CYSTICERCUS OVIS IN CANADIAN SHEEP: RISK FACTORS AND A TRANSMISSION MODEL TO ASSESS CONTROL MEASURES

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ABSTRACT

CYSTICERCUS OVIS IN CANADIAN SHEEP: RISK FACTORS AND A TRANSMISSION MODEL TO ASSESS CONTROL MEASURES

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This thesis investigated the epidemiology and control of *Cysticercus ovis* infection on Canadian sheep farms. Canadian slaughter data indicated an increase in sheep carcass condemnations due to *C. ovis* in 2007 and 2008. Trace-back of 237 carcasses condemned in Ontario, between 2009 and 2011, revealed they originated from 133 farms across Canada. A case-control study was performed (n=40 cases, 56 controls) to identify farm-level risk factors for carcass condemnations. Farm dogs scavenging deadstock (OR=4.04; 95% CI: 1.16–14.04) and failing to dispose of deadstock properly (OR=11.78; 95% CI: 2.93–47.40) were significantly associated with condemnations in multivariable analyses. A transmission model for *Taenia ovis* was created and control options were assessed. Model simulations predicted cestocide treatment of guardian dogs every fifth week, and proper deadstock disposal would reduce the risk of *C. ovis* infection in lambs.

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Introduction, literature review and objectives

1.0 Introduction

Cysticercus ovis is the intermediate stage of a canine tapeworm, *Taenia ovis*, which produces cystic lesions in the muscle of sheep. If numerous, these lesions can result in condemnation of the entire carcass. In recent years, abattoir data have revealed an increase in both the number and proportion of lamb condemnations due to *C. ovis* infection in Canada. The rise in carcass condemnations due to *C. ovis* suggests that the prevalence of this infection on Canadian sheep farms is likely increasing. Considering the financial consequences associated with lamb carcass condemnations due to *C. ovis* infection, it is essential that the epidemiology of this parasite in Canada be understood.

The following literature review provides an overview of the Canadian sheep industry, summarizes what is currently known about *C. ovis*, and explains the importance of the parasite around the world. At the end of the chapter, the rationale for this thesis, and its overall objectives, are described.

1.1 An overview of the Canadian sheep industry

Currently, the demand for sheep products (meat, milk products and wool) in Canada is great, and there is significant opportunity for flock expansion throughout the country driven primarily by the demand for lamb meat. Between 2005 and 2007, the per capita consumption of lamb in Canada increased by approximately 10%, with the average person consuming 1.24 kg annually in 2007 (Fleming, 2008). Since that time, the demand for lamb has continued to increase each year, culminating in record-setting lamb prices in April 2010 (Statistics Canada, 2011). As of July 2010, the total size of the Canadian

sheep flock was 1,042,200 head; ewes and replacement lambs comprised nearly 61% of the total, market lambs represented approximately 37%, and rams the remaining 2% (Statistics Canada, 2011). The Canadian sheep flock is distributed across the country, though not uniformly. Combined, the provinces of Quebec (30.3%), Ontario (27.3%), Saskatchewan (10.9%) and Alberta (15.8%) represent close to 85% of the national sheep flock (Statistics Canada, 2011). The Atlantic provinces (3.6%), Manitoba (6.7%) and British Columbia (5.8%) contribute to a lesser extent (Statistics Canada, 2011).

Sheep are frequently transported long distances across Canada for breeding and slaughter purposes. A market lamb is most often sold to slaughter either through an intermediary broker (e.g. auction mart) or direct to the abattoir, but occasionally will first be sold to a feedlot for finishing. Approximately 51% of the market lambs born in Canada will be transported into Ontario for slaughter (Statistics Canada, 2011); the market for lamb meat in Ontario is the greatest in Canada. This lucrative market is largely attributed to the thriving immigrant population in the Greater Toronto Area who tend to consume lamb as a higher proportion of their total meat consumption than the average Canadian population (Fleming, 2008).

Despite the favourable market for lamb in Canada, multiple factors have contributed to an overall decline in the Canadian sheep industry in recent years. Since 2004, the size of the Canadian ewe flock has decreased by more than 15%, with evidence of slight growth in 2011 (Statistics Canada, 2011). In May 2003, the United States, a major market for Canadian lambs, banned the importation of Canadian sheep after bovine spongiform encephalopathy was diagnosed in a Canadian cow. This lead to a fall in prices in 2004 as excess lambs flooded the Ontario markets. Market instability caused

producers to either exit the industry or decide not to expand their breeding flocks. As a consequence, a lower supply combined with an increasing demand has resulted in record high prices over the last six years. This high price for lamb meat has resulted in producers choosing to make short-term profit by marketing their lambs, instead of retaining some as replacement ewes. Additionally, limits on barn or pasture space, increased feed costs, labour costs, drug and vaccine costs, and predation issues may also have contributed to flock reduction. Regardless of the reason, the Canadian sheep industry cannot meet its current domestic market; in 2009, only 47% of the Canadian lamb market was supplied domestically (Statistics Canada, 2011). To make up the short-fall, Canada imports large quantities of foreign lamb, mainly from New Zealand (Statistics Canada, 2011). In the coming years, unless the Canadian sheep industry supplies more lamb, importers will serve an increasingly important role in satisfying the Canadian market. Eventually, insufficient numbers of domestic lamb may result in Canadian marketers relying solely on imported lamb to assure consistent quantities. Once the domestic markets are lost, it will be very difficult to recover them (Fleming, 2008).

An additional explanation for the decline in the Canadian sheep industry is the impact coyote predation has on lamb production. Losses due to predation are the number one reason producers leave the sheep industry (Canadian Sheep Federation, 2009). Predation can have a substantial impact on a producer's farming operation and profit. In 2008, the Canadian government provided $1.33 million (CAD) in compensation to livestock producers (from all sectors) who suffered losses due to predation (Canadian Sheep Federation, 2009). Lambs in particular are vulnerable to a coyote attack. In order to combat predation, producers have been forced to greatly modify their farm

management. For instance, the lambs of many Canadian producers are raised entirely indoors; a concept radically different from sheep production in Europe and New Zealand, where predation is not a major issue. In addition, it is very common for Canadian producers to use guardian animals, and in particular guardian dogs, for predation prevention. Guardian dogs are specifically bred and raised large-breed dogs that live with the flock 24 hours a day. Well trained guardian dogs can be very effective at reducing coyote predation (Ontario Ministry of Agriculture, Food and Rural Affairs, 2011).

