-person contact such as seen in daycare centres, senior homes, recreational water including swimming pools, splash pads and water parks and hospitals (Fayer, 1997; Fayer, 2004; Smith et al., 2006).

Transmission of Cryptosporidium spp. is enhanced by a variety of characteristics inherent to the parasite including: the large number of oocysts excreted by infected hosts (up to $10^{11}$ oocysts per gram of faeces); the low infective dose required to cause disease; multi-host specificity for some species of Cryptosporidium spp.; the persistence of oocysts in the environment; the small size of the oocysts, aiding their ability to pass through sand filters (Uga et al., 2000; Smith et al., 2006).

**1.2.4.2 Animal Transmission**

Transmission of Cryptosporidium spp. to cattle can be facilitated by a number of routes, including: wildlife and game ranched animals; the consumption of contaminated feed or water or mutual grooming (Olson et al., 2004).
1.2.5 Distribution

*C. parvum* in young calves and humans has been reported to be worldwide in distribution (Fayer, 1997). 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, disease) (Fayer, 1997). Typically children aged 1 to 5 years and immunosuppressed individuals are most susceptible to *C. parvum*, however *Cryptosporidium* spp. has been reported in individuals 3 days of age to 95 years old (Fayer, 1997, Fayer, 2004).

*C. andersoni* has been reported in older cattle in Canada, the U.S.A., Mexico, Brazil and Europe (Anderson, 1987; Fayer, 1997; Olson *et al.*, 1997a; Pena *et al.*, 1997; Maddox-Hyttel *et al.*, 2006). Until now *C. andersoni* had not been documented in Australian cattle (Ralston, 2007, unpublished).

*C. bovis* has been reported in pre- and post-weaned dairy calves in India, Zambia, China and the United States of America (Geurden *et al.*, 2006c; Feng *et al.*, 2007). *C. bovis* has also been reported in dairy cows in Georgia, U.S.A. (Feng *et al.*, 2007) and in mature sheep in Western Australia (Ryan *et al.*, 2005). A *C. bovis*- like genotype was reported in sheep in Maryland, U.S.A. (Satin *et al.*, 2007).
The Cryptosporidium cervine genotype was reported in lambs and ewes in Maryland, U.S.A. (Santin et al., 2007; Fayer et al., 2008) and Western Australia (Ryan et al., 2005). The deer-like genotype now referred to as C. ryanae in cattle has been reported world wide (Fayer et al., 2008; Feltus et al., 2008).

1.2.6 Pathophysiology and Pathogenesis

*C. parvum* infections in the small intestine usually result in diarrhoea which is associated with villous atrophy, shortening of microvilli and sloughing off of enterocytes (Clark and Sears, 1996; Fayer, 1997). It has also been proposed that *C. parvum* may release a toxin that also may cause profuse cholera-like watery diarrhoea (Clark and Sears, 1996). However, it is not clear if it is the toxin or some host immune response responsible for this pathogenesis (Clark and Sears, 1996). Asymptomatic infections have also been reported (Fayer, 2004).

*C. andersoni* in cattle invades the peptic and pyloric glands causing dilation of the glands, hypertrophy of the gastric mucosa and thinning of the epithelial lining (Olson et al., 2004; Masuno et al., 2006). These changes cause the impairment of protein digestion by increasing gastric pH and inhibition of proteolytic function (Olson et al., 2004).

Cattle infected with *C. bovis* are typically asymptomatic (Fayer et al., 2005).
1.2.7 Host Response and Immunology

1.2.7.1 *C. parvum*

Host response to a *C. parvum* infection in humans and animals can vary dramatically from the host being asymptomatic to causing serious illness or even death in immunocompromised individuals (Fayer, 2004). During infection, CD4 T cell numbers or functionality decreases and the disease worsens (Fayer, 2004). In severe cases life cycle stages of the parasite have been observed in the respiratory tract, liver, gall bladder, pancreas and other extraintestinal sites (Fayer, 2004). The most prevalent symptom of a *C. parvum* infection is voluminous, watery diarrhoea (Fayer, 2004).

1.2.7.2 *C. andersoni*

*C. andersoni* colonizes the abomasum of cattle and may persist for years (Olson *et al*., 2004). It has been reported to impair weight gain and decrease feed efficiency in feedlot cattle (Anderson, 1987; Ralston *et al*., 2003). Dairy cattle infected with the parasite have been reported to have a 3.2 Kg per day reduction in milk production (Estaban and Anderson, 1995). Recently a novel isolate of *C. andersoni* has been reported to infect immunocompetent and severe combined immunodeficient mice, Bactrian camel (*Camelus bactrianus*), Bobak marmot (*Marmota bobac*), European wisent (*Bison bonasus*), Mongolian gerbils (*Meriones unguiculatus*) and the southern
multimammate mouse (*Mastomys coucha*) (Kvac *et al.*, 2007), however these were not genotyped.

### 1.2.8 Zoonotic Potential

#### 1.2.8.1 *C. parvum*

*C. parvum* has been identified in 80 species of mammals and cross-transmission between species has been documented (Fayer, 1997). Environmental contamination is a concern with *C. parvum* oocysts due to the wide range of potential hosts that have access to watersheds that feed potable water sources for humans (Fayer, 1997). Recent molecular techniques have enhanced the ability to discriminate between different *Cryptosporidium* species and/or genotypes and it has been reported that cattle have not been conclusively identified as a source of any waterborne outbreaks within the U.S.A. or Canada with the exception of Cranbrook, British Columbia where bovine oocysts were identified (Olson *et al.*, 2004).

#### 1.2.8.2 *C. andersoni*

*C. andersoni* has been believed not to infect immunocompetent or HIV-infected humans (Olson *et al.*, 2004), however, there have been sporadic reports in the literature of *C. andersoni* infecting humans (Guyot *et al.*, 2001; Leoni *et al.*, 2006).
1.2.8.3 *C. bovis*

*C. bovis* has been reported not to infect humans (Fayer *et al*., 2005).

1.2.8.4 *C. ryanae*

*C. ryanae* is considered as cattle and sheep adapted and has not been reported in humans (Elwin and Chalmers, 2008; Fayer *et al*., 2008; Feltus *et al*., 2008).

1.2.9 *Cryptosporidium spp. and Cattle*

Calves generally begin shedding *C. parvum* oocysts between one and four weeks of age, however infection has been reported in calves as young as four days, but is rare in calves over one month of age (Xiao and Herd, 1994; Fayer, 1997; Huetink *et al*., 2001; Castro-Hermida *et al*., 2002a; Olson *et al*., 2004). The duration of infection is short, usually lasting for two weeks (Olson *et al*., 2004). Shedding of oocysts ranges between $10^5$ to $10^7$ per gram of faeces (Fayer, 1997). Prevalence of *C. parvum* in beef calves has been reported at 20% with dairy calves reported as high as 100% (Olson *et al*., 1997b; O’Handley *et al*., 1999).

*C. andersoni* infects older cattle and has been reported to decrease weight gains (10-50%), and milk production (3.2 kg/d) (Anderson, 1990, Esteban and Anderson, 1995). Overall prevalence in dairy cattle in California has been reported at 1.74% of 8539 samples and 85% in an Alberta beef feedlot
highlighting the variability among various classes of cattle (Anderson, 1990; Ralston et al., 2002).

*C. bovis* and *C. ryanae* have been reported to infect pre- and post-weaned calves and adult cattle with no sign of disease (Fayer et al., 2005; Geurden et al., 2006c; Feng et al., 2007; Fayer et al., 2008).

### 1.2.10 Prevention and Treatment

An oral vaccine for calves at birth was tested against experimental *C. parvum* challenge with positive results, however when field-tested the vaccine failed (Harp and Goff, 1998). A recombinant protein based vaccine for *C. parvum* was administered to cows prior to calving; it eliminated diarrhoea and reduced oocyst shedding in the calves as compared to a control group (Perryman et al., 1999).

Many chemotherapeutic products have been tested for efficacy against *Cryptosporidium* spp. but most are ineffective however, drugs such as paromomycin and decoquinate decrease oocyst shedding and reduce the frequency and severity of diarrhoea in lambs and calves (Fayer, 1997; Quilez et al., 2000; Olson et al., 2004). Halfuginone lactate (Halocur® Intervet) has recently been registered in Europe as a chemotherapeutic treatment for cryptosporidiosis in cattle, reducing the incidence and severity of diarrhoea,
but it does not prevent oocyst shedding (Olson et al., 2004). No products have been reported to be tested against *C. andersoni* (Olson et al., 2004).

Prevention of *C. parvum* infections includes disinfection, hygiene and adequate nutrition as a means of reducing or preventing animal to animal transmission (Fayer, 1997; Huetink *et al*., 2001). These measures can include strategies such as isolation of infected calves; disinfection of contaminated areas; a control programme for host rodents and provision of colostrums and nutritional supplements to calves (Fayer, 1997). Castro-Hermida *et al*. (2002b) reported that calves housed on cement floors had a 66% less risk of *C. parvum* infection than those housed on straw/earth floors due to the type of cleaning carried out, pressure washing versus sweeping. In light of the limited success with experimental vaccines and drug treatments, prevention is still the best medicine.

### 1.3 Western Australia and Alberta, Canada Feedlot Industries

Feedlots in Western Australia and Alberta, Canada are highly competitive and efficiently managed in order to supply quality product and compete in the global market place. Producers in both countries are supplying product of similar specifications into some of the same markets. The feedlot industries in both countries are an integral part of the economic and cultural fabric.
1.3.1 Western Australian Feedlots

1.3.1.1 General Industry

The feedlot industry in Western Australia is growing with the numbers of cattle on feed at the second highest level ever recorded, however, compared to other states in Australia the Western Australia feedlotting sector is relatively small (Burggraaf, 2004; Australian Lot Feeders Association (ALFA)/Meat and Livestock Australia (MLA), 2007). As of March 2007 Western Australia had a total one time feedlot capacity of 91,366 head with 68,932 head on feed at that time, the majority of which came from feedlots with a capacity of 1000 – 10,000 head (ALFA/MLA Feedlot Survey, Jan – March, 2007). Australia exports 66.2% of national beef production, with the balance being consumed domestically, making the industry very sensitive to global markets and requiring attention to cost of production to remain efficient and globally competitive (ALFA/MLA Feedlot Survey, Jan – March, 2007).

Only a small portion of Western Australian cattle are finished in feedlots. During the period December 2000 to June 2003 only 15% of the total cattle marketed had been lot fed, the balance had been finished on pasture (Burggraaf, 2004). The majority of cattle that enter a feedlot, have been weaned and backgrounded on pasture prior to arrival (Burggraaf, 2004). Backgrounded refers to weaned animals adjusted to pasture or feedlot conditions on a low ADG targeted for slow growth.
1.3.1.2 Feedlot Management

Generally cattle entering a Western Australian feedlot are transported from either their place of birth or the location where they were backgrounded (if different), placed in a new environment consisting of pens containing approximately 200 – 250 head of animals and usually mixed with unfamiliar animals from various locations (Burggraaf, 2004). This process may lead to cattle being exposed to potentially infective pathogens to which the animals may not have been previously exposed (Burggraaf, 2004). Upon entry into the feedlot animals are treated according to that feedlot’s particular herd health protocol which in Western Australia could include products protecting against *Clostridium botulinum* type C and D, *C. perfringens* type D, *C. tetani*, *C. novyi* type B, *C. septicum*, *C. chauvoei* and some endo and ecto parasites (ivermectin and monensin) but not *Cryptosporidium* spp. or *G. duodenalis*. Since animals arriving in the feedlot usually have been backgrounded on pasture previously, they tend to be 9 – 18 months of age at induction. Upon arrival at the feedlot animals must undergo a nutritional adjustment period to accustom them from their previous pasture-based diet to a total mixed ration consisting of feedstuffs such as grain (wheat, lupins, oats, barley) (up to 85% of the ration) and forage (ryegrass, clover, alfalfa, cereal straw, barley silage) (constituting the balance of the ration). Animal performance is monitored over the feeding period until the animals are ready for slaughter, which can vary from 2 to 24 months depending on the weight of the animal at the start of the feeding period, performance of the animal during the period and specifications
of the end market the animals are destined to fill. Australian cattle involved in the studies discussed in this thesis were all *Bos taurus*.

1.3.2 Alberta, Canada Feedlots

1.3.2.1 General Industry

The beef industry in Alberta is the largest single commodity source of farm cash receipts and contributes significantly to the Provincial and National economies (www.cattlefeeders.ca). Alberta’s feedlots have the capacity to finish more than 2.5 million head of cattle annually with 800,147 head on feed as of August 2007 (www.cattlefeeders.ca, www.canfax.ca). The feeding industry is comprised of 196 feedlots with a capacity of over 1000 head, 59% are 1000-5000 head, 23% are 5001-10,000 head, 7% are 10,001-15,000 head, 5% are 15,001-20,000 head and 6% are over 20,000 head (www.cattlefeeders.ca). Alberta is a major exporter of beef so global competitiveness is extremely important to the industry. The majority of cattle are finished in feedlots and very few are backgrounded on either grass or grain.

1.3.2.2 Feedlot Management

A typical slaughter animal cycle includes birth during Jan. to May, weaning Sept. – Dec. with the animals being transported, co-mingled and placed in feedlot pens of 200 – 300 head at approximately 6 months of age. Upon
induction into the feedlot cattle are treated according to that particular
feedlot’s herd health protocol which in our Alberta studies could include
products protecting against *Clostridium chauvoei, Cl. Septicum, Cl. Novyi, Cl.
Perfringens* type C and D, *Cl. Haemolyticum, Cl. Tetani*, Infectious Bovine
rhinotracheitis (IBR), Parainfluenza type 3 (PI3), Bovine viral diarrhoea
(BVD), Bovine respiratory syncytial virus (BRSV), *Pasteurella haemolytica*
and *P. somnus* and some endo and ecto parasites (ivermectin and monensin)
but not *Cryptosporidium* or *Giardia*. All animals receive an implant to
promote growth (e.g. trenbolone acetate, progesterone/estradiol,
testosterone/estradiol). Once inducted animals are placed on an induction
ration, usually consisting of 85% forage and the balance grain, to allow the
freshly weaned calves to adjust from their previous forage based diets to the
grain based diets they will receive in the feedlot. Finishing rations consist of
85 – 95% concentrate (barley, wheat, canola meal) and 5 – 15% roughage
(alfalfa, barley/pea silage, cereal straw). Animals’ performance is measured
over the feeding period until finish which can vary from 4 to 8 months,
typically depending on the weight of the animals at the start of the feeding
period, animal performance and end market. All cattle were *Bos taurus* in
origin.
1.3.3 Comparison of Factors in Western Australian and Alberta, Canada Feedlot Systems That May Impact *G. duodenalis* and *Cryptosporidium* spp. Infections

A comparison of parasite infections in Western Australia and Alberta, Canada feedlots will not only help us to better understand how these parasites might affect animal productivity but will also allow us to examine the differences and similarities between the two systems and perhaps what impacts, if any, they may have on infections.

Eighty-five percent of cattle in Western Australia are pasture finished whereas essentially all young cattle are feedlot finished in Alberta, Canada (Burggraaf, 2004). Since infection levels with the parasites are dependant on (oo)cyst availability and concentration in the environment, the intensive management of feedlot cattle in Alberta may lead to higher levels of infection (Olson *et al.*, 2000a).

Typically animals arriving into Western Australian feedlots are 9-18 months of age, have been previously weaned and grazed on pastures. Animals arriving in Alberta feedlots have just been weaned from their dams and are usually 6 months of age. The young age of these animals combined with the recent weaning process creates a great deal of stress which increases the prevalence of both *G. duodenalis* and *Cryptosporidium* spp. infections (Ralston *et al.*, 2002). The prevalence under both systems was compared.
Prior to the arrival at the feedlot, cattle from Western Australia and Alberta, Canada have usually travelled similar distances by truck, receive very comparable herd health protocols, are placed in pens with similar animal densities and receive very comparable rations.

Parasite prevalence in animals is not only associated with availability and concentration, but also with (oo)cysts’ viability which is enhanced under cool, moist conditions as reported by Olson et al. (2000a). Olson et al. (2000a) reported viable cysts at 4 °C for 11 weeks in water, 7 weeks in soil and 1 week in faeces as compared to 2 weeks in all three media at 25 °C. Cryptosporidium spp. remained viable for greater than 12 weeks at -4 and 4 °C (Olson et al., 2000a).