1.1.1 Canadian sheep identification program

An important aspect of animal health and management is having the ability to determine where an individual animal originated, regardless of its current location. Understanding the importance of this, in 2004 the Canadian sheep industry initiated the Canadian Sheep Identification Program (CSIP). The CSIP is an industry-run mandatory identification system that aids in identifying the farm of origin of animals, when an animal health or food safety emergency occurs (Canadian Food Inspection Agency, 2008). The program requires that all ovine animals in Canada be tagged with a unique identification number when leaving the farm of origin – in most cases, the location of their birth (Canadian Food Inspection Agency, 2008). Similarly, all imported animals must be tagged immediately after their arrival in Canada (Canadian Food Inspection Agency, 2008). Each animal carries the same identification number throughout its life, which is recorded and maintained in a database by the Canadian Cattle Identification Agency (Canadian Food Inspection Agency, 2008). The only exception to this program is in Quebec, where Agri-Traçabilité Québec manages the identification program (Canadian Food Inspection Agency, 2008); all other Canadian provinces use the CSIP. Currently,

there are four tag options approved for use with CSIP: Pink Allflex Dangle Tag, Allflex RFID button tag, Metal Ketchum Kurl-Lock #3, and the Shearwell Data SET tag. Two of these options, the Allflex RFID button tag and the Shearwell Data SET tag, are radio-frequency identification (RFID) tags (Canadian Sheep Federation, 2011a). There are fines associated with the transport of untagged animals ($1300 for a first offence, reduced to $650 if paid within 15 days (Canadian Sheep Federation, 2011b)); however, tags are commonly lost, and older tags can become illegible with time, occasionally making it difficult, even impossible, to identify animals.

The major limitation of the CSIP is its failure to allow complete traceability of animals. A true traceability system would allow each movement of an animal to be recorded (e.g. between farms and livestock trucks, through sales barns, and to slaughter); the CSIP is an incomplete trace-back program that only identifies the animal's farm of origin where it was tagged. The current trace-back system is therefore of limited use in tracing out animals at risk in a disease outbreak.

The limitations of the current identification program have caused the Canadian Food Inspection Agency to mandate the use of RFID tags to improve the traceability of sheep in Canada. After December 31 2012, all sheep must be tagged using RFID tags (Canadian Sheep Federation, 2011b). The use of RFID tags will allow the traceability of animals from birth through to slaughter, a task for which the current program is not designed.

1.2 An overview of *Cysticercus ovis*

The parasite *Cysticercus ovis*, commonly known as "sheep measles", is the metacestode stage of the canine tapeworm, *Taenia ovis* (Ransom, 1913). The lifecycle of

T. ovis is indirect (Gordon, 1939), meaning it is carried out in two or more different host species. The definitive host contains the adult stage of the parasite, and is the host in which the parasite reproduces. In contrast, the intermediate host contains only the immature, or larval, stage of the parasite. In the case of *T. ovis*, the definitive host is a canid – wild or domestic – while the intermediate host is a sheep or goat (Ransom, 1913).

1.2.1 *Taenia ovis*

Taenia ovis is the adult stage of *C. ovis* and inhabits the small intestine of canids. It is rare to find more than one adult inhabiting the intestine at a given time (Jackson and Arundel, 1971), and is unclear whether it is the canid's immune response, or intraspecific competition by *T. ovis*, that prevents establishment of other adult parasites. The tapeworm's scolex which, in adults, measures between 637 and 1092 µm in diameter, attaches to the intestinal mucosa using four suckers and an armed rostellum containing 24-36 hooks (Verster, 1969). It is the length of the rostellar hooks that allows differentiation between *T. ovis* and most other *Taenia* species (Verster, 1969); the one exception being *Taenia krabbei* which is covered in more detail in a later section of this chapter. The lengths of the large hooks of *T. ovis* range between 173 and 186 µm, while the small hooks range between 111 and 120 µm (Verster, 1969). All species of canids, wild and domestic, are considered equally susceptible to infection by *T. ovis* and capable of acting as the definitive host.

Canids can only become infected with the parasite following ingestion of viable larvae. Therefore, to become infected with *T. ovis*, canids must consume muscle infected with viable *C. ovis*. Following the ingestion of infective cysticerci, the prepatent period is between six and nine weeks (Ransom, 1913). Interestingly, anecdotal evidence from New

Zealand suggests a shorter prepatent period can occur when aggressive deworming protocols have been implemented (Heath and Lawrence, 1980).

Within the small intestine of the canid, *T. ovis* can reach a maximum length of 1.5 meters (Ransom, 1913). The adult cestode is composed of a scolex followed by segments collectively called a strobila. Each tapeworm segment, called a proglottid, is hermaphroditic in nature and, on average, contains 78,000 eggs (Arundel, 1972). Proglottids are continually budded from a germinal layer of cells in the cestode's neck region, immediately posterior to the scolex. Being hermaphroditic, each proglottid is capable of asexual or sexual reproduction involving nearby proglottids in the same strobila. (Taylor et al. 2007). The gravid proglottids travel posteriorly along the length of the worm as they grow and mature. The most mature proglottids are the largest segments, due to the abundance of eggs contained within, and are found at the posterior end of the cestode (Taylor et al. 2007). Mature proglottids break free of the worm and travel with the peristaltic movements of the canid's digestive tract.

While traveling through the intestine, proglottids typically release eggs through a small opening on the lateral portion of the segment called the genital pore (Arundel, 1972). Investigations by Gregory (1976) determined that in a 24-hour period, an infected canid with a patent *T. ovis* infection passes between one and three proglottids per adult cestode. However, detached proglottids found in the environment contain, on average, a few hundred eggs; indicating that the majority of the eggs are released within the intestine (Arundel, 1972). Overall, based on these various assumptions, a single parasite can release up to 250,000 eggs per day (Gregory, 1976); however, the true number of

viable eggs released per cestode per day is likely less than 250,000 as eggs in the intestine are subject to a variety of digestive enzymes.

The proglottids and eggs of *T. ovis* are released into the environment when a canid defecates. It has been reported that the eggs of *T. ovis* can remain viable in the environment for more than five months under controlled conditions, perhaps surviving up to six months (Sweatman and Henshall, 1962). The reported six month maximum was most likely based on studies investigating viability of eggs from *Taenia saginata* (Froyd, 1962; Storey and Phillips, 1985), a related zoonotic tapeworm of humans that uses cattle as the intermediate host. It is reasonable to assume the viability of eggs in the environment is similar between *T. ovis* and *T. saginata*. *Taenia* eggs can tolerate wide temperature ranges, and remain infective between -30°C and 37°C; however, they are highly susceptible to desiccation (Lawson and Gemmell, 1983).

Taenia ovis can survive in the canid's small intestine anywhere from five to nine months, after which partial immunity is developed to the parasite (Sweatman and Henshall, 1962). However, the duration of immunity is short and canids will often become re-infected if re-exposed. Lastly, it should be noted that infection with *T. ovis* rarely results in clinical disease, and in the vast majority of cases, the canid exhibits no morbidity effects (Taylor et al. 2007).

1.2.2 Dispersion of *Taenia* eggs

Taenia ovis eggs are most commonly transmitted to intermediate hosts through accidental ingestion while foraging in an environment contaminated with infective canid feces. The ability of intact proglottids to travel short distances away from feces has been well documented, and aids in the dispersion of eggs (Sweatman and Plummer, 1957).