Flies have been reported to be responsible for the potential transmission of both G. duodenalis and Cryptosporidium spp. (Graczyk et al., 1999; Graczyk et al., 2003; Szostakowska et al., 2004). Fly populations at the Western Australia feedlots were high, with flies covering the cattle’s backs whereas those in the Alberta feedlot were non-existent due to the winter/spring feeding period.
1.4 Current Knowledge

Currently, prevalence and impact on animal performance of *G. duodenalis, C. parvum* and *C. andersoni* in beef feedlot cattle has not been studied in Australia. Prevalence, infection patterns and impact on animal performance of these parasites in beef feedlot cattle in Canada has been explored, but only briefly in one study with 60 head of steers by Ralston et al., 2002. There have been several reports (Anderson, 1990; Olson and Buret, 2001) of weight loss in cattle infected with *Cryptosporidium* spp. and *G. duodenalis* but the impact of these parasites on feedlot cattle has not been reported.

1.5 Project Objectives

The objectives of this study were to collect information on prevalence, infection pattern and infection intensity in feedlot beef cattle in Alberta, Canada and Western Australia to better understand how these parasites might affect animal productivity, and to compare the similarities and differences of the epidemiology of the parasites between the two countries. This may help to identify geographical, nutritional and management factors that may be involved in the biology of these parasites.
Hypothesis 1 - *C. parvum, C. andersoni* and *G. duodenalis* exist in beef cattle in Western Australia and Alberta, Canada.

**Objectives:**

1. To determine the prevalence and infection patterns of *C. parvum, C. andersoni* and *G. duodenalis* in feedlot cattle in Western Australia and Alberta, Canada.

2. To determine and compare the genotypes of *G. duodenalis* and *Cryptosporidium* spp. utilizing molecular tools to assess the differences between Western Australia and Alberta, Canada and the public health significance of the occurrence of these parasites in feedlot cattle.

3. To relate the differences and similarities in feedlot conditions (nutrition, management) and to epidemiological factors between Western Australia and Alberta, Canada.
Hypothesis 2 - *C. parvum, C. andersoni* and *G. duodenalis* infections will have a negative affect on the performance of feedlot cattle.

**Objectives:**
1. To determine the prevalence and infection pattern of *C. parvum, C. andersoni* and *G. duodenalis* in feedlot cattle from weaning to slaughter in Alberta, Canada.
2. To determine the effect of the presence of an infection with *C. parvum, C. andersoni* and *G. duodenalis* infections on feedlot cattle average daily gain.
Chapter 2 General Materials and Methods
2.1 Sample Collection

Faecal samples (1-5g) were collected from cattle, either rectally or off the ground, after an animal was observed defecating. Larger faecal samples may have enhanced the detection of (oo)cysts but the 1-5g amount was chosen as a published methodology that was practical when collecting 7000+ samples from feedlot cattle in a commercial handling facility. A disposable latex glove was used to collect each sample in order to avoid cross contamination. Samples were placed in a pre-weighed 10 mL (Sarstedt, South Australia, Australia) or 15 mL centrifuge tube (VWR, Mississauga, Ontario, Canada) and stored at 4 °C until processing. Samples were typically processed 1 – 2 weeks after collection.

2.2 (Oo)cyst Purification and Enumeration

Faecal analysis was performed on samples arriving at the laboratory according to previously described procedures (Olson et al., 1997a; O'Handley et al., 1999). Samples were weighed to calculate faecal weight and then filtered through a 7.5 cm x 7.5 cm surgical gauze sponge (Nu-Gauze, Johnson & Johnson, Montreal, Quebec, Canada) and washed with phosphate buffered saline solution (PBSS: 0.9% NaCl, pH 7.2) and 5 – 7 mL of filtrate was expressed. The filtrate was then layered over 5 mL of 1M sucrose (specific gravity 1.13) and centrifuged at 800x g for 5 minutes in a fixed rotor centrifuge to concentrate (oo)cysts at the sucrose layer surface. The upper filtrate and sucrose surface were pipetted into a clean tube and centrifuged
again at 800x g for 5 minutes. The resulting supernatant was decanted and the pellet suspended in PBSS to 1 mL and placed in a 2 mL micro centrifuge tube (VWR Scientific Inc., Media, PA, USA) or 1.5 micro centrifuge tube (Quantum Scientific, Balcatta, Western Australia, Australia). The sucrose gradient flotation method does result in the minor loss of (oo)cysts, however the amount of debris that must be removed from cattle feces and the concentration of the (oo)cysts into a pellet for more sensitive detection has resulted in this published methodology being accepted within the scientific community.

The suspended pellet was then applied in two 0.015 mL spots on a fluorescence microscope slide (Erie Scientific Co., Portsmouth, NH, USA) and air-dried for 30 minutes. A *Giardia* – specific fluorescein isothiocyanate (FITC) – labelled monoclonal antibody solution (0.02 mL) (Giardi-a-glo, Waterborne, New Orleans, LA, USA) was applied to the left sample. A *Cryptosporidium* – specific FITC labelled monoclonal antibody solution (0.02 mL) (Crypt-a-glo, Waterborne, New Orleans, LA, USA) was applied to the right sample. The slide was then placed in a moist container and incubated at 37 °C for 45 minutes. Afterwards, the slide was removed from the incubator, dried and mounted with a fluorescent antibody mounting fluid (Aqua – polymount, Polysciences, Warrington, PA, USA) and a 22 mm x 22 mm micro coverslip (VWR Scientific Inc., Media, PA, USA).
(Oo)cysts were enumerated at 100x and 400x magnification, respectively, using a dry objective, with an epifluorescence microscope (Australia: Olympus BH-2, Olympus Optical Co. Ltd., Japan; Canada: Zeiss 9901, Zeiss, West Germany). The number of (oo)cysts per gram of faeces was calculated using the previously described formula (O’Handley et al., 1999) \( N = \frac{s}{\text{vol.} \times \text{wt.}} \).

Where \( N \) is the number of (oo)cysts per gram of faeces, \( s \) is the number of (oo)cysts counted on the slide, \( \text{vol.} \) is the volume of sample examined (0.015 ml) and \( \text{wt.} \) is the weight of the faecal sample originally processed (g). This procedure has a theoretical sensitivity of 66 (oo)cysts per gram of faeces when examining a 1 g faecal sample (O’Handley et al., 1999).

### 2.3 DNA Extraction, Amplification and Sequencing

#### 2.3.1 DNA Extraction

Faecal samples were processed as described in 2.2 and (oo)cysts were obtained in PBSS. DNA was extracted utilizing a QIAamp DNA Stool Mini Kit. Approximately 200 µl of the sucrose purified (oo)cysts of each sample were placed in micro-centrifuge tubes, 1.4 mL of buffer ASL was added to each sample and vortexed continuously for 1 minute. Samples were frozen in liquid nitrogen for 10 seconds and thawed in boiling water for 1 minute. This procedure was repeated 5 times (to rupture the (oo)cysts and release the DNA), as compared to the manufacturer’s recommendation of heating the samples for 5 minutes at 70 °C. The samples were vortexed for 15 seconds and centrifuged at 8500 x g for 1 minute to form a pellet. Supernatant (1.2
mL) was pipetted into a new 2 mL micro-centrifuge tube and the pellet was discarded. One InhibitEX tablet was placed into each sample and vortexed immediately and continuously for 1 minute. The suspension was incubated for 1 minute at room temperature to allow inhibitors to absorb to the InhibitEX matrix. The sample was then centrifuged for 3 minutes at 8500 x g to pellet inhibitors bound to InhibitEX. All of the supernatant was pipetted off into a new 1.5 mL micro-centrifuge tube and the pellet was discarded. The sample was then centrifuged at 8500 x g for 3 minutes. Proteinase K (15 µl) (Qiagen Pty. Ltd., Australia) was pipetted into a new 1.5 mL micro-centrifuge tube and then 200 µl of the supernatant was added. Buffer AL (200 µl) was added and the tube was vortexed for 15 seconds. The sample was then incubated at 70 °C for 10 minutes. After incubation, 200 µl of ethanol (100%) was added to the lysate and vortexed. The lysate was then transferred to a labelled QIAamp spin column with a 2 mL collection tube. The cap was closed and centrifuged at 8500 x g for 1 minute. The QIAamp spin column was then placed in a new 2 mL collection tube and the filtrate tube was discarded. The QIAamp spin column was opened and 500 µl of Buffer AW1 was added and the tube was centrifuged at 8500 x g for 1 minute. The QIAamp spin column was placed in a new 2 mL collection tube and the filtrate was discarded. The QIAamp spin column was opened and 500 µl of Buffer AW2 was added and centrifuged at 8500 x g for 3 minutes and the filtrate tube was discarded. The QIAamp spin column was placed in a new 1.5 mL micro-centrifuge tube and 50 µl of Buffer AE, as compared to the 200 µl recommended by the manufacturer, was
pipetted onto the QIAamp membrane (to increase the final template concentration for routine PCR). The sample was incubated for 1 minute at room temperature and then centrifuged at 8500 x g for 1 minute to elute the DNA.

2.3.2 DNA Amplification

2.3.2.1 Amplification of *Giardia* 18S rDNA

A fragment of the 18S rDNA was amplified using a nested PCR and sequenced as described by Hopkins *et al.* (1997). The 18S rDNA fragment was selected because it had a suitable balance of variation between species/genera, it has a good set of sequences published and was a reliable analysis. The primary master mix contained 1x reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM \((\text{NH}_4)_2\text{SO}_4\), 0.45% Triton X-100, 0.2 mg/ml gelatin), 0.05 µ/µl Tth+ (Fisher Biotech), 2.5 mM MgCl₂, 5% DMSO; 200 µM of each dNTPs, 500nM of each primer forward (RH11, 5’ – CATCCGGTCGATCCTGCC – 3’) and reverse (RH4, 5’ – AGTCGAACCCTGATTCTCGCCAGG – 3’), and 1 – 2 µl of DNA; made up to a final volume in water. Secondary reaction master mix was the same as the primary reaction master mix with the removal of DMSO (replaced with water) and different (internal) primers, forward (Giar18ser, 5’ – GACGCTCTCCCAAGGAC – 3’) and reverse (Giar18sir, 5’ – CTGCGTCACGCTGCTCG – 3’). Reactions were performed on a Perkin Elmer GeneAmp PCR System 2400 (Perkin Elmer, Foster City, California).
thermal cycler. Samples were kept on ice until the thermal cycler reached
temperature and then they were denatured for 5 minutes at 96 °C, followed by
10 cycles annealing temperature touchdown (from 59°C to 53°C at minus
0.5°C per cycle, with denaturation at 96°C for 30 s, annealing 59°C /53°C for
45 s and extension 72°C for 45 s, 59°C for 45 s, 72°C for 45 s) and a 45 cycle
regular programme (96°C for 30 s, 53°C for 45 s, 72°C for 45 s) with a final
extension of 72°C for 7 min. and a 15°C hold.
The secondary PCR cycle conditions were identical to the primary round.
PCR products were visualized utilizing agarose gel electrophoresis. The 1%
agarose gel consisted of agarose powder in 1x TAE buffer (40 mM Tris-HCl,
20 mM acetic acid, EDTA (pH 8.0). Gels were stained with 1% ethidium
bromide. 5 µl of sample mixed with 1 µl of loading dye were loaded and run
on a Power Gel Runner (Bio-Rad Model 200/2.0 Power Supply Pack) for 60
minutes at 80 volts. PCR fragment sizes were compared using a 100 base pair
DNA ladder. Sample bands on gels were visualized under UV light and
photographed. Samples producing bands of interest were loaded into a second
gel prepared and run as above, but with 20 µl of sample with dye. The
resulting agarose gel was placed under UV light and DNA bands were cut out
using a scalpel and placed in a 2 mL micro centrifuge tube and frozen until gel
purification.

Purification of PCR product from agarose gel to remove agarose and
sequencing inhibitors was done utilizing MOBIO Laboratories Inc. UltraClean
GelSpin DNA Purification Kit. Protocol was as follows, gel band weight was determined and the gel was placed into a spin filter basket with 3 volumes of GelBind buffer. The sample was incubated for 2 minutes at 55 °C, inverted once and incubated for 1 minute more until the gel melted. The spin filter was centrifuged for 10 seconds at 10,000 x g, the spin filter was removed and the collection tube was vortexed for 5 seconds to mix the flow through fluid. The liquid from the collection tube was reloaded onto the spin filter and centrifuged again for 10 seconds at 10,000 x g. The flow through liquid was then discarded and the filter basket replaced. Gelwash buffer (300 µl) was added and centrifuged for 10 seconds at 10,000 x g. Flow through was discarded and the filter basket was centrifuged again for 30 seconds at 10,000 x g. The filter basket was then transferred to a clean collection tube and 30 µl of elution buffer (10 mM Tris) was applied directly on to the centre of the spin filter membrane and centrifuged for 30 seconds at 10,000 x g, and the filter basket was discarded.

2.3.2.2 Amplification of Cryptosporidium 18S rDNA

A fragment of the 18S rDNA was amplified using a nested PCR as described by Xiao et al. (2000). The 18S rDNA fragment was selected because it had a suitable balance of variation between species/genera and it has a good set of sequences published. The primary master mix contained 1x reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM (NH₄)₂SO₄, 0.45% Triton X-100, 0.2 mg/mL gelatin), 0.05 µ/µl Tth+ (Fisher Biotech), 1.5 mM MgCl₂, 200 µM of
each dNTPs, 500 nM of each primer forward 18CRF2 (GACATATCATTCAAGTTTCTGACC) and reverse 18SCR2 (CTGAAGGAGTAAGGAACAACC) and 1-2 µl of DNA to a final volume in water. The secondary reaction master mix was the same as the primary reaction master mix with new (internal) primers, forward (18SCF1, 5’ – CCTATCAGCTTTTAGACGGTAGG – 3’) and reverse (18SCR1, 5’ – TCTAAGAATTTCACCTCTGACTG – 3’). Reactions were performed in a Perkin Elmer GeneAmp PCR System 2400 thermal cycler. Samples were kept on ice until the thermal cycler reached temperature and then they were denatured for 5 minutes at 94 °C, followed by 50 cycles of denaturation at 94°C for 30 s, annealing 56°C for 20 s and extension 72°C for 45 s with a final extension of 72°C for 7 min. and a 15°C hold. A second run was performed on the samples with the master mix altered to increase the magnesium concentration from 1.5 mM to 2.5 mM and the programme changed to reduce the annealing temperature from 56 °C for 20 sec to 50 °C for 45 sec. PCR product visualization and purification was the same as described in 2.3.2.1.

2.3.2.3 Amplification of Cryptosporidium Actin Gene

A fragment of the Cryptosporidium actin gene was amplified also using nested PCR as described by Ng et al. (2006). The actin gene was selected because it had a suitable balance of variation between species/genera and it has a good set of sequences published. For the first run the master mix contained 1x reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM (NH₄)₂SO₄,
0.45% Triton X-100, 0.2 mg/ml gelatin) and 0.05 µ/µl Tth+ (Fisher Biotech), 3.5 mM MgCl₂, 200 µM of each dNTPs, 500 nM of each primer forward (CRAct3F, 5’ GTKACWAAYTGGGAAGAYATGG 3’) and reverse (CRAct6R, 5’ GGDGCAACRACYTTRATCTTC 3’) and 1 µl of DNA made up to a final volume in water. The secondary master mix was the same except that it used the internal primer CRAct8F (5’ – CTGTDGGWAGYGARAGATTYAG – 3’) in place of CRAct3F. Samples were kept on ice until the thermal cycler reached temperature and then they were denatured for 5 minutes at 94 °C, followed by 50 cycles of denaturation at 94 °C for 30 s, annealing 58 °C for 20 s and extension 72 °C for 45 s with a final extension of 72 °C for 7 min. and a 15 °C hold.

The second run for the Cryptosporidium actin gene was similar to the first with the exception that the DNA polymerase enzyme was changed from Tth+ (Fisher Biotech) to AmpTaqGold (Applied Biosystems) and therefore the 1x reaction buffer was altered as well (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% (w/v) gelatin, Applied Biosystems). The thermal cycle protocol employed was the same as above except for an initial denaturation of 94 °C for 10 minutes, rather than 5 minutes. The gel was run at 90 volts for an hour.

The third run for the Cryptosporidium actin gene had the same master mix as the second run with the addition of a set of samples containing 2.0 µl of
sample. The thermal cycling conditions were altered to include an annealing temperature touchdown in the primary PCR – samples were again kept on ice until the thermal cycler reached temperature, then denatured for 10 minutes at 94°C, followed by 17 cycle annealing temperature touchdown (from 58°C to 53°C at – 0.3°C per cycle, with denaturation at 94°C for 30 s, annealing 58°C /53°C for 30 s and extension 72°C for 45 s), and a 50 cycle regular programme (94°C for 30 s, 53°C for 30 s, 72°C for 45 s), with a final extension of 72°C for 7 minutes and a 15°C hold.