However, field data have revealed that *Taenia* eggs can be widely distributed from areas where infective feces were initially deposited, beyond what can be accounted for by proglottid movement. It has been reported that within 10 days of deposition, *Taenia* eggs, which average 0.5 µm in diameter (Taylor et al. 2007), can be found up to 80 meters from their original location (Gemmell and Johnstone, 1976). This distribution could be explained by the movement of sheep, which may inadvertently pick up eggs on their fleece or hooves and carry them to other areas (Gemmell, 1972). Alternatively, it has been proposed that wind, rainwater, invertebrates, and even birds can act as mechanical vectors and transport eggs (Lawson and Gemmell, 1983; Lawson and Gemmell, 1985). At least two reports have suggested the transport of *Taenia* eggs via birds to a secluded location where canids were absent (Crewe and Crewe, 1969; Torgerson et al. 1995). There have also been reports of cestode eggs attached to flies captured in the field, suggesting that transmission can theoretically occur via this route (Lawson and Gemmell, 1985; Lawson and Gemmell, 1990); however, the role this transmission route has in the overall parasite lifecycle under natural conditions has yet to be determined.

1.2.3 Distinction between *Taenia ovis* and *Taenia krabbei*

Most *Taenia* species can be distinguished as adult parasites through differing host species, predilection sites, or morphological differences, particularly the number and size of the rostellar hooks. A notable exception, however, is distinguishing between *Taenia ovis* and *Taenia krabbei*. Morphologically, the two are indistinguishable (Sweatman and Henshall, 1962; Verster, 1969; Samuel et al. 2001), although it has been reported that the strobila of *T. krabbei* matures more quickly (Sweatman and Henshall, 1962). Intuitively, a faster development would favour *T. krabbei* because of the lower life expectancy of

wild canids. The predilection site within the intermediate host is also identical between the parasites, with cardiac and skeletal muscle becoming infected (Sweatman and Henshall, 1962). The only distinction between these two taeniids is subtle differences in intermediate host species. Unlike *T. ovis*, which largely relies on a domestic lifecycle involving canids and domestic sheep, the lifecycle of *T. krabbei* is sylvatic and normally involves wild canids as the definitive host, and cervids as the intermediate host (Freeman et al. 1961; Seesee et al. 1983; Samuel et al. 2001). The similarities between the two species have resulted in controversy about whether they are, in fact, a single species.

Sweatman and Henshall (1962) provided evidence, using cross-infection studies, that *T. ovis* and *T. krabbei* are distinct species. In their work, 10,000 eggs collected from *T. krabbei* were fed to sheep and goats, none of which developed characteristic lesions in their muscle (Sweatman and Henshall, 1962). Unfortunately, the experiment did not have cervid controls to demonstrate that the eggs were indeed viable, and failed to mention when and how they obtained *T. krabbei* eggs. A similar experiment was carried out by inoculating five deer and five sheep with between 5,000 – 150,000 *T. ovis* eggs each. As suspected, the sheep became heavily infected, but the cervids failed to develop lesions (Sweatman and Henshall, 1962). Although these results are generally accepted as proof that *T. ovis* and *T. krabbei* are not the same species (Samuel et al. 2001), exposing sheep to *T. krabbei* eggs should be repeated with cervid controls to validate the results.

1.2.4 *Cysticercus ovis*

The metacestode stage of *T. ovis* is called *Cysticercus ovis* and occurs within sheep or goats, the intermediate host. Infection occurs when these species inadvertently ingest the eggs of *T. ovis* from an environment contaminated with infective canid faeces

(Ransom, 1913). The consumed egg sheds a protective layer known as an embryophore. The activated embryo, known as an oncosphere, is then released and burrows through the intestinal wall using three pairs of keratinized hooks (Jabbar et al. 2010a). It then migrates via the bloodstream to the musculature (Ransom, 1913). At this point, each oncosphere loses its hooks and develops into a structure known as a cysticercus within the sheep's muscle. A cysticercus is a fluid-filled structure which contains a single, invaginated, larval parasite called a protoscolex (Taylor et al. 2007). Cysticerci are most commonly found in the animal's heart, diaphragm and masseter muscles, but other skeletal muscles are also routinely infected (Sweatman and Henshall, 1962). The cysticerci can appear as clear or white cysts within the muscle, hence the common name "sheep measles". Initial macroscopic detection of cysticerci in the intermediate host can occur by 13 days post infection (Ransom, 1913).

The exact mechanism *C. ovis* uses to migrate from the intestine to the muscle in sheep remains unclear. Experiments by Sweatman and Henshall (1962) showed that migrating larvae initially cause an acute inflammatory response during penetration of the intestine, and leave a trail of necrotic tissue and debris. This is consistent with the idea that larvae secrete enzymes that aid in the degeneration of host tissue and allow for migration. The duration of the inflammatory response is short, likely only occurring during initial migration to target tissues (Sweatman and Henshall, 1962). Unlike the closely related ovine parasites *Cysticercus tenuicollis* and *Echinococcus granulosus*, *C. ovis* fails to develop within the liver of the intermediate host despite almost certainly passing through it via the portal circulation during its migration to the musculature

(Ransom, 1913). Perhaps an unknown chemotactic signal from muscle tissue determines where *C. ovis* develops.

Although cysticerci may be visible 13 days following initial infection, 3-12 weeks is required before they become infective to the definitive host (Ransom, 1913). During this time, the cysticerci will continue to grow in size and can reach a maximum length and width of 10 mm and 5 mm, respectively (Sweatman and Henshall, 1962). This developmental period is required for the larvae to mature and develop the necessary structures, namely suckers and rostellar hooks, needed to successfully parasitize the definitive host (Sweatman and Henshall, 1962). Once infective, the cysticercus will only remain so for 4 to 8 weeks (Ransom, 1913), after which the larva within the lesion will die (Ransom, 1913). At this point the cysticercus becomes degenerate with a caseous, or sometimes calcified, centre (Ransom, 1913). Although the lesion in this state is no longer infective to canids, it will remain in the muscle of the host for potentially the remainder of the animal's life. The duration of the infective period in sheep is highly dependent on the immune response of the individual animal, and there can be great variation around the 2-3 month average. Adult sheep will develop a strong immune response against subsequent *C. ovis* infection, provided they are continually exposed to the parasite (Rickard and Bell, 1971; Rothel et al. 1996).

Ransom (1913) reported that the ingestion of large quantities of *T. ovis* could be fatal in lambs under experimental conditions. However, it has subsequently been determined that natural infection with *C. ovis* in sheep and goats produces no clinical signs and is not a concern from an animal health perspective (Taylor et al. 2007).

1.3 Ovine immune response to *Cysticercus ovis* infection

It has been suggested that within sheep, immunity to metacestodes occurs at two different stages of infection (Gemmell, 1962; Rickard et al. 1976). The initial immune response occurs within the intestine upon ingestion of cestode eggs from the environment (Gemmell, 1962). A second distinct immune response is induced as the larva penetrates the intestinal wall, and travels through the circulation to the predilection site (Gemmell, 1962). Early work on vaccine development by Blundell et al. (1968) involved exposing naive lambs to *C. ovis* through injection with artificially activated oncospheres of *T. ovis.* Artificially activated oncospheres are viable and can cause infection when injected; they are prepared *in vitro*, meaning the protective embryophore is removed from around the oncosphere, which allows them to bypass the digestive tract and be injected directly into the bloodstream. One month after injection, blood samples were collected from the exposed animals and the antibody titre to *T. ovis* was determined. A second group of lambs, naive to *C. ovis*, was then passively immunized individually with 100-120 mL of serum collected from the previously exposed animals. These animals were then immediately challenged with 2,500 *T. ovis* eggs. Eight weeks post-challenge, necropsies were performed and 3 mm tissue slices were carried out throughout the entire carcasses to reveal any *C. ovis* lesions in the muscle. The necropsies revealed that naive animals immunized with the serum from exposed animals developed significantly fewer *C. ovis* lesions ($p<0.05$) compared to non-immunized animals (Blundell et al. 1968). The transfer of immunity between animals via serum was suggestive of a protective antibody-mediated response to *T. ovis* infection (Blundell et al. 1968).

Evidence of humoral immunity against *C. ovis* infection has naturally led to inquiries about the antigens necessary to induce such a response. It is known that living *T. ovis* larvae are more effective at inducing immunity in sheep compared to dead larvae (Gemmell 1962; Rickard and Bell, 1971). If the parasite antigen(s) responsible for activating the host's immune response was a structural antigen, dead larvae in sheep should be equally effective at inducing the immunity. However, because living larvae were more immunogenic, it was believed that an excretory or secretory (ES) antigen elicited the immune response (Rickard and Bell, 1971). Presumably, the ES antigen is actively transported by a living oncosphere; meaning it is transported outside the larva for reasons that remain unknown. Following oncosphere death, transport would cease, reducing stimulation of the immune system. Work by Rickard and Bell (1971) strongly supports the existence of ES antigens and the active role they play in eliciting host immune responses.

Following several exposures to *T. ovis* eggs, an immune response is produced that destroys subsequent parasites while they are penetrating the intestinal mucosa, or shortly thereafter (Gemmell, 1972). In 1957 it was reported that a naive lamb exposed to as few as 50 viable eggs from *T. hydatigena* at a single time developed resistance to re-infection (Sweatman, 1957). Furthermore, as the challenge infection to a lamb increased, the strength of the immune response against re-infection increased (Gemmell, 1969). Throughout these experiments it is important to recognise that challenging a lamb involved directly placing *Taenia* eggs into the lamb's rumen. In the field, determining the number of eggs necessary to develop a protective immune response is difficult to quantify, and depends greatly on host and environmental factors. Immunity is not

lifelong, and within 5-12 months following the last exposure to eggs, sheep are again susceptible to infection (Rickard et al. 1976).

Researchers have observed a statistically significant positive correlation between the *T. ovis* challenge level, and the titre of circulating anti-45W IgG (both IgG_1 and IgG_2) in the host (Rothel et al. 1996). It has been documented that IgG_1 is the primary immunoglobulin transferred to lambs via colostrum (Watson at al. 1994). Several studies have examined transfer of immunity from ewes to their offspring via colostrum (Gemmell, 1972; Rickard and Arundel, 1974; Lawrence et al. 1993). Investigations into passive transfer of antibodies have been very encouraging and have shown that both naturally and artificially infected ewes are capable of transferring immunity to their lambs (Rickard and Arundel, 1974; Lawrence et al. 1993). The likely mechanism of maternal immunity against *T. ovis* infection in lambs involves an IgG_1 response transferred from the dam (Gemmell, 1972; Rickard and Arundel, 1974; Lawrence et al. 1993). Investigation into the duration of maternal immunity has suggested that it is protective up to six weeks of age, after which the susceptibility of lambs to infection increases (Rickard and Arundel, 1974; Heath et al. 1979).

1.3.1 Shared immunity between *Cysticercus ovis* and other cestodes

In light of their biological similarities, there has been substantial research investigating the prospect of immunological cross-protection between *C. ovis* and other cestodes whose life cycles involve sheep as an intermediate host. Studies investigating whether hydatid cysts of *E. granulosus* provide cross-protection against *C. ovis* or *C. tenuicollis* have been unsuccessful. Furthermore, there is no evidence suggesting that infection with *E. granulosus* protects against other cestode infections (Rickard and

Williams, 1982; Lawson, 1994). Thus, different genera of cestodes, despite similar lifecycles, are not sufficiently homologous to produce cross protection in their hosts.

Studies examining cross-protection between *C. ovis* and *C. tenuicollis* in sheep have produced conflicting results. Two early studies concluded that a partial immune response to *C. ovis* can be achieved through prior exposure to activated embryos of *T. hydatigena* (Gemmell 1965; Gemmell, 1969; Varela-Diaz et al. 1972). However, work by Rickard et al. (1976) failed to replicate these findings. Currently, it is generally agreed that *C. ovis* and *C. tenuicollis* share some antigenic properties that could result in partial cross-protection in the intermediate host under natural conditions (Lawson, 1994). Whether modern vaccines can replicate cross-protection has yet to be determined.

1.4 Lamb carcass condemnation due to *Cysticercus ovis*

The cystic lesions caused by *C. ovis* in the musculature of infected sheep are not zoonotic (Ransom, 1913), but are aesthetically undesirable in meat destined for human consumption (Arundel, 1972). Throughout the world, sheep or lamb carcasses heavily infected with *C. ovis* at slaughter are routinely condemned for human consumption; lightly or moderately infected carcasses may be trimmed and passed (Food and Agriculture Organization, 2000). In areas where the prevalence of *C. ovis* infection is high, the resulting financial consequences associated with high numbers of condemned lamb carcasses can have a substantial negative impact on the local sheep industry.

The absence of clinical signs of *C. ovis* infection in both the definitive and intermediate host has been cited as a significant reason for the parasite's detrimental impact on sheep industries (Rickard et al. 1995). Detection of *C. ovis* infection in a lamb can only be undertaken at carcass inspection during the slaughtering process. By the time

infection is detected at an abattoir, usually the animal has already been purchased from a producer whose farm may, or may not be, the origin of the infection. With lambs occasionally being transported from farm to farm prior to slaughter, the absence of clinical signs means that there is no simple way of determining on which farm the infection originated. The current national identification program for sheep does not allow for identification of offending farms by those buying the lambs, in order to penalise them for producing infected animals. The adoption of a sheep identification program that allows complete traceability would help rectify this problem.

In Canada, and other countries including Australia, New Zealand, and the United States, the inspection of lamb carcasses at an abattoir is based on guidelines for meat inspection developed by the Food and Agriculture Organization (FAO) of the United Nations. These guidelines recommend that a lamb carcass be condemned due to *C. ovis* infection if it is heavily infected with the parasite. A carcass is considered heavily infected if lesions are discovered in two of the usual inspection sites including the masseter muscle, tongue, oesophagus, heart, diaphragm or exposed musculature, and in two sites during incision into the shoulder and the rounds (Food and Agriculture Organization, 2000). If a carcass is lightly or moderately infected, the FAO recommendation is to trim the carcass; the cysts are removed from the carcass, and the meat is held for 10 days at -10°C before being passed (Food and Agriculture Organization, 2000). Research has shown that at this temperature, all viable *C. ovis* lesions will be killed, ensuring the carcass is free of any infective parasites that may have been missed with trimming (Whitten, 1971). Heating the carcass to at least 72°C is also effective at destroying living parasites (Arundel, 1972).