The secondary PCR had a thermal cycling of 94°C for 10 minutes, followed by 50 cycles at 94°C for 30 s, 53°C for 30 s and 72°C for 45 s with a final extension of 72°C for 7 minutes and a 15°C hold.

The fourth run for PCR amplification of the Cryptosporidium actin gene had a master mix the same as the second run with one set of reactions containing 2 µl of template and a second set with 3 µl of template. The secondary reaction contained the usual 1 µl of primary PCR product. Thermal cycling times were the same as the third run. PCR product visualization and purification was the same as 2.3.2.1.
2.3.2.4 QPCR *Cryptosporidium* 18S rDNA and Melting Curve Analysis

The primers 5’ AAGCTCGTAGTTGGATTTCTG 3’ and 5’ CTTTAAGCACTCTAATTCTCTCAAAG 3’ were used to amplify a fragment of *Cryptosporidium* 18S rDNA that varied from 128 to 152 bp in size depending upon the presence of insertions/deletions characteristic of the particular *Cryptosporidium* species examined. PCR reactions consisted of 1 µl of DNA template, 0.5 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems, CA, USA) with compatible 1x PCR buffer II, 2.5 mM MgCl₂, 200 µM of each deoxynucleoside triphosphate (Fisher Biotec, Western Australia), 0.4 µM of each primer (Sigma Proligo, NSW, Australia) and 3.3 µM SYTO9 (Molecular Probes, OR, USA), which was made up to a final volume of 25 µl with sterile distilled water. Thermocycling was carried out in a RotorGene 3000 (Corbett Research, NSW, Australia) controlled through the PC software Rotor-Gene 6, version 6.0, build 25 (Corbett Research, NSW, Australia). The thermocycling protocol consisting of 10 min at 95°C, followed by 50 cycles consisting of (1) 95°C for 15 s, (2) a 10 cycle touchdown from 64°C to 55°C for 15 s with the remaining 40 cycles set at 55°C for 15 s and (3) 68°C for 20 s. Fluorescence data was captured in real-time on the FAM/Sybr channel (410 nm excitation and 510 nm detection) at Step 3 (68°C for 20 sec) with two gain values of 5 and 3. The amplification stage was completed by a 5 min hold at 68°C.
Melting curve analysis

Immediately at the completion of the amplification stage, a DNA melting curve analysis from 65°C to 90°C was performed at 0.5°C increments, with a 60 s hold on the first step followed by 30 s holds for each step afterwards. Fluorescence data was again monitored on the FAM/Sybr channel at each step. Melt peaks were displayed with the digital filter set to “none” following differentiation of the fluorescence data with respect to temperature by the afore-mentioned software.

2.3.3 DNA Sequencing for *Giardia* 18S rDNA, *Cryptosporidium* 18S and *Cryptosporidium* Actin Gene

Samples were sequenced using dye terminator chemistry with the Big Dye v3.1 Kit (Applied Biosystems) and run on an ABI 3730 48 capillary machine. Purified PCR products were placed into a 10 µl sequencing reaction consisting of 2.0 µl dye terminators and enzyme, 1.0 µl of 5x sequencing buffer, 0.25 µl of 12.5 µM primer and a maximum of 7 µl PCR product (concentrated samples diluted with water). Samples were kept on ice until the thermal cycler reached temperature and then they were denatured for 2 minutes at 96°C, followed by 25 cycles of 96°C for 10 s, 53°C for 5 s, 60°C for 4 min, finished with a 15°C hold. Sequencing was performed in both the forward and reverse direction for each sample.
A post-reaction purification (ethanol precipitation) was performed on each sample. Sequencing products were transferred to 0.5 mL tubes, then each had 1 µl of 125 mM EDTA (disodium salt) pipetted in followed by 1 µl of 3M sodium acetate (pH 5.2) and finally 25 µl of 100% room temperature ethanol. The contents of the tube were mixed by aspiration and expulsion from a pipette and left for 20 minutes (incubation) at room temperature. The samples were then micro-centrifuged at 8500 x g for 30 minutes. The supernatant was discarded and the pellet was washed with 125 µl of 70% ethanol (micro-centrifuged at 8500 x g for 5 minutes, supernatant discarded). The samples were then either left upside down overnight to airdry or were placed in a speedvac vacuum drier 15 minutes. Samples were submitted for sequencing on an ABI 3730 48 capillary machine. Genetic sequences were analysed using SeqEd v 1.0.3 (Applied Biosystems). Sequences were aligned using CLUSTAL W (Thompson et al., 1994), and compared to those in Genbank.
Chapter 3

Western Australia Preliminary Study of Point Parasite Occurrence in Beef Feedlot Cattle and Calves Prior to Weaning
3.1 Introduction

*C. parvum* and *G. duodenalis* have been documented in dairy cattle in Western Australia (O’Handley *et al.*, 2000; Becher *et al.*, 2004). Becher *et al.* (2004) collected samples weekly from birth to weaning in dairy calves and reported a prevalence of 48 and 89% for *C. parvum* and *G. duodenalis* respectively. The authors also noted no significant association between parasite occurrence and season or management practices (housing) (Becher *et al.*, 2004).

O’Handley *et al.* (2000) collected faecal samples from 2 to 10 week old dairy calves in Western Australia and reported a 58% prevalence of *G. duodenalis*. However, no reports of *G. duodenalis* or *Cryptosporidium* spp. have been published for beef cattle in Western Australia.

3.1.1 Aim

The purpose of this preliminary study was to determine if these parasites existed in Western Australian beef calves and feedlot cattle prior to developing a subsequent more comprehensive study involving parasite prevalence.
3.2 Materials and Methods

Samples were collected from eight groups of cattle to establish the existence of *G. duodenalis* and *C. andersoni* in beef cattle in Western Australia. Four cattle operations were selected within a 600 km radius of Perth, Western Australia that employed typical area management techniques (as described in 1.3) that were also similar to those found in Alberta, Canada to facilitate comparison of parasite prevalence. Since these groups of cattle were on privately owned operations and this was only a preliminary survey for the existence of these parasites, some generalizations in management techniques (i.e. nutritional regimes, herd health protocols) have been made to protect the confidentiality of the sensitive information of these producers. The groups referred to in this Chapter are in no way related to those groups of cattle referred to in Chapter 4.

3.2.1 Animals

3.2.1.1 Location 1 Group 1

Twenty crossbred steers (Gelbvieh x Murray Grey x Simmental x Hereford) single source, farm raised, weighing approximately 400 kg and eleven months of age were sampled at an 1800 head feedlot near Mt. Barker, Western Australia. These steers were born in March 2003, weaned in December 2003 and sampled on February 12, 2004. Feedlot pens housed 200 individuals and had bunk feeders.
3.2.1.2 Location 1 Group 2

Thirteen calves, still nursing their dams and approximately two months of age, were sampled from two herds on grass near Mt. Barker, Western Australia, October 19, 2004.

3.2.1.3 Location 2 Group 3

Eleven crossbred steers, sourced directly off multiple farms, weighing approximately 450 kg, 12 months of age and on feed for 60 – 70 days were sampled February 12, 2004 from two pens (Figure 3.1). Feedlot pens were approximately 200 – 250 head in size and had bunk feeders in a feedlot near Borden, Western Australia.

Figure 3.1 Cattle sampled in feedlot Location 2 Group 3
3.2.1.4 Location 2 Group 4

Thirteen, 18 month old feedlot heifers, sourced directly off multiple farms, weighing 423 ± 23 kg and on feed for 80 days were sampled October 20, 2004 in a feedlot near Borden, Western Australia (Figure 3.2). These heifers were ten days away from slaughter.

Figure 3.2  Cattle sampled Location 2 Group 4

3.2.1.5 Location 3 Group 5

Eleven crossbred steers, multiple sourced, weighing approximately 400 kg and 12 months of age were sampled from two pens February 13, 2004 from a feedlot near Vasse, Western Australia (Figure 3.3). Feedlot pens were approximately 25 head in size and had bunk feeders.
3.2.1.6 Location 3 Group 6

Six, three year old Angus cows and three of their three month old nursing calves were sampled on October 21, 2004 while grazing grass near Vasse, Western Australia (Figure 3.4). Another group of three 13+ year old cows and six of their three month old nursing calves were also sampled on the same date (Figure 3.5).
Figure 3.4 Three year old cows with calves sampled at Location 3 Group 6

Figure 3.5 Thirteen + year old cows with calves sampled at Location 3 Group 5

3.2.1.7 Location 4 Group 7

Ten Angus steers, sourced directly off multiple farms, weighing approximately 900 kg and 36 months of age were sampled prior to slaughter.
on February 25, 2004 from a feedlot near Three Springs, Western Australia (Figure 3.6). Animals would have been on feed for approximately 24 months.

3.2.2 Diets and Feeding

Animals were fed rations consisting of roughages including ryegrass, silage and grains including barley, oats and wheat with the exception of Groups 2 and 6 which grazed perennial grass pastures. Typical feedbunks (Figure 3.7 and 3.8). Typical pasture (Figure 3.9).
Figure 3.7 Feed bunk at Location 2 Group 3

Figure 3.8 Feed bunk for cattle on feed at Location 3 Group 5
3.2.3 Faecal Collection and Analysis

3.2.3.1 Location 1 Group 1; Location 2 Group 3; Location 3 Group 5; Location 4 Group 7

One time faecal samples (1 – 5g) were collected off the ground after steers were observed defecating. Using a disposable latex glove for each faecal pat the sample was immediately taken from the upper middle part of the pat to avoid contamination by surrounding soil or faeces, placed in a centrifuge tube and stored at 4 °C until further processing.

Faecal analysis was performed according to previously described procedures in section 2.1 and 2.2 (Olson et al., 1997a; O’Handley et al., 1999) with the exception that faecal samples were not weighed. Cysts and oocysts were enumerated at 100x and 400x magnification, respectively, using a dry
objective, with an epifluorescence microscope (Olympus, BH-2, Olympus
Optical Co. Ltd. Japan). The number of (oo)cysts per slide were counted. Five
_**G. duodenalis**_ microscopic positive samples were molecularly characterised
and reported in Table 4.1 in Chapter 4.

### 3.2.3.2 Location 1 Group 2

Faecal samples were collected and processed from the calves as described in
section 3.2.3.1 with the exception that they were placed in weighted centrifuge
tubes and the number of (oo)cysts per gram of faeces was then calculated
using a previously described formula (O’Handley et al., 1999). The sensitivity
of this detection method was 66 (oo)cysts per gram of faeces (O’Handley et
al., 1999).

### 3.2.3.3 Location 2 Group 4

Faecal samples (1-5g) were collected rectally, using a disposable latex glove,
from each heifer once. Samples were stored and processed as described in
3.2.3.2.

### 3.2.3.4 Location 3 Group 6

Faecal samples (1-5g) were collected and processed from cows and calves as
described in 3.2.3.2.
3.2.4 Data Analysis

*Giardia* cyst count geometric means were calculated for positive animals using Prism Version 2.0 software column statistics.

3.3 Results

The estimates of occurrence of parasites for the 8 sampling groups at the 4 locations is summarized in Table 3.1.

<table>
<thead>
<tr>
<th>Location</th>
<th>Group</th>
<th>Animal Class</th>
<th>Giardia</th>
<th>Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location 1</td>
<td>Group 1</td>
<td>Steers</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Location 1</td>
<td>Group 2</td>
<td>Calves</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Location 2</td>
<td>Group 3</td>
<td>Steers</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Location 2</td>
<td>Group 4</td>
<td>Heifers</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Location 3</td>
<td>Group 5</td>
<td>Steers</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Location 3</td>
<td>Group 6</td>
<td>Cows</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Location 3</td>
<td>Group 6</td>
<td>Calves</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Location 4</td>
<td>Group 7</td>
<td>Steers</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Animals were not infected with *Cryptosporidium* spp. at any of the locations or in any of the groups. *G. duodenalis* was identified at 3 of the 4 locations and in animals from 5 of the 8 groups sampled. The range and geometric means of *G. duodenalis* cysts shed per gram of faeces of infected animals for
the 3 groups for which intensity of infection data was collected were as follows: Location 1 Group 2 (128.21 to 1538.46; geometric mean 444.1); Location 2 Group 4 (16.54 to 33.06; geometric mean 23.38); Location 3 Group 6 Calves (15.5 to 22.08; geometric mean 18.08).

3.4 Discussion

This is the first study to examine the occurrence of *G. duodenalis* and *Cryptosporidium* spp. in beef cattle in Western Australia. Two studies from Western Australia have been published, one demonstrating the point prevalence of *G. duodenalis* in dairy calves (O’Handley *et al.*, 2000) and the second documenting longitudinal prevalence of both *G. duodenalis* and *Cryptosporidium* spp. in dairy calves (Becher *et al.*, 2004). Since these parasites have not been previously reported in feedlots and beef cow/calf operations in Western Australia, a preliminary survey of occurrence was justified to confirm their existence prior to developing a more comprehensive study involving these parasites’ prevalence.

The occurrence of *G. duodenalis* detected in the two groups of beef calves (Groups 2 and 6) in the current study was 15 and 33% with geometric means of 441 and 18 cysts/gram of faeces respectively. These calves were between 2 and 3 months of age at the time of sampling.
The results from this preliminary study confirmed the existence of *G. duodenalis* in both beef calves and feedlot cattle in Western Australia for the months of February and October. The existence of *C. andersoni* in beef animals is still unconfirmed by this study. A more comprehensive study to determine the prevalence of *C. andersoni* and *G. duodenalis* in beef feedlot cattle is warranted.
Chapter 4

Prevalence of *Giardia duodenalis* and
*Cryptosporidium andersoni* in Beef Feedlot Cattle

in Western Australia
4.1 Introduction

The prevalence studies described in Chapter 3 confirmed the existence of *G. duodenalis* in both beef calves and beef feedlot cattle in Western Australia. The occurrence of *C. andersoni* in beef feedlot cattle was not established in the preliminary studies reported in Chapter 3. The current study was designed to examine the point prevalence and molecular characteristics of *G. duodenalis* and *C. andersoni* in beef feedlot cattle in Western Australia.

Current literature has reported *G. duodenalis* Assemblages A and E in dairy calves from Western Australia (O’Handley et al., 2000; Becher et al., 2004), however literature reporting assemblages of *G. duodenalis* in Western Australian feedlot cattle and beef calves does not exist. The current study examines these aspects of parasitic infections.

4.1.1 Aim

Based on the establishment of *G. duodenalis* occurrence in Western Australian cattle discussed in Chapter 3, it was decided to focus on beef cattle in one particular feedlot, with faecal samples being collected several times during the production year on different pens of cattle. Since this was a commercial feedlot, samples could only be collected at times when the animals were being processed through the handling facility for other management procedures.
4.2 Materials and Methods

4.2.1 Animals

4.2.1.1 Group 1 (K1-K90)

Ninety crossbred steers (Angus x Shorthorn x Simmental) single source, farm raised, weighing 505 ± 40.8 kg and between 18 to 24 months of age were sampled from one pen at a 6000 head feedlot on Nov. 2, 2004 (Figure 4.1). These animals had been in the feedlot for approximately 15 months prior to sampling.

Figure 4.1 Group 1 (K1-K90) Cattle on Nov. 2, 2004
4.2.1.2 Group 2 (KE221-KE350)

One hundred and thirty crossbred steers (Hereford x Angus x Murray Grey x Shorthorn) single source, farm raised, weighing $492 \pm 42.5$ kg and between 18 to 24 months of age were sampled from one pen on Nov. 6, 2004. These animals had been in the feedlot for approximately 15 months prior to sampling.

Figure 4.2 Animal restraint system at feedlot
4.2.1.3 Group 3 (J1 – J23)

Twenty-three crossbred steers were separated from Group 2 based on the weight of those steers being less than 420 kg. These “poor gaining” calves were sampled and weighed on Nov. 6, 2004 with a mean weight of 381 ± 23.5 kg. The calves were sourced from the same location as Group 1.

4.2.1.4 Group 4 (J)

One hundred and sixty-one head of crossbred steers (Angus x Shorthorn x Simmental) were sourced from the same location as Group 1, they were faecal sampled on Feb. 7, 2005.