1.4.1 Sensitivity and specificity of *Cysticercus ovis* detection

Several studies have been conducted to estimate the sensitivity of routine meat inspection in detecting *C. ovis* infection in lamb carcasses (McNab and Robertson, 1972; Heath et al. 1985). From an epidemiological perspective, sensitivity is the probability that an infected animal will be correctly classified as infected during routine meat inspection (Dohoo et al. 2009). The gold standard for detecting *C. ovis* infection in lamb carcasses is to finely slice the animal's musculature into 3-4 mm slices and search for lesions; though too laborious for routine meat inspection, this is the only way to ensure all lesions are accounted for (Sweatman and Henshall, 1962). Using this gold standard as the referent test, an Australian study determined the sensitivity of routine meat inspection for detecting *C. ovis* to be 50% (McNab and Robertson, 1972). Accordingly, only 50% of lamb carcasses infected with *C. ovis* are actually detected at the abattoir. Therefore, using abattoir data to estimate the true prevalence of this infection in a population will result in significant underestimation (McNab and Robertson, 1972).

Specificity refers to the probability of carcasses truly without *C. ovis* infection being classified as uninfected (Dohoo et al. 2009). In other words, specificity refers to the likelihood of lamb muscle lesions, which are not actually *C. ovis*, being incorrectly classified as *C. ovis* infection (false positives) during meat inspection. The parasites *Sarcocystis ovifelis* (previously *S. gigantea*) and *C. tenuicollis* are capable of producing lesions in lambs resembling *C. ovis* (Ransom, 1913; Food and Agriculture Organization, 2000). However, there are significant differences that make misidentification unlikely. The lesions of *C. tenuicollis* are found exclusively in the liver and peritoneal cavity, rather than skeletal muscle, and commonly are associated with larval tracts and scarring

in the liver (Taylor et al. 2007). In sheep and goats, *S ovifelis* is primarily found in the musculature of the tongue and oesophagus and are slightly larger (1.5 cm), on average, than *C. ovis* (Taylor et al. 2007). Overall, despite their physical resemblance, infection with *C. tenuicollis* and *S. ovifelis* can be differentiated from *C. ovis* based on their location within the host and histological appearance. The bacterium *Corynebacterium pseudotuberculosis*, the causative agent of caseous lymphadenitis (CL), can also produce abscesses within the musculature of a lamb (Food and Agriculture Organization, 2000). Typically, however, the abscesses characteristic of CL are larger than *C. ovis* lesions and are found almost exclusively in the draining lymph nodes and not in the muscle (Food and Agriculture Organization, 2000). It has been documented that, in addition to pathogens, foreign bodies and injections can occasionally produce a muscle abscess; however, the location and size of a single abscess, would be difficult to confuse with *C. ovis* (Ransom, 1913; Food and Agriculture Organization, 2000).

The only absolute method of identifying *C. ovis* infection is through microscopically measuring the rostellar hooks of the protoscolex (Verster, 1969). However, based on the size and location of the lesions within the lamb's musculature, it is generally believed that misidentification of *C. ovis* is unlikely to occur even during routine meat inspection. Therefore, despite a lack of studies estimating the specificity of detecting *C. ovis* infection at slaughter, it is believed to be very good.

1.5 Treatment of *Cysticercus ovis* and *Taenia ovis* infection

As mentioned, the muscle lesions characteristic of *C. ovis* infection do not cause clinical disease in a flock or herd, meaning there are no measureable effects attributed to infection. However, because the infection is often financially important, a treatment to

kill or remove the parasitic lesions from the host's musculature has long been desired by producers. Currently, there is no practical option for the treatment of *C. ovis* in the intermediate host (Taylor et al. 2007). Research has shown that treating pigs, the intermediate host of the cestode *Taenia solium*, with 30 mg/kg of oxfendazole kills all viable cysts with the exception of those in the brain (Sikasunge et al. 2008). This dose is six times the usual dose administered to sheep to kill gastrointestinal nematode parasites (5 mg/kg). Additionally, there have been several studies that have investigated the use of anthelmintics to kill other metacestode infections in sheep, and the results of these inquires vary. Specifically, treating sheep with oxfendazole, or a combination of praziquantel/oxfendazole or praziquantel/albendazole, has been shown to have a modest effect against the metacestode stage of *E. granulosus, C. tenuicollis* and *Coenurus cerebralis* (Gemmell and Johnstone, 1983; Gavidia et al. 2010). However, there is at least one report suggesting that treatment with praziquantel (50 mg/kg) is nearly 100% effective against metacestodes in sheep, including *C. ovis* (Andrews et al. 1983). Importantly, treatments did nothing to remove the lesions from the tissues. Further investigation into anthelmintic treatment for metacestode infections in sheep is warranted.

The use of licensed cestocides is nearly 100% successful in treating *T. ovis* infections in canids (Andrews et al. 1983). However, until recently, there was no product licensed specifically for *T. ovis* in Canada; the only option was to use a product licensed for other *Taenia* species (e.g. *Taenia pisiformis*). The following cestocides are currently licensed for use in dogs in Canada for *Taenia* infections: praziquantel (Drontal Plus® (Bayer, Animal Health Division), Droncit® (Bayer, Animal Health Division), Dolpac®

(Vétoquinol)); nitroscanate (Lopatol® (Novartis Animal Health)); epsiprantel (Cestex® (Pfizer Animal Health)), and fenbendazole (Panacur® (Intervet/Schering-Plough Animal Health)) (Health Canada, 2010). In February 2010, Dolpac® became the first drug licensed in Canada specifically for *T. ovis* infection (Health Canada, 2010) – an indication of increasing awareness about this parasite in Canada.

1.6 Recombinant vaccine development

Previous work has revealed that *T. ovis* oncospheres provide a rich source of immunogenic antigens (Rickard and Bell, 1971; Rickard et al. 1977; Rickard and Williams, 1982). Thus, the host immune system generates a strong humoral response against the oncosphere (Rickard and Bell, 1971; Rothel et al. 1996). Johnson et al. (1989) isolated mRNA for several proteins found on the surface of *T. ovis* oncospheres and developed various recombinant vaccines. After a clinical trial involving these vaccines, necropsies on lambs challenged with *T. ovis* revealed that immunization with the GST-45W protein provided the greatest protection (Johnson et al. 1989). A second trial, incorporating adjuvant, found that 50µg of GST-45W in saponin was the most successful combination, providing 94% protection in lambs against challenge infection (Johnson et al. 1989). This report described the first successful development of a highly effective recombinant vaccine against a parasite infection in its natural host (Johnson et al. 1989), and has since been used as a model for the development of many other recombinant parasite vaccines (Lightowlers, 2006) .

1.6.1 Field trials with the 45W vaccine

Though a major breakthrough, the work by Johnson et al. (1989) did not investigate the vaccine's efficacy under field conditions. The first field trial, carried out

by Harrison et al. (1993a), examined both naturally and artificially challenged lambs following two vaccinations administered subcutaneously in the dorsal neck region at 6 and 12 weeks of age. Following necropsy and thin-tissue slicing to count lesions, the vaccine was determined to reduce cysticerci counts by 98% compared to controls under field conditions (Harrison et al. 1993a). Other large field trials were conducted on five farms in Western Australia and 14 farms in New Zealand (Harrison et al. 1993b). Local abattoirs kept a record of the vaccinated animals and compared them with suitable controls from the same farms. There is no information on how the abattoirs examined carcasses for lesions, but it was likely at the regular processing speed of 7 seconds per carcass (Lawrence et al. 1996). Irrespective of farm location, vaccinated lambs consistently had fewer cysticerci (mean 1.8) compared to the control lambs (mean 92) at $p<0.05$ (Harrison et al. 1993b).

In Australian field trials that assessed the efficacy of *C. ovis* vaccines, 4 out of 5 enrolled farms had *C. ovis* present, with an estimated within-flock prevalence ranging between 2 and 30% at slaughter prior to the study. Of the four farms with *C. ovis* infection, two gave the first vaccination when lambs reached 6-7 weeks of age, after maternal immunity was presumed to have waned, and had great success; the vaccine reduced the detected number of lesions by 85-100% compared to controls (Harrison et al. 1993b). The remaining two farms, which gave the first vaccination when lambs reached 9-11 weeks of age, had less success which was attributed to a greater exposure time after passive immunity had waned and prior to vaccination (Harrison et al. 1993b).

In similar trials involving 14 farms in New Zealand, where the farm-level prevalence of infection in lambs at slaughter was between 0.3-13%, the vaccine resulted

in an 83% reduction in the number of cysticerci detected at slaughter (Harrison et al. 1993b). Four of the New Zealand farms, in addition to lamb vaccination at docking and weaning, opted to additionally vaccinate pregnant ewes to enhance passive immune transfer. The vaccine's efficacy under these conditions resulted in only a 29% reduction in the number of lesions found in the lambs compared to controls (Harrison et al. 1993b). These findings are consistent with those of Lawrence et al. (1993), who found that the presence of maternal antibodies hinders the efficacy of vaccination in lambs. From these data, it is reasonable to assume that in order to maximize protection in lambs, vaccination should not occur until after maternal immunity has waned, most likely at six weeks of age.

1.6.2 To16 and To18 vaccine development

In addition to the original recombinant 45W protein discovered by Johnson et al. (1989), two other unrelated immunogenic recombinant proteins have been isolated and proven effective against *C. ovis* infection in lambs (Harrison et al. 1996). These proteins, called To16 and To18, have been the subject of clinical trials by Harrison et al. (1996) and provided a 92% and 99% reduction in the number of cysticerci, respectively, in vaccinated lambs compared to controls ($p<0.01$). Analysis of the structural components of the three host-protective proteins (45W, To18, To16) indicated that they are not closely related (Harrison et al. 1996). The development of three vaccines, each incorporating unique recombinant proteins, would potentially alleviate selection pressure against a single vaccine and lessen the risk of development of vaccine-resistant *C. ovis*.

1.6.3 Biological function of recombinant proteins

The exact function of the recombinant proteins' natural counterparts in the biology of an oncosphere remains unknown (Harrison et al. 1996; Waterkeyn et al. 1997). Genome sequencing has failed to reveal a homolog that could provide insight on function (Harrison et al. 1996). Immunogenic proteins of *Taenia* species are believed to be excretory or secretory in nature (Rickard and Bell, 1971); however, Lightowlers et al. (2003) caution that a somatic protein that is released upon lysis of the parasite cannot be excluded as the source of host-protective antigens. Regarding function, one study suggested that *T. ovis* antigens may be necessary for the initial attachment of the egg to the lamb's intestinal wall (Bork and Doolittle, 1993). However, work by Waterkyn et al. (1997) indicated that the parasite continues to secrete host-protective antigens at a time when attachment to the lamb's intestine is no longer required, possibly indicating another biological function. A recent study that microscopically examined oncospheres using immunohistochemical staining with labelled streptavidin–biotin failed to locate any of the *T. ovis* host-protective antigens on the surface of newly activated oncospheres (Jabbar et al. 2010b). Examination of the oncosphere surface nine days later, revealed moderate to dense staining, but at day 15 there were no antigens present (Jabbar et al. 2010b). These data suggest that host-protective antigens on the surface of an oncosphere are only present for a short time following activation, after which they disappear and the oncosphere is no longer susceptible to vaccine-mediated immune attack (Jabbar et al. 2010b).

1.6.4 Vaccine commercialization

Despite the successful development of a vaccine against *C. ovis*, both in terms of efficacy (Johnson et al. 1989; Harrison et al. 1996) and large-scale manufacturing (Dempster et al. 1996), the product has never been available for commercial use anywhere in the world (Lightowlers, 2006). The primary motivation for *C. ovis* vaccine development was the financial consequences associated with high numbers of condemnations, and the impact the infection was having on lamb export, particularly in Australia and New Zealand. By the time the vaccine had been developed, proven effective, and made ready for licensing, it was decided that the financial returns associated with the vaccine would be insufficient to warrant further marketing (Rickard et al. 1995). Considering the prevalence of infection on some Australian farms was reported to be as high as 30% during the early 1990s (Harrison et al. 1993b), the halt in vaccine licensing may appear abrupt. However, *C. ovis* infection rarely results in financial losses for the individual producer (Rickard et al. 1995). Furthermore, even with known *C. ovis* infection on their farm, producers may be able to market their animals because there is typically no accountability for condemnations at slaughter. Holding producers accountable is further complicated by the transport of animals between farms and feedlots prior to slaughter. When the lamb is raised on more than one property, it can be difficult to determine exactly where a condemned carcass was originally infected. For these reasons, the monetary losses caused by *C. ovis* infection are most frequently absorbed by the abattoir where the infection is first detected. The lack of accountability, which means less risk of consequences for individual producers, results in producers unwilling to spend money on a novel vaccine that requires multiple injections per year,

regardless of its efficacy. In order to increase the incentive to vaccinate against *C. ovis*, it may be necessary for abattoirs to require proof of proper vaccination before purchasing animals.

1.7 National *Cysticercus ovis* control programs

Cysticercus ovis is generally considered a sporadic infection (Taylor et al. 2007) that has been reported in every inhabited continent of the world (Ransom 1913; Drabble, 1934; Gordon 1939; McCleery and Wiggins, 1960; Soehl 1984; Cardoso and Oliveira, 1994; Al-Qureishy, 2008; Eichenberger et al. 2011). However, official records of *C. ovis* condemnations are rarely kept, and the prevalence of infection in most countries is not known. Undoubtedly, *C. ovis* is not equally distributed around the world; certain countries have experienced greater trouble with the parasite compared to others. Furthermore, the low sensitivity of detecting the parasite in lambs at slaughter (50%) almost certainly means that higher prevalences occur than those reported in the literature.