4.2.1.5 Group 5 (JJ2-JJ211)

Ninety-eight crossbred steers (Angus x Murray Grey x Hereford) single source, farm raised, weighing 477 ± 23.5 kg and 12 months of age were sampled from one pen of 200 head on Nov. 9 and Nov. 28, 2005 (Figures 4.2, 4.3). These animals had been in the feedlot for approximately five months prior to sampling.
4.2.2 Diets and Feeding

All groups of animals received the same rations. Animals were fed free choice hay for 24 hours at induction to the feedlot. Rations contained no Rumensin or Tylan and animals were not implanted with growth promotants. Due to confidentiality full ration disclosure is not available, but animals went through a 4 step programme with increasing levels of energy starting at 10.3 Mcal and ending at 11.7 Mcal. Protein was constant at approximately 14%. The ration was a mixture of barley silage, lupins, oats, oat silage and barley straw.
4.2.3 Faecal Collection and Analysis

Faecal samples (1-5g) for all Groups were collected rectally (Figure 4.4) and analysis was performed according to previously described procedures in Chapter 2.

4.2.4 DNA Extraction, Amplification and Sequencing

DNA was extracted, amplified and sequenced utilizing procedures outlined in section 2.3. Only a subset of microscopic positive samples in Table 4.1 was tested by PCR to verify parasite presence. Sample 346 for Cryptosporidium 18S rDNA was amplified using QPCR and compared using melting curve analysis utilizing the procedures outlined in sections 2.3.1 and 2.3.2.4. Samples tested from Australia included the following detailed in Table 4.1.
including some samples collected from studies in Chapter 3 (M43, M44, V14, V26, P10).

Table 4.1  DNA sequenced samples from Australia

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Animal Group</th>
<th>Fragment Tested</th>
<th>Result</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>M43</td>
<td>3.2.1.4 Feedlot Heifer 18 months</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage A</td>
<td>NA – mixed</td>
</tr>
<tr>
<td>M44</td>
<td>3.2.1.4 Feedlot Heifer 18 months</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage B</td>
<td>DQ448643</td>
</tr>
<tr>
<td>V14</td>
<td>3.2.1.6 Nursing Cow 13+ yrs</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage C</td>
<td>EF157972</td>
</tr>
<tr>
<td>V26</td>
<td>3.2.1.6 Nursing Cow 13+ yrs</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage D</td>
<td>DQ448642</td>
</tr>
<tr>
<td>P10</td>
<td>3.2.1.2 Nursing Calf 2 months</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage B&amp;E</td>
<td>NA – mixed</td>
</tr>
<tr>
<td>J12-1</td>
<td>4.2.1.3 &lt;420 kg Feedlot Steer 18-24 mo</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage E</td>
<td>DQ448641</td>
</tr>
<tr>
<td>J19-1</td>
<td>4.2.1.3 &lt;420 kg Feedlot Steer 18-24 mo</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage E</td>
<td>EF157973</td>
</tr>
<tr>
<td>287</td>
<td>4.2.1.2 Feedlot Steer &gt;420 kg 18 – 24 mo</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage E</td>
<td>EF157975</td>
</tr>
<tr>
<td>316</td>
<td>4.2.1.2 Feedlot Steer &gt;420 kg 18 – 24 mo</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage A</td>
<td>NA - incomplete</td>
</tr>
<tr>
<td>346</td>
<td>4.2.1.2 Feedlot Steer &gt;420 kg 18 – 24 mo</td>
<td><em>Giardia</em> 18S</td>
<td>Assemblage E</td>
<td>EF157975</td>
</tr>
<tr>
<td>346</td>
<td>4.2.1.2 Feedlot Steer &gt;420 kg 18 – 24 mo</td>
<td><em>Crypto</em> 18S</td>
<td><em>C. andersoni</em></td>
<td>NA QPCR</td>
</tr>
<tr>
<td>J3-1</td>
<td>4.2.1.3 &lt;420 kg Feedlot Steer 18-24 mo</td>
<td><em>Crypto</em> 18S</td>
<td><em>C. andersoni</em></td>
<td>EF150369</td>
</tr>
</tbody>
</table>
4.2.5 Data Analysis

Initial weights were analysed using Prism Version 2.0 software Unpaired t test (P<0.05 considered significantly different). Geometric means for *G. duodenalis* cysts and *C. andersoni* oocyst counts were calculated for positive animals using Prism Version 2.0 software column statistics.

4.3 Results

4.3.1 Group 1 (K1 – K90)

The prevalence of *G. duodenalis* and *C. andersoni* (Figure 4.5) was 2 and 8% respectively. The range and geometric means of *G. duodenalis* cysts shed per gram of faeces was 29.37 to 149.80; geometric mean 66.33. *C. andersoni* oocysts shed per gram of faeces was 22.99 to 262.70; geometric mean 72.95.
4.3.2 Group 2 (KE221 – KE350)

The prevalence of *G. duodenalis* (Figure 4.6) and *C. andersoni* was 8 and 5% respectively. The range and geometric means of *G. duodenalis* cysts shed per gram of faeces was 20.51 to 1333.0; geometric mean 100.10. *C. andersoni* oocysts shed per gram of faeces was 48.48 to 2000.00; geometric mean 234.90.
4.3.3 Group 3 (J1 – J23)

These feedlot steers were removed from Group 2 because they weighed less than 420 kg at induction into the feedlot on Nov. 6, 2004. The prevalence of *G. duodenalis* and *C. andersoni* was 22 and 26% respectively which was significantly higher than the other groups (P<0.07). The range and geometric means of *G. duodenalis* cysts shed per gram of faeces was 20.51 to 284.40; geometric mean 69.58. *C. andersoni* oocysts shed per gram of faeces was 48.48 to 2000.00; geometric mean 307.20.

4.3.4 Group 4 (J)

The one hundred and sixty-one steers in this group only had faecal samples collected on Feb. 7, 2005 revealing a 0.6 and 0% prevalence of *G. duodenalis*.
and *C. andersoni* respectively. Only one animal was infected with *G. duodenalis* shedding 25.84 cysts per gram of faeces.

### 4.3.5 Group 5 (JJ2 – JJ211)

The prevalence of *G. duodenalis* and *C. andersoni* was 2 and 0% respectively for the first sampling on Nov. 9, 2005 and 8 and 0% respectively for the second sampling on Nov. 28, 2005. The range and geometric means of *G. duodenalis* cysts shed per gram of faeces for Nov 9 and Nov 28 were 45.98 to 256.40 and 20.62 to 538.70; geometric mean 6108.60 and 156.10 respectively.

### 4.3.6 Molecular Characterization

Of the microscopic positive isolates a subset of 21 samples were analysed utilising PCR, 50% (10) were successfully sequenced at the *Giardia* 18S rDNA locus (Figure 4.7). The genotyped isolates represented samples from 4 different farms. Isolate P10 from Chapter 3 group 3.2.1.2 was a mixed Assemblage B and E sequence (and therefore not deposited in GenBank).

Isolates from Chapter 3 group 3.2.1.4, M43 and M44, were zoonotic Assemblages A (with some ambiguous bases and also therefore not deposited in Genbank) and B (DQ448643) respectively. Isolates from Chapter 3 group 3.2.1.6, V14 and V26, were dog related Assemblages C (EF157972) and D (DQ448642) respectively. Isolates from Chapter 4 group 4.2.1.2, (KE)287 and (KE)346 were hoofed livestock Assemblage E (EF157974) and (EF157975) respectively, whilst Chapter 4 isolate (KE)316 was zoonotic Assemblage A.
Finally isolates from group 4.2.1.3, J12-1 and J19-1, were also both hoofed livestock Assemblage E (DQ448641) and (EF157973) respectively.

Figure 4.7 Determination of the genotype of *Giardia duodenalis* in cattle faeces using *Giardia* 18S rDNA. From left to right, the gel shows the 100 bp ladder in lane 1, and some of the (*Giardia* 18S rDNA) PCR positive samples (from Australia and Canada) in lanes 2 to 15 – the PCR fragment measures 175 base pairs.

- Assemblage E Sample J12-1 Animal Group 4.2.1.3 (lane 12)
- Assemblage D Sample V26 Animal Group 3.2.1.6 (lane 13)
- Assemblage B Sample M44 Animal Group 3.2.1.4 (lane 14)
- Assemblage B&D Sample P10 Animal Group 3.2.1.2 (lane 15)
Of the 12 microscope positive Cryptosporidium spp. samples, PCR and sequencing was only successful on 1 isolate, J3-1 (*C. andersoni*, GenBank accession number EF150369) at the 18S rDNA locus (Figure 4.8). Although a couple of other samples initially appeared weakly PCR positive at the 18S rDNA or Actin loci (Figure 4.9), their subsequent sequencing reactions were negative or were not specific to *Cryptosporidium* spp. Sample 346 was subjected to QPCR and melt curve analysis that indicated that it was *C. andersoni*. 
Figure 4.8 PCR for *Cryptosporidium* spp. at the 18S rDNA locus. From left to right, the gel shows the 100 bp ladder (lane 1), lanes 2-4 show PCR results (samples Animal 551/T7, Animal 551/T116, J3-1).
Figure 4.9  PCR for *Cryptosporidium* spp. at the Actin locus. From left to right, the gel shows the 100 bp ladder (lane 1), lanes 2 – 9 show PCR results from using 1 µl template (samples A551/T7 – Canadian (Lethbridge Research Centre), KE346, KE320, J21-1 and J3-1, plus positive control, negative control and master mix control). Lanes 10 – 17 show PCR results from the same samples and controls except with 2 µl of template.
4.4 Discussion

Prevalence of *G. duodenalis* for all groups of cattle involved in this study ranged from 0.6 – 22%. In a point prevalence study by Olson *et al.* (1997b) beef cattle greater than 6 months of age had a *G. duodenalis* prevalence of 11%. *C. andersoni* prevalence for all Groups in the current study ranged from 0 – 26%, compared to those results of 11.8% reported in California feedlot cattle by Anderson (1991). However, both *G. duodenalis* and *C. andersoni* prevalence in the current point prevalence study are much lower than those reported by Ralston *et al.* (2002) of 82 and 85% respectively in the 257 day longitudinal study highlighting the underestimation of infection levels with point prevalence studies (Buret *et al.*, 1990).

Isolates from 4 different farms were genotyped by PCR amplification and sequencing of a 130 to 175 bp fragment of the 18S-rDNA gene. The beef calves sampled (10) were infected with *G. duodenalis* Assemblage A (2 samples, zoonotic genotype), Assemblage B (1 sample, zoonotic genotype), Assemblage C (1 sample, dog related genotype), Assemblage D (1 sample, dog genotype), Assemblage E (4 samples, hoofed livestock-specific genotype) and a mixed infection of Assemblages B and E (1 sample). These results differ from those reported by Applebee *et al.* (2003) where beef calves, aged 2 to 10 weeks, from 9 farms were sampled and genotyped resulting in 97.4% positive for the *G. duodenalis* livestock genotype (Assemblage E) and one isolate was Assemblage A (2.6%). O’Handley *et al.* (2000) also found Assemblage E to
be the most prevalent in a study of dairy calves, aged 2 to 10 weeks, in Alberta, Canada and Western Australia where 80% (12/15) were infected with Assemblage E and 20% with Assemblage A. In a study in Western Australia that sampled 31 dairy calves at less than 12 weeks of age, all *G. duodenalis* isolates were categorized as Assemblage E (Becher *et al.*, 2004). In the current study one animal was 8 weeks old, similar to the above reported studies, and was positive for a mixed infection of Assemblages B and E. Trout *et al.* (2005) sampled dairy calves 3 to 11 months of age and reported 87% of the *G. duodenalis* positive animals infected with Assemblage E and 13% with Assemblage A. Trout *et al.* (2006) also sampled older dairy heifers between 12 and 24 months of age and reported 91% positive for Assemblage E and 9% Assemblage A. Geurden *et al.* (2008) reported that Assemblage E occurred in 53% (n=53) of Belgium calves less than 10 weeks of age, 16% (n=16) Assemblage A and 31% (n=31) mixed infection Assemblage A/E, the first report of mixed infections in calves. As these studies demonstrate, cattle typically harbour one of two genotypes of *G. duodenalis*; the livestock genotype (Assemblage E) and to a lesser incidence (<20%) Assemblage A (the most common genotype affecting humans) (Thompson, 2004; Trout *et al.*, 2004; Trout *et al.*, 2007). Results of the current study with 2 beef animals infected with Assemblage C and D suggest that perhaps these animals were infected by dogs while on pasture or that these are not infections but rather cysts passing through the animals. However, the number of genotyped samples is so small that it would be difficult to draw any conclusions as to the
source of the Assemblage C and D infections and further study would be warranted to validate these unusual findings.

The one animal infected with Assemblage B is a significant finding. Typically farm animals are more commonly infected with Assemblages A and E, however Aloisio et al. (2006) has reported Assemblage B to cause severe weight loss and impaired feed efficiency in 30-90 day old naturally infected lambs. Due to G. duodenalis Assemblage B zoonotic potential, its existence in cattle has public health implications (Thompson, 2004).

Isolates from one farm were genotyped, one by PCR amplification and sequencing, the other using QPCR and melting curve analysis. QPCR and melting curve analysis was used to indicate the temperature at which PCR products melt and then compare to reference peaks to determine species. The feedlot steers (2) sampled were infected with C. andersoni. This is the first time that C. andersoni has been reported in Australian cattle.
Chapter 5

Prevalence and Impact on Performance of

*Giardia duodenalis* and *Cryptosporidium Andersoni* in Single Source Alberta, Canada

Feedlot Beef Steers
5.1 Introduction

*G. duodenalis* and *Cryptosporidium* spp. are two of the most common protozoan parasites in humans and animals and are distributed worldwide (Wolfe, 1992; Scott *et al.*, 1994). *G. duodenalis* develops in the small intestine of cattle after faecal-oral ingestion, remaining either subclinical or causing malabsorptive diarrhoea, increased intestinal motility, decreased weight gain and impaired feed efficiency (Kirkpatrick, 1989; Olson *et al.*, 1995; Marshall *et al.*, 1997; Olson *et al.*, 2004). *G. duodenalis* has been reported in calves 5 – 10 weeks of age and can persist for months with intermittent shedding of cysts (Xiao, 1994; O’Handley *et al.*, 1997; 1999). The prevalence of *G. duodenalis* in cattle populations has been reported as high as 100% in calves and 82% in mature feedlot cattle (Xiao, 1994; O’Handley *et al.*, 1997; 1999; Ralston *et al.*, 2002).

*C. andersoni* can chronically infect the abomasum of older calves and mature cattle, and has been reported to reduce milk production in dairy cows by approximately 3.2 kg d\(^{-1}\) and to reduce weight gains in some feedlot steers by 10 – 50% (Anderson, 1987; 1990; 1991; Estaban *et al.*, 1995). The prevalence of *C. andersoni* in cattle populations ranges between 1.6 and 85% with intermittent oocyst shedding (Anderson, 1990; 1991; Estaban *et al.*, 1995; Bukhari and Smith., 1996; Fayer *et al.*, 1997; Ralston *et al.*, 2002).

### 5.1.1 Aim

Documented longitudinal studies of beef feedlot cattle’s protozoan parasite prevalence and infection patterns are limited. The objective of this study was to determine whether these parasites have a detrimental impact on animal performance in order to assess if therapeutic intervention is warranted.

### 5.2 Materials and Methods

#### 5.2.1 Animals

Three hundred six month old crossbred steers (Hereford x Angus) (198 ± 20 kg initial body weight) were blocked by weight (to average pen weights) and assigned to 30 pens at the feedlot facility of Agriculture and Agri-Food Canada Research Station in Lethbridge, Alberta, Canada. The steers were derived in October at approximately six months of age from a single source located within 100 kms of the research facility where they had resided from birth until weaning at the initiation of this 314 d study. Thirty pens (10 steers
per pen) were assigned to each of six antibiotic treatments as part of a companion study that examined the effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* in feedlot cattle. Treatments included 1) control, no antibiotics; 2) chlortetracycline and sulfamethazine (each at 350 mg/head.d); fed as Aureo S® - 700G, Alpharma Inc., NJ USA; 3) chlortetracycline (11 ppm; fed as Aureomycin® - 100G, Alpharma); 4) monensin (25 ppm, fed as Rumensin®, Elanco Animal Health, AB., Canada); 5) tylosin phosphate (11 ppm, fed as Tylan®, Elanco Animal Health); 6) virginamycin (250 mg/head.d); fed as V-Max®, Pfizer Animal Health, NY, USA). Adjacent pens were supplied with water by a common trough that was available *ad libitum* to the animals. All animals were cared for according to the Guidelines for Care and Use of Experimental Animals (Canadian Council on Animal Care 1993).