Compared to other countries, the sheep industries in Australia and New Zealand have endured the most adversity from *C. ovis* infection, and much of what is currently known about the parasite can be attributed to research in these countries. In New Zealand, throughout the first half of the 20th century, *C. ovis* infection was rare (Lawson, 1994); and *T. ovis* was almost exclusively a parasite of dogs working at abattoirs, with an estimated prevalence of 6% in 1958. *Taenia ovis* was rarely seen in farm dogs during this same time period (Gemmell, 1958b). There was, however, a high prevalence of *E. granulosus* in farm dogs, which became a substantive issue for both human and sheep health throughout New Zealand (Gemmell 1958b; Lawson, 1994). In 1959, the first formal control program against *E. granulosus*, known as the Hydatids Act, was initiated

in New Zealand (Lawson, 1994). The Hydatids Act resulted in the formation of a regulatory body known as the National Hydatids Council (NHC) which had a mandate to develop and implement new policy in an attempt to control the infection (Lawson, 1994). The NHC initiated an educational campaign focusing on control measures that were specifically directed at sheep producers. This educational campaign also consisted of mandatory treatment of farm dogs using arecoline hydrobromide, a purgative that effectively functioned as a cestocide (Lawson, 1994). In the early 1960s, following the creation of the NHC, there was a sudden increase in *C. ovis* infection reported throughout the country (McNab and Robertson, 1972).

Concurrently in Australia, warnings about escalating numbers of sheep carcass condemnations due to cestode infections were issued to producers (Arundel, 1972). Approximately 6.0% and 5.0% of sheep livers were being condemned due to *E. granulosus* and *C. tenuicollis*, respectively; however, at this time, the number of carcass condemnations caused by *C. ovis* infection remained only around 0.0044% (Arundel, 1972). Again, the true prevalence of *C. ovis* infection was likely much greater due to low test sensitivity at slaughter, but nevertheless, such low numbers of condemnations failed to inspire much change in farm management to prevent *C. ovis* infection. Furthermore, because of the nearly identical lifecycles of these parasites, the elevated numbers of *E. granulosus* and *C. tenuicollis* infections were indicative of farm management practices that were likely contributing to *C. ovis* infection. In 1967, the extent of the problem with *C. ovis* infection in Australian mutton became clearer when the United States Department of Agriculture (USDA) introduced a new program to sample imported meat (Arundel, 1972). The new program improved detection of *C. ovis* lesions in mutton and resulted in

an 18% increase in rejected Australian mutton shipments to the USA (Arundel, 1972). During the first six months of 1968 alone, 12.5% of the total shipment of Australian mutton to the USA was rejected because of *C. ovis* infection, costing Australia an estimated $US 1,540,000 in sales (Arundel, 1972).

Indirect losses associated with the new USDA requirements also drastically affected the Australian sheep industry during this time. Other countries, including Canada, would not accept the Australian mutton rejected by the USA, resulting in it being downgraded to pet food or being transported back to Australia for use in local markets (Arundel, 1972). Moreover, insurance companies started increasing their premiums to cover the cost of higher rejection rates (Arundel, 1972). During 1970, the USA and Canada completely banned all imports of Australian mutton. The occurrence of *C. ovis* infection played a significant role in the ban, but caseous lymphadenitis was also partly responsible (Arundel, 1972). Losing the USA and Canadian markets decreased the export of Australian mutton by 45% (Arundel, 1972). Immediate changes were implemented at Australian abattoirs to better allow the detection of *C. ovis* prior to export (Arundel, 1972). The improvements immediately increased the quality of Australian meat being exported, and the bans on Australian mutton were lifted.

In New Zealand during the 1960s, the prevalence of *C. ovis* infection in slaughtered ewes rose substantially throughout the decade and was estimated to be 6.0% in 1968 (McNab and Robertson, 1972). The increase in prevalence was surprising because a control program was in place against *E. granulosus*. Furthermore, the prevalence of *C. tenuicollis* had declined dramatically upon initiation of the Hydatids Act in 1959 (Lawson, 1994). A central point in the NHC's educational campaign was to

discontinue the feeding of sheep offal to farm dogs (Lawson, 1994). Dog food can be expensive and, often times, feeding scraps of offal from slaughtered animals is a more cost-effective way of feeding farm dogs. By discontinuing the feeding of ovine offal to farm dogs, producers effectively reduced the transmission of *E. granulosus*, whose hydatid cysts are primarily found in sheep liver and lungs. Similarly, the Hydatids Act was also effective against *C. tenuicollis*, which is also found in sheep liver. To compensate for no longer feeding offal, the carcasses of cull ewes became the staple diet for the great majority of farm dogs, resulting in increased exposure to *C. ovis* (Lawson, 1994). In addition, there was some evidence that supported the idea of cross-protection between *C. tenuicollis* and *C. ovis* (Gemmell 1965; Gemmell, 1969; Varela-Diaz et al. 1972); the documented decline in the prevalence of *C. tenuicollis* possibly resulted in greater susceptibility of sheep to *C. ovis* infection. Another factor that allowed the prevalence of *C. ovis* to increase, while *E. granulosus* declined, was the high fecundity of *T. ovis*. During the early years of the Hydatids Act, dogs were dosed regularly with arecoline hydrobromide as part of the surveillance program (Lawson, 1994). This drug is effective as a purgative but only moderately effective as a cestocide (Gemmell, 1958a). Frequently, multiple proglottids were removed with this treatment, but scolices were often left in place, allowing further reproduction. The high fecundity of *T. ovis* (Gregory, 1976) resulted in enormous egg production compared to *E. granulosus* (Gemmell et al. 1987). With no protocol in place to control *C. ovis*, combined with the ease at which dogs were infected, and the parasite's high fecundity, *C. ovis* infection increased substantially in New Zealand.

Realizing what was occurring in Australia because of *C. ovis*, and fearing they too would lose their export market, in 1970, New Zealand added *T. ovis* to the Hydatids Act (Lawson, 1994). This made it illegal to feed dogs sheep meat that had not been frozen at -10°C for ten days or cooked to 72°C (Lawson, 1994). In 1972, the NHC introduced mandatory deworming of farm dogs every six weeks using niclosamide, which was subsequently replaced with praziquantel in 1978 (Lawson, 1994). Following the introduction of mandatory deworming, the number of condemnations declined. However, after 1976, the number of infections seen at abattoirs began to once again slowly increase (Lawson, 1994). There was speculation that decreased producer compliance regarding mandatory deworming was responsible for the rise in infection (Lawson, 1994). There was also evidence that mandatory deworming had selected for *T. ovis* with prepatent periods less than six weeks, allowing it to reproduce prior to the required treatment schedule (Heath and Lawrence, 1980). Nevertheless, the prevalence of *C. ovis* infection stabilized and remained relatively constant in New Zealand over the next 15 years (Lawson, 1994).