### 5.2.2 Diets, Feeding and Weighing

Animals were fed a typical Canadian forage-based backgrounding diet for the first 115 d consisting of 70% barley silage, 25% barley grain and 5% supplement (DM basis). Adaptation to a grain-based finishing ration occurred over a 21 d transition period cumulating in a diet consisting of 85% barley, 10% barley silage and 5% supplement (DM basis). Cattle were maintained on this diet for an additional 178 d. Steers were fed once daily, consuming all provided feed. Every 28 d each animal was weighed in the morning prior to feeding.
5.2.3 Faecal Collection and Analysis

Faecal samples (1 – 5 g) were collected rectally, using a disposal latex glove, from each steer on five days (d15, d57, d92, d183, d225) during the 314d study. Three samples (d15, d57, d92) were collected during the silage-based backgrounding period and two samples (d183, d225) were collected during the grain-based finishing diet period. Faecal samples were placed in weighed centrifuge tubes and stored at 4°C until further processing.

Faecal analysis was performed according to previously described procedures (Olson et al., 1997a; O’Handley et al., 1999). Cysts and oocysts were enumerated at 100x and 400x magnification, respectively, dry objective, with an epifluorescence microscope (Olympus, BX60, Olympus Optical Co. Ltd. Japan). The number of (oo)cysts per gram of faeces was then calculated using a previously described formula (O’Handley et al., 1999). The sensitivity of this detection method was 66 (oo)cysts per gram of faeces (O’Handley et al., 1999).

5.2.4 Data Analysis

Proportions of parasitized and non-parasitized animals were compared using Prism Version 2.0 software Fishers exact test (P < 0.05 considered significantly different). Geometric mean (oo)cyst counts for parasitized animals on each sample date were compared to those recorded on the first
sample date (d15) using Prism Version 2.0 software Unpaired t test ($P < 0.05$ considered significantly different). Average daily gain (ADG) values were analysed using Prism Version 2.0 software Unpaired t test ($P < 0.05$ considered significantly different). Performance data comparing infected and non-infected animals for *G. duodenalis* and *C. andersoni* was done within treatment groups to eliminate the effects of antibiotic treatments on performance between treatment groups. Parasite prevalence and (oo)cyst shedding data was not segregated into treatment groups but rather analysed as a whole (300 head) since treatment antibiotics had no observed or documented influence on parasitic infection. Infected and non-infected animals were defined two ways for the purposes of ADG analysis. The first definition was: an animal was considered infected if it had one or more parasite-positive samples for the period of interest (e.g. backgrounding period Days 0-115). The second definition was: an animal was considered infected if it had >50% parasite-positive samples for the period of interest. The animals considered positive under the first definition would reflect those animals, which were either intermittent or chronic shedders of (oo)cysts. The second definition would reflect those animals that were more chronic shedders of (oo)cysts.

### 5.3 Results

The prevalence of *G. duodenalis* and *C. andersoni* in the 300 feedlot steers for the duration of the 314d study is summarized in Fig. 5.1. There was a decrease ($P < 0.05$) in the percentage of *G. duodenalis*-infected steers on Day 57.
compared to the first sample date (d15) of the trial, while *C. andersoni* infected steers did not differ significantly from the first sample date throughout the duration of the trial. Overall prevalence (as defined as one or more positive samples for a particular animal during the duration of the study e.g., $\geq 1/5$) of *G. duodenalis* and *C. andersoni* was 82 and 12%, respectively. Combined *G. duodenalis* and *C. andersoni* infections occurred at the same time in 5% of the steers, and infection with only *G. duodenalis* and only *C. andersoni* were 76 and 1%, respectively. These parasites could not be detected in 17% of the animals for the duration of the study.

The number of *G. duodenalis* cysts and *C. andersoni* oocysts shed per gram of faeces in the positive animals did not vary significantly throughout the trial period (Fig. 5.2). Some animals continued to shed *G. duodenalis* cysts and *C. andersoni* oocysts at $10^3$ and $10^4$ per gram of faeces respectively until the end of the study.
**Figure 5.1** Percentage of steers shedding *G. duodenalis* cysts per sample date (square ■). Percentage of steers shedding *C. andersoni* oocysts per sample date (triangle ▲).

* indicates significant difference ($P < 0.05$) from first sampling date (d15).

![](image1.png)

**Figure 5.2** Mean number of *G. duodenalis* cysts excreted per gram of faeces by infected steers (square ■).

Mean number of *C. andersoni* oocysts excreted per gram of faeces by infected steers (triangle ▲).

![](image2.png)
ADG was compared between *G. duodenalis* infected and non-infected animals over three set periods of time (Backgrounding/Transition period d0 – 136, Finishing period d137 – 314, Overall d0 – 314) and under the two previously described definitions of infected (Table 5.1 and 5.2). Similar comparisons were made between *C. andersoni* infected and non-infected animals.

A comparison between *G. duodenalis* infected and non-infected steers’ ADG demonstrated no overall differences (*P* > 0.05) with the exception of a lower (*P* < 0.05) ADG for non-infected steers compared to infected (defined as ≥ 1 parasite positive samples during the period) for treatment 5. A *C. andersoni* comparison between infected (defined as ≥ 1 parasite positive samples during the period) and non-infected animals demonstrated no overall differences (*P* > 0.05). Infected (defined as > 50% parasite positive samples during the period) and non-infected steers ADG comparison was not possible as none of the study animals met the criteria.
Table 5.1  Performance data of *G. duodenalis* and *C. andersoni*  
infected feedlot steers (infected = ≥ 1 parasite positive samples during the period)

<table>
<thead>
<tr>
<th></th>
<th>Giardia duodenalis</th>
<th>Cryptosporidium andersoni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection Status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>Non-Infected</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>SE</td>
</tr>
</tbody>
</table>
| Period 1 Days 0 to 136 
Trt 1 (n) ADG (kg d⁻¹) | (36) 1.05          | (14) 1.09                |
|                  | 0.03               | 0.03                      |
|                  | (30) 1.10          | (20) 1.12                |
|                  | 0.02               | 0.03                      |
|                  | (35) 1.05          | (15) 1.02                |
|                  | 0.02               | 0.04                      |
|                  | (36) 1.07          | (14) 1.06                |
|                  | 0.02               | 0.03                      |
|                  | (39) 1.05          | (10) 1.02                |
|                  | 0.02               | 0.04                      |
|                  | (37) 1.02          | (12) 1.02                |
|                  | 0.02               | 0.07                      |
| Period 2 Days 137 to 314 
Trt 1 (n) ADG (kg d⁻¹) | (33) 1.28          | (17) 1.25                |
|                  | 0.03               | 0.04                      |
|                  | (23) 1.31          | (27) 1.30                |
|                  | 0.03               | 0.03                      |
|                  | (16) 1.23          | (34) 1.30                |
|                  | 0.04               | 0.03                      |
|                  | (34) 1.23          | (16) 1.25                |
|                  | 0.03               | 0.04                      |
|                  | (16) 1.20          | (33) 1.18                |
|                  | 0.04               | 0.03                      |
|                  | (25) 1.24          | (24) 1.22                |
|                  | 0.03               | 0.03                      |
| Overall Days 0 to 314 
Trt 1 (n) ADG (kg d⁻¹) | (44) 1.20          | (6) 1.18                 |
|                  | 0.02               | 0.03                      |
|                  | (36) 1.24          | (14) 1.25                |
|                  | 0.02               | 0.04                      |
|                  | (37) 1.19          | (13) 1.16                |
|                  | 0.02               | 0.04                      |
|                  | (41) 1.17          | (9) 1.19                 |
|                  | 0.02               | 0.04                      |
|                  | (43) 1.15a         | (6) 1.046                |
|                  | 0.02               | 0.06                      |
|                  | (41) 1.14          | (8) 1.13                 |
|                  | 0.02               | 0.06                      |
|                  |                    |                           |
|                  | (6) 1.15           | (2) 1.24                 |
|                  | 0.04               | 0.04                      |
|                  | (8) 1.14           | (12) 1.15                |
|                  | 0.06               | 0.03                      |
|                  | (12) 1.15          | (12) 1.15                |
|                  | 0.03               | 0.03                      |
|                  | (6) 1.11           | (6) 1.11                 |
|                  | 0.08               | 0.08                      |
|                  | (43) 1.14          | (43) 1.14                |
|                  | 0.02               | 0.02                      |

*a-b* values are means, means followed by different letters within a row for a particular parasite differ (*P* < 0.05).

Trt is treatment group.

ADG is average daily gain in kilograms per day.
Table 5.2  Performance data of *G. duodenalis* infected feedlot steers

(infected = > 50% parasite positive samples during the period)

<table>
<thead>
<tr>
<th></th>
<th>Infected</th>
<th>SE</th>
<th>Non-Infected</th>
<th>SE</th>
</tr>
</thead>
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<tr>
<td><strong>Period 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 136</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trt 1 (n) ADG (kg d⁻¹)</td>
<td>(15) 1.09</td>
<td>0.04</td>
<td>(35) 1.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Trt 2</td>
<td>(5) 1.03</td>
<td>0.04</td>
<td>(45) 1.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 3</td>
<td>(13) 1.04</td>
<td>0.03</td>
<td>(37) 1.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 4</td>
<td>(18) 1.06</td>
<td>0.03</td>
<td>(32) 1.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 5</td>
<td>(7) 1.03</td>
<td>0.03</td>
<td>(42) 1.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 6</td>
<td>(19) 1.02</td>
<td>0.03</td>
<td>(30) 1.02</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Period 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 137 to 314</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trt 1 (n) ADG (kg d⁻¹)</td>
<td>(9) 1.27</td>
<td>0.06</td>
<td>(41) 1.27</td>
<td>0.03</td>
</tr>
<tr>
<td>Trt 2</td>
<td>(6) 1.25</td>
<td>0.04</td>
<td>(44) 1.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 3</td>
<td>(8) 1.22</td>
<td>0.07</td>
<td>(42) 1.29</td>
<td>0.03</td>
</tr>
<tr>
<td>Trt 4</td>
<td>(14) 1.27</td>
<td>0.05</td>
<td>(36) 1.23</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 5</td>
<td>(1) N/A</td>
<td>N/A</td>
<td>(48) N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Trt 6</td>
<td>(8) 1.22</td>
<td>0.05</td>
<td>(41) 1.23</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 314</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trt 1 (n) ADG (kg d⁻¹)</td>
<td>(17) 1.21</td>
<td>0.03</td>
<td>(34) 1.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 2</td>
<td>(7) 1.21</td>
<td>0.03</td>
<td>(43) 1.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 3</td>
<td>(13) 1.14</td>
<td>0.03</td>
<td>(37) 1.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 4</td>
<td>(22) 1.18</td>
<td>0.02</td>
<td>(28) 1.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 5</td>
<td>(3) 1.13</td>
<td>0.02</td>
<td>(46) 1.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Trt 6</td>
<td>(15) 1.16</td>
<td>0.03</td>
<td>(34) 1.13</td>
<td>0.02</td>
</tr>
</tbody>
</table>

### 5.4 Discussion

Overall prevalence of *G. duodenalis* in the 300 feedlot steers over the 314 d study was 82%. A point prevalence study by Olson *et al.* (1997b) of 104 beef cattle greater than 6 months of age from 15 different Canadian geographical locations reported a *G. duodenalis* prevalence of 11%, which is considerably lower than that found in the present study. McAllister *et al.* (2005) reported a *G. duodenalis* point prevalence of 8.7% in beef cows from 39 separate farms.
located in Ontario, Canada. Buret *et al.* (1990a) observed a 10.4% prevalence of *G. duodenalis* 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 the fact that only one faecal sample was collected from each animal. Faecal samples were collected only once in the studies by Olson, McAllister and Buret, whereas animals in this study had 5 samples collected over a 314 d study period. The five individual samples collected per animal gave a more accurate assessment of actual prevalence of *G. duodenalis* in cattle over 6 months of age as compared to single samplings per animal. Ralston *et al.* (2002) reported the only documented longitudinal study of *G. duodenalis* prevalence in 60 beef feedlot cattle showing a prevalence of 82%, consistent with what was determined in the current study. Prevalence of *G. duodenalis* throughout the study was constant with the exception of the d57 sample date that was significantly (*P* < 0.05) lower than the rest. This drop may be due to animals clearing their infection or reduction in shedding after the initial co-mingling and stress that occurred upon introduction to the feedlot. The subsequent rise in prevalence at d92 may be due to reinfection 35d after clearing the initial infection. This pattern is similar to that reported by Ralston *et al.* (2002) where *G. duodenalis* prevalence peaked at d41 post feedlot arrival and decreased throughout the duration of the study. When comparing the two studies the Day 0 prevalence in Ralston *et al.* (2002) must be ignored to account for the current study’s first sampling not occurring until d15 which reflects the increase in the prevalence of *G. duodenalis*. This
increase may be related to the effects of stress at weaning and introduction of animals to the feedlot causing a temporary relaxation of immunity (Xiao et al., 1994).

Cyst excretion levels in infected steers were constant throughout the study, not varying significantly from those on d15 (first sampling date). The maximum cysts shed per gram of faeces by an individual animal was $10^5$. Ralston et al. (2002) reported a fluctuation in cyst shedding throughout the longitudinal beef feedlot study period showing an intermittent cyst shedding pattern, with cysts shed per gram of faeces also reaching $10^5$. The difference in cyst shedding patterns could be due to the intensity of sampling where the current study had 5 samplings over 314d and Ralston et al. (2002) had 12 samplings over 257d, perhaps documenting more clearly the intermittent nature of shedding of *G. duodenalis* cysts. At the last sample (d225) 32% of the steers were still shedding an average of $10^3$ cysts per gram of faeces suggesting that infected animals may continue to shed cysts into maturity. Ralston et al. (2002) also reported continuous cyst shedding throughout the course of their study. These results raise the concern of the potential for meat to become contaminated with *G. duodenalis* cysts at the time of slaughter (Moriarty et al., 2005; Smith et al., 2007; Duffy et al., 2008).

*G. duodenalis* infected and non-infected steers for either definition of infection during any of the three periods of interest had no significant
difference in ADG. The exception was the overall period for Treatment (Trt) four where infected (defined as 1 or more positive samples) animals had a significantly ($P < 0.05$) higher ADG than non-infected. Olson et al. (1995) reported a significant reduction in rate of gain and impairment in feed efficiency in market lambs infected with $G. \text{duodenalis}$ compared to non-infected lambs, attributed to malabsorption and maldigestion of carbohydrates, fats and vitamins. These reductions were not found in the present study. However, Ralston et al. (2002) reported similar results to the current study highlighting the need for further investigation into the impact of variables such as animal species, age, level of infection, environment challenge, other diseases and ration type on animal performance to clarify the variability in reported results.

$C. \text{andersoni}$ prevalence in the beef steers was 12% over the 314d study period. Anderson (1991) reported the prevalence of $C. \text{andersoni}$ in cattle to be 4.7% in the United States. In another study of feedlot cattle in California, prevalence was reported at 11.8% by Anderson (1991). Both of these studies involved one time sampling of the cattle with similar results to our longitudinal study. Ralston et al. (2002) reported a $C. \text{andersoni}$ prevalence of 85% over a 257d study, considerably higher than the 12% found in this longitudinal study. Infrequent sampling, different supply sources of cattle (resulting in variability of prior exposure to pathogens) and stress in this study as compared to Ralston et al. (2002) may account for decreased prevalence
detection. Both studies had cattle housed at the Lethbridge Research Station, Alberta, Canada with similar health and nutrition management systems.

Prevalence of *C. andersoni* throughout this study remained constant and did not differ significantly (*P* > 0.05) from the first sample date. Conversely, Ralston *et al.* (2002) reported a reduction (*P* > 0.05) in prevalence of *C. andersoni* infected steers from d97 to the conclusion of the study. This difference may be due to the above mentioned reasons.

*C. andersoni* oocyst shedding of infected steers did not vary significantly (*P* > 0.05) from that of the first sample date. Ralston *et al.* (2002) reported an increase (*P* < 0.05) in oocysts per gram of faeces from d132 to d257. This variation between the two studies may again be due to herd specific factors such as previous exposure to pathogens and stress. The current study had steers shedding $10^4$ oocysts per gram of faeces on the final sample date indicating that some animals infected may continue to shed large numbers of oocysts into maturity.

Overall, *C. andersoni* infected versus non-infected steers had no significant (*P* < 0.05) difference in ADG. Impaired performance in cattle infected with *C. andersoni* has been reported by Anderson (1990). Estaban *et al.* (1995) reported a 3.2 kg d$^{-1}$ reduction in milk production by dairy cows shedding *C. andersoni* oocysts compared to non-infected cows. Anderson (1990) described
cattle with *C. andersoni* as having hypertrophy of gastric mucosa, hyperplasia of mucus neck cells, thinning of lining epithelium and dilation of gland lumens. Anderson (1990) suggests that these pathological changes may impair protein digestion. Plasma pepsinogen concentrations of *C. 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. The maldigestion of protein may be due to its reliance on pepsin that is derived from pepsinogen and activated at an acidic pH. Gastric pH has been reported to increase with *C. andersoni* infection and consequently the conversion of pepsin to pepsinogen may be impaired.

The present study describes the prevalence, infection pattern and (oo)cyst shedding of *G. duodenalis* and *C. andersoni* in beef feedlot steers. The effect of these parasites on animal performance requires further study, utilizing larger numbers of animals to establish a more consistent baseline for impact on ADG and to clarify the variations reported in the literature.
Chapter 6

A Longitudinal Study of Prevalence, Infection Pattern and Impact on Performance of *Giardia duodenalis* and *Cryptosporidium andersoni* in Alberta, Canada Feedlot Beef Steers
6.1 Introduction

The study described in Chapter 5 documents prevalence and impact on performance of *G. duodenalis* and *Cryptosporidium* spp. in feedlot beef cattle in Alberta, Canada. The current study in Alberta, Canada is also a simultaneous study of two groups of feedlot beef cattle where prevalence of these parasites is determined and their impact on animal performance is evaluated. A subset of samples from animals testing microscope positive for *G. duodenalis* were genotyped using PCR. Several of the *Cryptosporidium* spp. microscope positive samples were also sequenced using PCR to confirm *C. andersoni*.

6.1.1 Aim

The current study was designed to expand the sampling carried out in the study described in Chapter 5 and to allow for the molecular analysis of parasite positive microscope samples to evaluate zoonotic potential and compare to the Australian samples in the study described in Chapter 4.

6.2 Materials and Methods

6.2.1 Animals

6.2.1.1 Pre-Weaned Calves

Fifty-nine and fifty-seven crossbred, March - April 2004 born steer and heifer calves were sampled on Sept. 15 and Oct. 5, 2004 respectively, at their ranch.
of birth located approximately 100 kms from the Agriculture & Agri-Food Canada Research Station in Lethbridge, Alberta, Canada (Figures 6.1a and 6.1b). These calves were then weaned, transported to the Agriculture and Agri-Food Canada Research Station in Lethbridge, Alberta, Canada and allocated as a portion of the feedlot animals in both TGAPS and KK0429 groups.
Figures 6.1a and 6.1b Pre-weaned calves
6.2.1.2 TGAPS

Two hundred and forty crossbred steers (249 ± 26 kg initial body weight) were blocked by weight and assigned to 24 pens (ten head per pen) at the feedlot facility of Agriculture and Agri-Food Canada Research Station in Lethbridge, Alberta, Canada Figure 6.2). The steers were derived from a single source located within 100 km of the research facility where they had resided from birth until weaning at the initiation of this 225 d study. Five pens (10 steers per pen) were assigned to each of four antibiotic treatments and 4 pens were used for treatment 5 as part of a companion study that examined the effect of sub therapeutic administration of antibiotics on the prevalence of antibiotic resistant *Enterococci, E. coli* and *Camplylobacter* in feedlot cattle. Treatments included 1) control, no antibiotics; 2) chlorotetracycline and sulfamethazine (AS7000) (each at 350 mg/hd/d); 3) chlorotetracycline (11 ppm; fed as Aureomycin® 100G, Alpharma); 4) tylosin phosphate (11 ppm, fed as Tylan®, Elanco Animal Health); 5) chlorotetracycline (350 mg/hd/d). Adjacent pens were supplied with water by a common trough that was available *ad libitum* to the animals.
6.2.1.3 KK0429

Three hundred and twelve crossbred steers (243 ± 25 kg initial body weight) were blocked by weight and assigned to 24 pens (13 head per pen) at the feedlot facility of Agriculture and Agri-Food Canada Research Station in Lethbridge, Alberta, Canada (Figure 6.3). The steers were derived from a single source located within 100 km of the research facility where they had resided from birth until weaning at the initiation of this 225 d study. Six pens (13 steers per pen) were assigned to each of four feed rations as part of a companion study that examined the effect of ration protein source in feedlot cattle. Treatments included: 1) control; 2) canola; 3) Urea; 4) SoyP. Adjacent
pens were supplied with water by a common trough that was available *ad libitum* to the animals.

All animals were cared for according to the Guidelines for Care and Use of Experimental Animals (Canadian Council on Animal Care 1993).

**Figure 6.3 KK0429 steers**

6.2.2  Diets, Feeding and Weighing

6.2.2.1 Pre-weaned Calves

Animals were grazing a native fescue grass pasture and water was supplied by runoff dugouts *ad libitum*. No animal weights were collected.
6.2.2.2 TGAPS

Animals were fed a typical Canadian forage-based backgrounding diet for the first 80 d consisting of 70% barley silage, 25% barley grain and 5% supplement (DM basis). Cattle were then subsequently transitioned from the silage-based diet to a grain-based diet (85% barley, 10% barley silage, 5% supplement, DM basis) over a 21 d period and maintained on this diet for an additional 124 d until the conclusion of the study. Steers were fed once daily, consuming all provided feed. Each animal was weighed monthly in the morning prior to feeding.

6.2.2.3 KK0429

Animals were fed a backgrounding diet for the first 80 d consisting of 55% barley silage, 39% barley grain and 6% supplement (DM basis). Cattle were then subsequently transitioned from the backgrounding diet to a finishing diet (84% barley, 9% barley silage, 7% supplement, DM basis) (Figure 6.4) over a 21 d period and maintained on this diet for an additional 124 d until the conclusion of the study. Steers were fed once daily, consuming all provided feed. Each animal was weighed monthly in the morning prior to feeding.
6.2.3 Faecal Collection and Analysis

6.2.3.1 Pre-Weaned Calves

Faecal samples (1-5g) were collected from faecal pats after calves were observed to defecate, using a disposal latex glove on Sept. 15 (n=59) and Oct. 5 (n=57), 2004 prior to calves being weaned. Faecal samples were placed in weighed centrifuge tubes and stored at 4°C until further processing. Faecal analysis and (oo)cyst enumeration was performed according to previously described procedures (Olson et al., 1997a; O’Handley et al., 1999).
6.2.3.2 TGAPS and KK0429

Faecal samples (1-5g) were collected rectally, using a disposal latex glove, from each steer on a monthly basis during the 225 d study. Three samples (Nov., Dec., Jan.) were during the silage-based backgrounding period; one sample (Feb.) during the transition period and five samples (Mar., Apr., May, Jun., Jul.) during the grain-based finishing diet period. Faecal samples were placed in weighed centrifuge tubes and stored at 4°C until further processing. Faecal analysis and (oo)cyst enumeration was performed according to previously described procedures (Olson et al., 1997a; O’Handley et al., 1999).

6.2.4 DNA Extraction, Amplification and Sequencing

DNA was extracted, amplified and sequenced utilizing procedures outlined in section 2.3. Only the subset of microscopic positive samples in Table 6.1 were tested by PCR to verify parasite presence and to genotype *G. duodenalis*.
Table 6.1 DNA Sequenced Samples from Alberta, Canada

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Animal Group</th>
<th>Fragment Tested</th>
<th>Result</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal 373</td>
<td>KK0429</td>
<td>Giardia 18S</td>
<td>Assemblage E</td>
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</tr>
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<td>Jan 26/05</td>
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<td></td>
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<td>Giardia 18S</td>
<td>Assemblage E</td>
<td>DQ448635</td>
</tr>
<tr>
<td>Apr 13/05</td>
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<td>KK0429</td>
<td>Giardia 18S</td>
<td>Assemblage E</td>
<td>DQ448636</td>
</tr>
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<tr>
<td>Animal 535</td>
<td>TGAPS</td>
<td>Giardia 18S</td>
<td>Assemblage A</td>
<td>DQ448637</td>
</tr>
<tr>
<td>Mar 21/05</td>
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<tr>
<td>Animal 536</td>
<td>TGAPS</td>
<td>Giardia 18S</td>
<td>Assemblage A</td>
<td>DQ448638</td>
</tr>
<tr>
<td>Mar 21/05</td>
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<tr>
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<td>TGAPS</td>
<td>Giardia 18S</td>
<td>Assemblage A</td>
<td>DQ448639</td>
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<tr>
<td>Feb 21/05</td>
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<td>Animal 545</td>
<td>TGAPS</td>
<td>Giardia 18S</td>
<td>Assemblage E</td>
<td>DQ448640</td>
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<tr>
<td>Apr 18/05</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Animal 551</td>
<td>TGAPS</td>
<td>Crypto 18S</td>
<td>C. andersoni</td>
<td>DQ448631</td>
</tr>
<tr>
<td>Jul 11/05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal 551</td>
<td>TGAPS</td>
<td>Crypto 18S +</td>
<td>C. andersoni</td>
<td>DQ448632</td>
</tr>
<tr>
<td>Jun 14/05</td>
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<td>Actin</td>
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</tr>
</tbody>
</table>

6.2.5 Data Analysis

6.2.5.1 TGAPS

Proportions of parasitized and non-parasitized animals were compared using Prism Version 2.0 software Fishers exact test (P < 0.05 considered significantly different). Geometric mean (oo)cyst counts for parasitized animals on each sample date were compared to those recorded on the first
sample date (Nov. 04) using Prism Version 2.0 software Unpaired t test ($P < 0.05$ considered significantly different). Average daily gain (ADG) values were analysed using Prism Version 2.0 software Unpaired t test ($P < 0.05$ considered significantly different). Performance data comparing infected and non-infected animals for *G. duodenalis* and *C. andersoni* was done within treatment groups to eliminate the effects of antibiotic treatments on performance between treatment groups. Parasite prevalence and (oo)cyst shedding data was not segregated into treatment groups but rather analysed as a whole (240 head) since treatment antibiotics had no observed or documented influence on parasitic infection. Infected and non-infected animals were defined two ways for the purposes of ADG analysis. The first definition was: an animal was considered infected if it had one or more parasite-positive samples during the period of interest (*e.g.* backgrounding period Days 0-80). The second definition was: an animal was considered infected if it had >50% parasite-positive samples for the period of interest. The animals considered positive under the first definition would reflect those animals, which were either intermittent or chronic shedders of (oo)cysts, and the second definition would reflect those animals that were more chronic shedders of (oo)cysts.

### 6.2.5.2 KK0429

Proportions of parasitized and non-parasitized animals were compared using Prism Version 2.0 software Fishers exact test ($P < 0.05$ considered significantly different). Geometric mean (oo)cyst counts for parasitized
animals on each sample date were compared to those recorded on the first sample date (Nov. 04) using Prism Version 2.0 software Unpaired t test ($P < 0.05$ considered significantly different). ADG values were analysed using Prism Version 2.0 software Unpaired t test ($P < 0.05$ considered significantly different). Performance data comparing infected and non-infected animals for *G. duodenalis* and *C. andersoni* was not segregated into treatment groups but rather analysed as a whole (312 head) for each feeding period as protein source had no observed influence on ADG, parasite prevalence and (oo)cyst shedding data was also not segregated into treatment groups but rather analysed as a whole (312 head) since protein source had no observed or documented influence on parasitic infection. Infected and non-infected animals were defined two ways for the purposes of ADG analysis. The first definition was: an animal was considered infected if it had one or more parasite-positive samples during the period of interest (e.g. backgrounding period Days 0-80). The second definition was: an animal was considered infected if it had >50% parasite-positive samples during the period of interest. The animals considered positive under the first definition would reflect those animals, which were either intermittent or chronic shedders of (oo)cysts, the second definition would reflect those animals that were more chronic shedders of (oo)cysts.
6.3 Results

6.3.1 Pre-weaned Calves

The prevalence of *G. duodenalis* and *C. andersoni* in the first pre-weaning sampling on Sept. 15, 2004 was 5.1 and 0% respectively. Prevalence of *G. duodenalis* and *C. andersoni* on the subsequent sampling Oct. 5, 2004 was 8.8 and 0% respectively. *G. duodenalis* infected animals were shedding an average of $10^3$ oocysts per gram of faeces.

6.3.2 TGAPS

6.3.2.1 Protozoal Prevalence

The prevalence of *G. duodenalis* and *C. andersoni* in the 240 feedlot steers for the duration of the 225d study is summarized in Fig. 6.5. The percentage of *G. duodenalis*-infected steers increased ($P<0.05$) on Day 85 through Day 225 compared to the first sample date (d1) of the trial, peaking on Day 113. *C. andersoni* infected steers did not differ significantly from the first sample date throughout the duration of the trial. Overall prevalence (as defined as one or more positive samples for a particular animal during the duration of the study e.g., $\geq 1/9$) of *G. duodenalis* and *C. andersoni* was 39.0 and 2.9%, respectively. Combined *G. duodenalis* and *C. andersoni* infections occurred at the same time in 0.8% of the steers, and infection with only *G. duodenalis* and only *C. andersoni* was 38 and 2%, respectively. These parasites could not be detected in 59% of the animals for the duration of the study.
The number of *G. duodenalis* cysts and *C. andersoni* oocysts shed per gram of faeces in the positive animals did not vary significantly throughout the trial period (Fig. 6.6). Some animals continued to shed *G. duodenalis* cysts and *C. andersoni* oocysts at an average of $10^3$ and $10^4$ per gram of faeces respectively until the end of the study.

**Figure 6.5** Percentage of TGAPS steers shedding *G. duodenalis* cysts per sample date (square □). Percentage of steers shedding *C. andersoni* oocysts per sample date (triangle ▲). * indicates significant difference ($P < 0.05$) from first sampling date (d1).
**Figure 6.6** Mean number of *G. duodenalis* cysts excreted by infected TGAPS steers (square □). Mean number of *C. andersoni* oocysts excreted by infected TGAPS steers (triangle ▲).

**6.3.2.2 Average Daily Gain**

ADG was compared between *G. duodenalis*-infected and non-infected animals over three set periods of time (Backgrounding period d0 – 80, Finishing period d81 – 225, Overall d0 – 225) and under the two previously mentioned definitions of infected (Table 6.2). Similar comparisons were made between *C. andersoni* infected and non-infected animals.

A comparison between *G. duodenalis*-infected and non-infected steers’ ADG demonstrated no overall differences (*P*>0.05). A *C. andersoni* comparison between infected (defined as ≥ 1 parasite positive samples during the period) and non-infected animals demonstrated no overall differences (*P*>0.05).
Infected (defined as > 50% parasite positive samples during the period) and non-infected steers’ ADG comparison was not possible as none of the study animals met the criteria.

**Table 6.2 Performance data of *G. duodenalis* infected feedlot steers**

*(infected = ≥ 1 parasite positive samples during the period)*

<table>
<thead>
<tr>
<th>Period</th>
<th>Infected (kg d⁻¹)</th>
<th>SE</th>
<th>Non-Infected (kg d⁻¹)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 80</td>
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<td></td>
</tr>
<tr>
<td>Control (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS700</td>
<td>(2) .70a</td>
<td>0.08</td>
<td>(48) .80a</td>
<td>0.02</td>
</tr>
<tr>
<td>T11</td>
<td>(4) .88a</td>
<td>0.04</td>
<td>(46) .91a</td>
<td>0.02</td>
</tr>
<tr>
<td>A11</td>
<td>(1) N/A</td>
<td>N/A</td>
<td>(48) N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>A44</td>
<td>(3) .77a</td>
<td>0.10</td>
<td>(47) .77a</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(2) .82a</td>
<td>0.04</td>
<td>(38) .87a</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 81 to 225</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS700</td>
<td>(18) 1.32a</td>
<td>0.05</td>
<td>(32) 1.40a</td>
<td>0.05</td>
</tr>
<tr>
<td>T11</td>
<td>(19) 1.33a</td>
<td>0.04</td>
<td>(31) 1.31a</td>
<td>0.05</td>
</tr>
<tr>
<td>A11</td>
<td>(20) 1.34a</td>
<td>0.04</td>
<td>(30) 1.33a</td>
<td>0.04</td>
</tr>
<tr>
<td>A44</td>
<td>(21) 1.32a</td>
<td>0.03</td>
<td>(29) 1.36a</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(8) 1.71a</td>
<td>0.41</td>
<td>(32) 1.22a</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 225</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS700</td>
<td>(19) 1.30a</td>
<td>0.03</td>
<td>(31) 1.31a</td>
<td>0.03</td>
</tr>
<tr>
<td>T11</td>
<td>(22) 1.33a</td>
<td>0.03</td>
<td>(28) 1.29a</td>
<td>0.05</td>
</tr>
<tr>
<td>A11</td>
<td>(20) 1.29a</td>
<td>0.03</td>
<td>(30) 1.28a</td>
<td>0.03</td>
</tr>
<tr>
<td>A44</td>
<td>(22) 1.29a</td>
<td>0.03</td>
<td>(28) 1.27a</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(9) 1.38a</td>
<td>0.06</td>
<td>(31) 1.18a</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*a-b* values are means, means followed by different letters within a row for a particular parasite differ (*P* < 0.05).
6.3.2.3 Molecular Results

Of the five microscope positive isolates tested, 80% (4) were successfully sequenced at the *Giardia* 18S rDNA-rDNA locus. Three samples were Assemblage A (zoonotic: DQ448637, DQ448638, DQ448639) and one sample was Assemblage E (hoofed livestock: DQ448640). Of the three microscope positive *Cryptosporidium* samples, PCR and sequencing was only successful on 2 isolates (*C. andersoni*, GenBank accession numbers DQ448631 and DQ448632).

6.3.3 KK0429

6.3.3.1 Protozoal Prevalence

The prevalence of *G. duodenalis* and *C. andersoni* in the 312 feedlot steers for the duration of the 225d study is summarized in Fig. 6.7. There was variability throughout the study period in the percentage of *G. duodenalis*-infected steers. *C. andersoni*-infected steers did not differ significantly from the first sample date throughout the duration of the trial. Overall prevalence (defined as one or more positive samples for a particular animal during the duration of the study e.g., ≥1/9) of *G. duodenalis* and *C. andersoni* was 43.6 and 3.5%, respectively. Combined *G. duodenalis* and *C. andersoni* infections occurred at the same time in 1.3% of the steers, and infection with only *Giardia* and only *C. andersoni* were 42.6 and 2.2%, respectively. These parasites could not be detected in 52.9% of the animals for the duration of the study.
The number of *G. duodenalis* cysts and *C. andersoni* oocysts shed per gram of faeces in the positive animals did not vary significantly throughout the trial period (Fig. 6.8). Some animals continued to shed *G. duodenalis* cysts and *C. andersoni* oocysts at an average of $10^3$ and $10^4$ per gram of faeces respectively until the end of the study.

**Figure 6.7** Percentage of KK0429 steers shedding *G. duodenalis* cysts per sample date (square ■). Percentage of steers shedding *C. andersoni* oocysts per sample date (triangle ▲). * indicates significant difference ($P < 0.05$) from first sampling date (d1).
**Figure 6.8** Mean number of *G. duodenalis* cysts excreted by infected KK0429 steers (square ■). Mean number of *C. andersoni* oocysts excreted by infected KK0429 steers (triangle ▲).

6.3.3.2 Average Daily Gain

ADG was compared between *G. duodenalis*-infected and non-infected animals over three set periods of time (Backgrounding period d0 – 80, Finishing period d81 – 225, Overall d0 – 225) and under the two previously mentioned definitions of infection (Table 6.3). Similar comparisons were made between *C. andersoni*-infected and non-infected animals (Table 6.4).

A comparison between *G. duodenalis*-infected and non-infected steers’ ADG demonstrated no overall differences (*P*>0.05). A *C. andersoni* comparison between infected (defined as ≥ 1 parasite positive samples during the period) and non-infected animals demonstrated no overall differences (*P*>0.05). Infected (defined as > 50% parasite positive samples during the period) and
non-infected steers ADG comparison was only possible during the backgrounding period, as none of the study animals met the criteria during the other periods.

**Table 6.3 Performance data of *G. duodenalis* infected feedlot steers**

(infected = ≥ 1 parasite positive samples during the period) and (infected = ≥ 50% parasite positive samples during the period)

<table>
<thead>
<tr>
<th>Period</th>
<th>Infected</th>
<th>SE</th>
<th>Non-Infected</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1 (infected ≥ 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 80</td>
<td>(90) 0.76a</td>
<td>0.01</td>
<td>(221) 0.77a</td>
<td>0.01</td>
</tr>
<tr>
<td>Period 2 (infected ≥ 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 81 to 225</td>
<td>(86) 1.49a</td>
<td>0.03</td>
<td>(225) 1.47a</td>
<td>0.04</td>
</tr>
<tr>
<td>Overall (infected ≥ 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 225</td>
<td>(136) 1.41a</td>
<td>0.01</td>
<td>(175) 1.38a</td>
<td>0.03</td>
</tr>
<tr>
<td>Period 1 (infected &gt;50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 80</td>
<td>(16) 0.81a</td>
<td>0.03</td>
<td>(295) 0.77a</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*a-b* values are means, means followed by different letters within a row differ (*P* < 0.05).
Table 6.4  Performance data of *C. andersoni* infected feedlot steers  (infected = \( \geq 1 \) parasite positive samples during the period)

<table>
<thead>
<tr>
<th>Period</th>
<th>Infected</th>
<th>SE</th>
<th>Non-Infected</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1 (infected ( \geq 1 ))</td>
<td>(10) 0.72(a)</td>
<td>0.03</td>
<td>(301) 0.77(a)</td>
<td>0.01</td>
</tr>
<tr>
<td>Days 0 to 80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n ) ADG (kg d(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (infected ( \geq 1 ))</td>
<td>(9) 1.42(a)</td>
<td>0.05</td>
<td>(302) 1.39(a)</td>
<td>0.02</td>
</tr>
<tr>
<td>Days 0 to 225</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n ) ADG (kg d(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( a-b \) values are means, means followed by different letters within a row differ \( (P< 0.05) \).

6.3.3.3 Molecular Results

Of the eight microscope-positive isolates tested, 75\% (6) were successfully sequenced at the *Giardia* 18S rDNA-rDNA locus. All samples were Assemblage E (hoofed livestock: DQ448629, DQ448630, DQ448633, DQ448634, DQ448635, DQ448636). Of the four microscope-positive *Cryptosporidium* samples tested, PCR and sequencing was not successful on any of the samples.

6.4 Discussion

6.4.1 Pre-Weaned Calves

The prevalence of *G. duodenalis* in the pre-weaning sampled calves on the two sample dates was 5.1 and 8.8\%. These calves were 6 to 7 months of age at the time of sampling and still nursing their dams on range. There was no *Cryptosporidium* present in any of the calves on either sampling date. Trout *et*
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*al.* (2005) reported a *G. duodenalis* point prevalence of 42% in calves 6 months of age and 33% in calves 7 months of age from 14 dairy farms in 7 States of the United States. These results are substantially higher than the levels found in our study, likely due to the extensive pasture based management system of our pre-weaned beef calves. This would limit their exposure to *G. duodenalis* cysts for infection, as compared to the intensive management system of dairy farms resulting in higher animal density and greater exposure to cysts. Santin *et al.* (2004) reported *Cryptosporidium* species in 30.4% of 6 month old dairy calves on 15 farms in 7 US States however, there was no *Cryptosporidium* in our study’s sampling dates, again, reflecting the role that management systems play in the difference in prevalence of parasites between beef and dairy calves.

### 6.4.2 TGAPS and KK0429

Overall, prevalence of *G. duodenalis* in the TGAPS and KK0429 feedlot steers over the 225 day study was 39.0 and 43.6% respectively. This prevalence is considerably higher than the point prevalence previously reported by Olson *et al.* (1997b), McAllister *et al.* (2005) and Buret *et al.* (1990a) ranging from 8.7 to 11%. Comparing the current study’s *G. duodenalis* prevalence to the longitudinal studies’ prevalence reported by Ralston *et al.* (2002) and that of the study reported in Chapter 5 (both at 82%) the prevalence in the current study is much lower. All three studies were
conducted at the Lethbridge Research Station in Alberta, Canada and at the same time of year (Nov. – May). Animals were derived from a single source in all studies and in the Chapter 5 study the steers came from the same source as the current study. There are several variables that were not accounted for, that may have influenced the *G. duodenalis* prevalence. Weather conditions during the feeding period may have contributed to cysts remaining viable for longer periods of time, thereby resulting in animals having a higher exposure rate. Also, general health status of the steers during the feeding period (such as if animals were immunocompromised by other concurrent infections such as pneumonia or coccidiosis), may have made the general population more susceptible to infection by *G. duodenalis*. The TGAPS prevalence of *G. duodenalis* increased significantly (*P*<0.05) on Day 85 through Day 225 as compared to the first sample date, peaking on Day 113. The KK0429 prevalence of *G. duodenalis* was variable throughout the 225 day study, with a significant (*P*<0.05) increase from d15 – 141 (compared to Day 0) with a drop on d113. The only other longitudinal studies of beef feedlot cattle were Ralston *et al.* (2002) and the Chapter 5 study which both demonstrate a relatively constant percentage of infected animals throughout the study periods. In the current study, a low percentage of animals infected with *G. duodenalis* were observed initially and rose throughout the study period. This pattern suggests that the animals had a low exposure to *G. duodenalis* prior to the start of this study compared to the other two previously mentioned studies,
and when animals were stressed through shipping, weaning and co-mingling at the feedlot, infection percentages increased.

Cyst excretion levels by TGAPS and KK0429 infected steers were constant throughout the study, not varying significantly from those on d0 with the exception of KK0429 steers decreasing on d50. The maximum cysts shed per gram of faeces by an individual animal was $10^3$. Cyst shedding throughout the study period remained constantly low with no significant ($P>0.05$) fluctuations. These results differ from the cyst shedding fluctuations reported by Ralston et al. (2002) but are similar to those reported in Chapter 5. A variable that could influence cyst shedding would include animal health status, which if depressed could account for higher numbers of cysts to be shed per gram of faeces by infected steers.

*G. duodenalis* infected and non-infected steers showed no significant difference in ADG for any of the three periods of interest (Backgrounding period d0-80, Finishing period d81-225 and Overall d0-225). These results are similar to those reported by Ralston et al. (2002), however Aloisio et al. (2006) reported severe weight loss in lambs (30 – 90 days of age) infected with *G. duodenalis* Assemblage B, whereas of the 4 TGAPS samples tested in our study, 3 were Assemblage A and one was Assemblage E. Six KK0429 samples were tested and all were Assemblage E. Aloisio et al. (2006) notes that the severity of the clinical signs and pathogenic effects observed in the
study still need to be proven to be linked to the genetic background of the parasite. These differences highlight the need for further controlled study to determine if assemblage could influence performance impact.

In the current TGAPS study 75% (3) of the tested samples were Assemblage A and 25% (1) was Assemblage E. In the KK0429 study 100% (6) of the tested samples were Assemblage E. There are no published studies documenting genotypes of *G. duodenalis* from infected beef feedlot steers, however, Trout *et al.* (2005) found in a study of post-weaned dairy calves that 87% (206) of the isolates were Assemblage E and 13% (31) were Assemblage A, but noted that the genotypes present varied greatly from farm to farm. Trout *et al.* (2006) also reported genotypes of *G. duodenalis* in 1-2 year old dairy cattle where 91% of the isolates were Assemblage E and 9% were Assemblage A, again stating that the genotypes present varied greatly from farm to farm. Animals were housed at the same location for both studies, and were originally sourced from the same farm. Becher *et al.* (2004) reported that zoonotic genotypes of *G. duodenalis* may only be present transiently, in cattle under conditions where the frequency of transmission with the livestock genotype is high and competition is likely to occur. The sample size used for molecular typing in both the TGAPS and KK0429 was very small so they might not have been representative of the predominant genotype in each group. Also, the samples were collected at one point in time so whether the Assemblage A detected in the majority of the TGAPS samples was transient
or in fact the continual Assemblage present is unknown. Currently, the exact
contribution of parasite genetic variability on symptomatology is unclear due
to the contrasting results linking Assemblage A to mild, intermittent diarrhoea
and Assemblage B to severe, acute or persistent diarrhoea or **vice versa**
(Caccio *et al.*, 2005).

Overall prevalence of *C. andersoni* in the TGAPS and KK0429 feedlot steers
over the 225 day study was 2.9 and 3.5% respectively. This is considerably
lower than the 12% reported in the Chapter 5 study and the 85% reported by
Ralston *et al.* (2002), both longitudinal studies. The prevalence of *C.
andersoni* in this study is similar to the point prevalence reported by Anderson
(1991) in the U.S.A. of 4.7%, highlighting the variability in prevalence
between locations, and between point prevalence and longitudinal prevalence.

The prevalence of *C. andersoni* throughout these studies remained constant
and did not differ significantly (*P* > 0.05) from the first sample date, similar to
those results reported in Chapter 5. The number of *C. andersoni* oocysts shed
per gram of faeces in the positive animals did not vary significantly
throughout the trial period, similar to those results published in Chapter 5.

*C. andersoni* infected and non-infected steers overall had no significant (*P* <
0.05) difference in ADG similar to our results in Chapter 5. Impaired
performance in cattle infected with *C. andersoni* has been previously reported by Anderson (1990) and Estaban *et al.* (1995).

Two microscope positive *C. andersoni* samples underwent PCR and were confirmed by sequencing as *C. andersoni* (GenBank DQ448631, DQ448632).

The present study describes the prevalence, infection pattern, (oo)cyst shedding, impact of infections on animal performance and molecular characterization of *G. duodenalis* and *C. andersoni* in beef feedlot steers in Alberta, Canada. The study highlights the need for further study into *G. duodenalis* genotypes and their effect on animal performance to elucidate if varying isolates could be responsible for the conflicting reports of severe weight loss in animals infected with *G. duodenalis*. 
Chapter 7 General Discussion and Conclusions
7.1 Background

Little has been documented on the prevalence and impact on performance in beef feedlot cattle by of *Giardia duodenalis, Cryptosporidium andersoni* and *Cryptosporidium parvum*. Important questions remain to be answered on how these parasites affect animal health/production and their role in the transmission of disease to humans. Also, do these parameters differ between Western Australia and Alberta, Canada, two geographically and climatic regions of the world, and if so, why?

The research conducted in this thesis was designed to address the following through a series of studies; prevalence, infection patterns and impact on beef feedlot cattle performance of *Cryptosporidium* spp. and *G. duodenalis* on Western Australia and Alberta, Canada; the impact of variations in management systems and nutrition; and genotyping from *G. duodenalis* positive animals to assess the parasites potential infectivity to humans and other animals.

The first of these studies was undertaken as a step towards understanding the prevalence and infection patterns of *Giardia duodenalis, Cryptosporidium andersoni* and *Cryptosporidium parvum* in feedlot cattle in Western Australia and Alberta, Canada over a two year period. None of the cattle sampled in Alberta, Canada or Western Australia were infected with *C. parvum*. This
result is not unexpected as the literature reports *C. parvum* mostly in cattle less than one month of age and the youngest cattle sampled in these studies were two months of age (Olson *et al*.*., 2004). These calves were also on extensive pastures, allowing for separation which would reduce their exposure to *C. parvum* oocysts if any were present in the environment. Also, the beef calves sampled were nursing their dams and therefore should have received adequate colostrum at birth, and not be immunosuppressed. There are reports of *C. parvum* in mature cattle (Villacorta *et al*.*., 1991) but genotyping was not performed and this was most likely *C. bovis*, a related but distinct parasite. *C. bovis* was not observed in the cattle sampled in either Canada or Australia, however it has been reported in Zambian beef calves under three months of age (Geurden *et al*.*., 2006c). This may be due to the fact that *C. bovis* prevalence has been reported to be much lower in older animals, > 2 years, (0-3.3%) (Feltus *et al*.*., 2008). Many of the animals in our Australian studies were these older animals. The younger animals in the Alberta, Canada studies were all derived from a single ranch and that herd may have been naïve to *C. bovis*.

*C. andersoni* was present in three out of the twelve groups (598 head) sampled in Western Australia with the prevalence ranging from 0 – 26%. All three groups (852 head) sampled in Alberta, Canada were positive with a prevalence ranging from 2.9 – 12%. This is the first documented evidence that *C. andersoni* is present in the Western Australian feedlot cattle population. In
the Western Australian study, one group of 23 head of light weight steers, segregated from their contemporaries due to their lower weights, had a four times higher prevalence of *C. andersoni* than their heavier pen mates. If the prevalence of this group was removed from the analysis, then the prevalence of *C. andersoni* between Alberta, Canada and Western Australia cattle is very similar.

Unfortunately, due to the one time sampling of the Western Australian cattle, the establishment of an infection pattern for comparison to those in Alberta, Canada was not possible. Molecular characterization of positives from Alberta, Canada and Western Australia confirmed *C. andersoni*.

*G. duodenalis* was present in ten out of the twelve groups sampled in Western Australia with prevalence ranging from 0 – 22%. All three groups sampled in Alberta, Canada were positive with a prevalence ranging from 39 – 82%. The prevalence of *G. duodenalis* is substantially higher in Alberta, Canada as compared to Western Australia. Since management systems between Western Australia and Alberta, Canada feedlots are very similar, the author suggests that perhaps environmental conditions are responsible for the higher prevalence in Alberta, Canada. The studies conducted in Alberta, Canada occurred between Nov. – June in southern Alberta where conditions are usually moist and cool, but often times not freezing, thereby allowing cysts to remain viable for longer periods of time increasing the potential for infection.
of other animals. The studies in Western Australia occurred during the summer months when conditions are very dry and hot, thereby rendering the cysts non-viable and minimizing environmental contamination and infection spread. It has been shown that *Giardia* cysts are readily inactivated by desiccation, high temperatures and light (Olson *et al.*, 2000a). Perhaps prevalence of Western Australian cattle during the cooler, wetter winter months would be higher.

Also of interest, the cattle sampled in Western Australia were on average 6 – 8 months older than those sampled in Alberta, Canada which may have contributed to their lower prevalence of *G. duodenalis* since age has been shown to affect prevalence of *Giardia* (Coklin *et al.*, 2007).

Molecular characterization in a subset of the Alberta, Canada *G. duodenalis* positive samples (10) revealed 30% (3) genotype A, and 70% (7) genotype E. The same characterization of the Western Australian samples (10) showed 20% (2) genotype A, 40% (4) genotype E, 10% (1) genotype B, 10% (1) genotype C, 10% (1) genotype D and 10% (1) genotype B and E. Based on these results, 30% of the samples from Alberta, Canada and 40% from Western Australia have the potential to be zoonotic. It is important not to extrapolate these results too far, considering they represent only 20 samples, which is a very small population. However, compared to the Alberta, Canada samples the Western Australian samples did exhibit greater variability in
genotypes, perhaps due to the extensive use of dogs on many of the cattle operations or the more extensive pasture situations exposing cattle to other species faeces as a source of contamination. The greater variability in Western Australian cattle may also be due to the larger number of herds sampled as compared to Alberta, Canada. The variation between Alberta, Canada and Western Australian samples is interesting and suggests further study is required to better characterize exactly which genotypes are most prevalent in feedlot cattle and to more accurately assess the actual zoonotic risk.

The second of these studies explored the impact on animal performance of *C. andersoni* and *G. duodenalis* in Alberta, Canada over a two-year period. None of the cattle sampled either in Western Australia or Alberta Canada were infected with *C. parvum*, making the assessment on animal performance impossible.

*C. andersoni* was present in 25% of the cattle groups studied in Western Australia and 100% of the groups in Alberta, Canada. All three Alberta, Canada studies collected performance data, however, there was no significant difference between infected and non-infected steers average daily gain in the feedlot. The literature routinely references performance impairment in *C. andersoni* infected animals; however, this has not been documented in commercial scale feedlots where potential control of the parasite would have economic implications (Anderson, 1990; 1998). Therefore further study of *C.
*Andersoni* in feedlot beef cattle to assess impacts on performance would be valuable to the cattle industry.

*G. duodenalis* was present in 83% of the cattle groups in Western Australia and 100% of the groups in Alberta, Canada. Performance data was established for the three groups of cattle analysed from Alberta, Canada and there was no statistical difference in animal performance between the infected and non-infected steers. The limitations of this study must be taken into consideration when evaluating these results and accept that confounding factors are present in commercial conditions that can be controlled in experimental settings. These results are contrary to those found by Olson *et al.* (1995), where weight loss was reported in lambs infected with *G. duodenalis*. Performance effects associated with *G. duodenalis* in ruminants are a result of malabsorption and maldigestion of carbohydrates, fats and vitamins resulting from a reduced microvillus brush border surface area and reducing intestinal brush border disaccharidase levels (Olson *et al.*, 1995). Younger animals’ tend to be more deleteriously affected by giardiasis (Olson *et al.*, 2004). However, large scale commercial feedlot studies have not been attempted, therefore, further research of this magnitude is required to determine if giardiasis impacts performance on a commercial feedlot scale and if it warrants control measures to provide economic benefits back to the producer.
7.2 *Giardia duodenalis* Was More Prevalent in the Alberta, Canada Studies than the Western Australia

The research presented in Chapter 3 is the first occurrence study (n=87) of *G. duodenalis* and *Cryptosporidium* spp. in feedlot cattle undertaken in Western Australia. Results showed that *G. duodenalis* occurrence in five groups of feedlot cattle tested were 5, 9, 15, 0 and 0%. None of the groups demonstrated an infection with *Cryptosporidium* spp. A subsequent study discussed in Chapter 4 involving five groups of Western Australian feedlot cattle (n=502) also reported a low *G. duodenalis* prevalence, 2, 8, 22, 0.6 and 8%.

Comparing these results to those reported in Chapter 5 and 6 where feedlot cattle in Alberta, Canada were sampled multiple times and *G. duodenalis* prevalence of 82, 39 and 44% were reported, *G. duodenalis* appears to be more prevalent in the Alberta, Canada conducted studies as compared to those done in Western Australia. Previous comparisons between point prevalent studies and longitudinal studies have identified that the methodology can influence prevalence favouring the longitudinal studies (Ralston *et al.*, 2002). However, point prevalence results for Alberta, in Chapter 5 and 6 fluctuated above 10% prevalence on 39% of the sample dates, whereas Western Australian data was above 10% occurrence on only 20% of the sample dates. This demonstrates that the prevalence of *G. duodenalis* in the Alberta, Canada feedlot cattle studies versus Western Australia studies is approximately double.
Possible explanations for a higher prevalence of *G. duodenalis* in Alberta, Canada are the age of the cattle in the feedlots, animal densities, management and environmental conditions. Cattle tested in Alberta feedlots were 6 – 14 months of age whereas Western Australian feedlot cattle were 9-36 months of age. Xiao (1994) and others reported that there is a greater prevalence of *Giardia* in younger cattle, potentially explaining the higher prevalence observed in Alberta, Canada. Typically Western Australian cattle are raised for the majority of their production cycle on the range under extensive management techniques and Alberta, Canada cattle are raised under more intensive conditions. This combined with Alberta’s cooler wet climatic conditions (allowing for longer cyst viability) during the spring, summer and fall (Olson *et al.*, 2000a), increases the chance of cattle under feedlot conditions becoming infected with *Giardia*, and may explain the greater prevalence of the parasite in Alberta feedlot cattle. The cold winter conditions in Alberta, Canada would cause some killing of cysts but typically cysts can tolerate cold wet conditions as those found during Alberta winters more successfully than the intense heat and dry conditions prevalent in Western Australia.
7.3 First Report of C. andersoni in Australian Feedlot Cattle

The study of G. duodenalis and C. andersoni in beef feedlot cattle in Western Australia presented in Chapter 4 provides a unique assessment of the prevalence of C. andersoni in five large groups of feedlot cattle (n=502). Data presented showed a prevalence of C. andersoni in three out of the five groups of cattle at 8, 5 and 26%. C. andersoni has not been previously reported in Australian cattle, however it has been reported in one sheep in Western Australia (Ryan et al., 2005).

Two samples were genotyped, one by PCR amplification and sequencing, the other using qPCR and melting curve analysis. Both confirmed the microscopy designation of C. andersoni. The confirmation of C. andersoni in cattle of Western Australia is significant as the parasites’ presence has not been previously reported in Australia. It is however important to realize that C. andersoni was confirmed microscopically but only two samples were successfully sequenced as C. andersoni.

7.4 C. parvum, C. bovis or C. ryanae did not Exist in Either the Western Australia or Alberta, Canada Feedlot Cattle Studies

The research presented in Chapter 3 was an occurrence study of eight groups of Western Australian cattle (7 groups of mature animals, 1 group of calves) totalling 87 head. None of the samples tested positive for C. parvum. The prevalence study of five groups of Western Australian feedlot cattle (n=502)
in Chapter 4 also had all samples from these cattle test negative for \textit{C. parvum}. The author found no \textit{C. parvum}, \textit{C. bovis} or \textit{C. ryanae} positive animals at any point in the research for this thesis in the Western Australian study animals. Results reported in Chapter 5 from Alberta, Canada feedlot cattle (1 group \(n = 300\)) sampled five times over a 314d feeding period revealed all samples tested negative for \textit{C. parvum}, \textit{C. bovis} or \textit{C. ryanae}. The longitudinal study reported in Chapter 6 of Alberta, Canada feedlot cattle documented a total of 552 head, sampled nine times over the feeding period, had no samples test positive for \textit{C. parvum}, \textit{C. bovis} or \textit{C. ryanae}. Again, no animals in the Alberta, Canada studies tested positive for \textit{C. parvum}, \textit{C. bovis} or \textit{C. ryanae}.

The absence of \textit{C. parvum} from both the Alberta, Canada and Western Australian studies was expected, as the literature reports \textit{C. parvum} mostly in cattle under one month of age and all of the cattle in these studies were between six and thirty-six months of age (with the exception of one group of calves at two months of age) (Olson \textit{et al.}, 2004). Becher \textit{et al.} (2004) has documented \textit{C. parvum} in Western Australian dairy calves’ but they were less than one month of age. The absence of \textit{C. bovis} and \textit{C. ryanae} from both the Alberta, Canada and Western Australian studies was somewhat unexpected as the literature reports that these two species of \textit{Cryptosporidium} are more prevalent in older animals (Fayer \textit{et al.}, 2005; Feltus \textit{et al.}, 2008). A possible explanation would be that the overall prevalence of these parasites in the
mature cattle population is reported to be 1 – 2 % and our lack of observance could be a function of sample size (Feng et al., 2007) or perhaps their occurrence is regional.

7.5 *G. duodenalis* and *C. andersoni* Infections did not have a Significant Impact on Cattle Performance in the Alberta, Canada Feedlot Studies

Research presented in Chapters 5 and 6 on longitudinal prevalence of *G. duodenalis* and *C. andersoni* in beef feedlot cattle in Alberta, Canada documented the prevalence of *G. duodenalis* at 82% (n=300), 39% (n=240) and 44% (n=312) in three samplings respectively, with no significant difference in ADG between infected and non-infected animals. *C. andersoni* prevalence was 12, 3 and 3.5% in the three groups of cattle with no statistically significant impact on animal performance.

Literature has reported some detrimental impacts on performance of *G. duodenalis* and *C. andersoni* infections in cattle and sheep (Anderson, 1990; Olson et al., 1995; Aloisio et al., 2006). Based on reports in the literature and the results of this study, showing no impacts of these infections on animal performance, one must conclude that more than one variable is involved in whether or not infection affects animal performance. These variables could include intensity of infection, animal age, overall health (immune status, co-infections) and nutritional status of the animal. Perhaps when a combination
of these variables comes into play there is a more substantial and therefore statistically significant reduction in ADG. When mild infections occur in older, healthy animals, the impact on ADG may not reach statistical significance.

Unfortunately, due to the limitations of doing research on privately owned cattle in a commercially operated feedlot, blood samples were not obtainable for analysis to determine active infections to certain viral pathogens that could be attributed to reduced weight gain (e.g. Bovine Viral Diarrhoea, Bovine Immunodeficiency Virus). However, animals appeared to be clinically normal at the time of sample collections, they had been treated for other endo and ecto parasites and no illness was suspected. Subclinical parasitic diseases such as Ostertagiosis and Coccidiosis have been shown to affect performance (Ploeger et al., 1990; McAllister et al., 1996; Niezen et al., 1998). All animals were dewormed at the time of entry into the feedlot.

### 7.6 The Majority of *G. duodenalis* Positive Feedlot Cattle in the Studies are Non-zoonotic Genotypes

A small subset (n=10) of the *G. duodenalis* positive samples collected in the study discussed in Chapter 4 (of feedlot cattle in Western Australia) were molecularly characterized utilizing PCR at the *Giardia* 18S rDNA locus. The genotyped isolates represented samples from four different farms and five different Assemblages. Fifty percent of the samples sequenced were
Assemblage E which is a hoofed livestock genotype, reported not to be infective to humans (Thompson, 2004). One sample each of Assemblage B, C, D and two samples of Assemblage A were also sequenced. Of these Assemblages, only A and B (30%) have been reported to be transmissible to humans (Thompson, 2004). These results are similar to those reported by Trout et al. (2005) and Trout et al. (2006) who found, in a study of post-weaned dairy calves, that 87% (n=206) of the isolates were Assemblage E and 13% (n=31) were Assemblage A and in 1-2 year old dairy cattle 91% were Assemblage E and 9% were Assemblage A. O’Handley et al. (2000) also reported that dairy calves from Western Canada and Western Australia positive for G. duodenalis sequenced 80% (n=8, n=4 respectively) Assemblage E and 20% Assemblage A (n=2, n=1 respectively).

The molecular results of these studies must be cautiously interpreted due to the small sample set however they are encouraging for the cattle industry concerned with the potential zoonotic transmission of G. duodenalis to humans. The majority of mature cattle sampled were not infected with a previously reported zoonotic Assemblage of G. duodenalis. Despite the small size of the subset samples molecularly characterized in these studies the results are similar to those reported in much larger studies (Trout et al., 2005; Trout et al., 2006).
7.7 Nutritional and Management Impacts

The effect of animal nutrition and management on parasite prevalence and animal performance is difficult to assess due to the magnitude of these variables. Experimental design of the studies reported in Chapters 3, 4, 5 and 6 was not developed to test these two parameters to measure their impact on parasite prevalence or ADG. Also, due to the constraints of utilizing privately owned cattle in a commercially operated feedlot, management and nutrition of the cattle could not be deviated from the feedlot’s standard operating procedures.

The prevailing conditions have been reported and contrasted between the Alberta, Canada and Western Australia studies to suggest possible hypotheses for the variations seen in parasite prevalence between the two countries. The suggested impacts of these variables are not definitive due to the nature of the studies’ design, but the role they may play in infection patterns, prevalence and impact on performance must not be discounted and warrants further controlled study.

7.8 Limitations of Conducting Research in Commercial Feedlots in Two Countries

Conducting research in commercial feedlots has both advantages and challenges. Commercial feedlots allow for the sampling and data collection of large groups of cattle managed under identical systems, providing consistent
environments and a reduction of variables. Commercial feedlots also source, house and manage the cattle at their expense, allowing for minimal expenses to be incurred for the animal portion of the study. If the study had to own and manage the cattle, study costs would be prohibitive.

The challenges of conducting research in commercial feedlots include the fact that they must respond to market signals that may involve selling pens of cattle part way through a study, or conversely not filling pens with cattle until after the start of the study. Researchers must also be sensitive to the feedlot’s management schedule that involves only sampling cattle when they are moved through a chute for other processes typical to that particular operation. This means that data collection may not occur as often or as consistently as the researcher might desire. Also, the performance data collected is limited to average daily gain and excludes feed efficiency, dry matter intake or carcass quality characteristics.

The challenges of conducting research in two different countries across the world simultaneously include the inability to have day to day control over the studies and to react quickly to changes in the study protocol precipitated by the feedlot owner’s response to market changes.

Overall, the opportunity to compare parasitic infections in commercial feedlot cattle in two countries far outweighs the challenges associated with this type
of study. It does however; result in data that might not be as complete as if the studies had been conducted in the controlled environment of a research station.

7.9 Future Studies

The impact of *C. andersoni* and *G. duodenalis* on beef cattle performance on a commercial scale is still unclear. The literature documents instances where performance of animals is impacted by these parasitic infections, but to date this has not been replicated on a commercial sized feedlot operation (Anderson, 1990; Olson and Buret, 2001). Therefore, it is important to clarify the relationship between the parasitic infection and animal performance to adequately address the question of whether the treatment of these parasites in a commercial feedlot setting would be economically viable.

Molecular characterization of *G. duodenalis* is a relatively recent advancement in science. Currently, very little genotyping of *G. duodenalis* in commercial feedlots has been completed. Therefore, it is important to establish the predominant genotypes of *G. duodenalis* in feedlot cattle to more accurately assess the potential zoonotic risk.
Chapter 8 References


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