In 1990, following the success of the Hydatids Act, the efforts to control *E. granulosus* and *C. ovis* were separated as it was thought that producers could maintain control of *C. ovis* without government support (Lawson, 1994). However, in 1991, the number of carcasses infected with *C. ovis* at New Zealand abattoirs tripled to nearly 4.0% (Simpson, 2009). In fear of losing their sheep exports, a new industry-based, non-regulatory program called Ovis Management Limited was created by the Meat Industry Association of New Zealand, and continues to exist to this day. The purpose of Ovis Management Ltd. is to educate producers about *C. ovis* and the necessary control

strategies, including the need to deworm farm dogs at least every six weeks (preferably every four weeks) (Ovis Management Ltd., 2011). Since the formation of Ovis Management Ltd., the number of *C. ovis* infections detected in sheep in New Zealand has declined to approximately 0.6% (Simpson, 2009).

1.8 Status of *Cysticercus ovis* in Canada

There is little published information on *C. ovis* infection in Canada. *Cysticercus ovis* is not a reportable, immediately notifiable or annually notifiable disease in Canada (Canadian Food Inspection Agency, 2010). As a result, there are few federal or provincial records available about rates of infection in Canada. The information that does exist suggests that *C. ovis* has occurred sporadically across Canada since at least the early 1980s, and likely longer (Soehl, 1984). In the United States, there have been documented *C. ovis* infections sporadically appearing in sheep carcasses since as early as 1912 (Ransom, 1913; Jensen et al. 1975). With the close proximity between Canada and the USA, along with the importation of live sheep into Canada from the USA, it is likely that there were infections in Canadian sheep prior to the 1980s that went unrecorded. The earliest documented instance of *C. ovis* infection in Canada was in 1981 when five geographically distinct farms in Nova Scotia were found to have lambs infected at slaughter (Soehl, 1984). The source of infection was eventually determined to be a farm from which all of the infected farms had recently purchased dogs (Soehl, 1984). This farm routinely offered mutton to their dogs while training them; it was later determined that the farm also had sheep infected with *C. ovis* (Soehl, 1984). Since then, infections have continued to appear sporadically in sheep at abattoirs across the country; however, it remains difficult to determine where these infected animals originated.

1.8.1 Impact of wildlife on *Cysticercus ovis* in Canada

An interesting aspect of *C. ovis* infection in Canada is the potential involvement of wild canids and small ruminants in a sylvatic lifecycle. The role of wildlife in transmission of the parasite in Canada is currently not known. A study in the late 1960s, investigating parasites of wild Canadian Bighorn sheep (*Ovis canadensis*), found no evidence of *C. ovis* infection in this population, although this does not prove that they cannot become infected (Uhazy and Holmes, 1971).

The potential for wild canids to act as a definitive host for *T. ovis* is a unique scenario in Canada that is not seen in other areas of the world where *C. ovis* has caused problems. There have been several studies investigating internal parasites of wild canids in Canada (Freeman et al. 1961; Samuel et al. 2001). On several occasions, the parasite *T. krabbei* was reported in coyote and wolf populations, with an estimated prevalence of 2% and 7%, respectively (Freeman et al. 1961; Seesee et al. 1983; Samuel et al. 2001). *Taenia ovis* was never reported in coyotes and wolves, probably because their primary food source (wildlife) limits their exposure (Samuel et al., 2001). However, the inability to differentiate between *T. krabbei* and *T. ovis* using morphological criteria means that it cannot be assumed a diagnosis of *T. krabbei* in wild canids is always correct. It is possible that some *T. krabbei* infections identified in wild canids are actually *T. ovis*, acquired through the consumption of domestic sheep. If this is the case, then the prevalence in wild canids could be significantly greater now that the prevalence of *C. ovis* infection has increased on Canadian sheep farms. In both North America and Australia, fecal analysis and post-mortem examinations have never revealed *T. krabbei* or *T. ovis* in foxes (Ryan, 1974; Samuel et al. 2001). It is thought that the normal diet of

foxes, primarily rodents, limits exposure to both *T. ovis* and *T. krabbei*, and therefore they do not significantly contribute to transmission.

Of the wild canids in Canada, the coyote has had the greatest impact on sheep production through predation. Although current research is lacking, the frequency of predation on Canadian sheep farms suggests that coyotes are almost surely being exposed to *C. ovis* given the increased prevalence seen in slaughtered sheep compared to earlier years. Besides predation, the improper disposal of deadstock by producers often likely results in carcass scavenging by coyotes, potentially leading to *C. ovis* exposure. While coyote exposure to *C. ovis* is probable, the risk of transmitting the parasite to sheep remains unclear. For example, it is not known how frequently coyotes defecate on pasture while preying on sheep. For a coyote, the pasture could be considered a high-risk environment, producing stress that effectively eliminates the urge to defecate (Brent Patterson, Ministry of Natural Resources, personal communication 2010). There has been speculation that coyotes use round bales to scout hunting locations, and that defecation could occur on them, but this has not been proven (Barling et al. 2001). If coyotes are not defecating on feed, whether it is pasture or round bales, then the risk of them transmitting *C. ovis* to sheep is minimal. Further research is needed in this area to determine the impact coyotes have on *C. ovis* transmission in Canada.

1.9 Thesis rationale and objectives

In recent years, anecdotal data from Canadian abattoirs have revealed a significant increase in the number of lamb condemnations due to *C. ovis* infection. This rise suggests that the prevalence of this infection on Canadian sheep farms is increasing. While there have been numerous studies investigating the parasite in New Zealand and Australia,

little research has been carried out elsewhere. The epidemiology of *C. ovis* in Canada remains unknown, but is likely to be significantly different to New Zealand and Australia because of differences in flock management, and the presence of wild canids in Canada. Considering the financial consequences associated with lamb carcass condemnations due to *C. ovis* infection, as illustrated by situations in New Zealand and Australia, it is essential that the risk factors for infection of Canadian sheep be identified. Understanding the risk factors and the impact that farm dog management, wildlife predation, and deadstock disposal have on the transmission of C. *ovis* will also allow for the development of an effective control or eradication program(s) for Canadian sheep flocks.

The primary objectives of this thesis were:

1) To determine the geographical distribution of farms in Canada that have had lamb carcass condemnation(s) due to *Cysticercus ovis* infection.

2) To determine farm-level risk factors associated with carcass condemnation due to *Cysticercus ovis* infection in slaughtered lambs or sheep.

3) To develop an infectious disease transmission model for *Cysticercus ovis* that will predict the spread of the parasite on Canadian sheep farms based on various input parameters, and allow for the assessment of control options.

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Chapter Two

Distribution of, and risk factors associated with, sheep carcass condemnations due to *Cysticercus ovis* infection on Canadian sheep farms

2.1 Introduction

Cysticercus ovis is the intermediate stage of the canine cestode, *Taenia ovis*, with sheep and goats being the intermediate host species. Within skeletal and cardiac muscle of the intermediate host, *C. ovis* appears as a thin-walled, fluid-filled, cyst-like structure approximately 1 cm in diameter (Ransom, 1913). Transmission of the parasite occurs when definitive hosts, wild and domestic canids, ingest viable *C. ovis* lesions through the consumption of sheep and goat meat (Sweatman and Henshall, 1962). In the small intestine of the definitive host, *C. ovis* matures into an adult tapeworm and, via the host's defecation, releases large numbers of eggs into the environment that may later be consumed by intermediate host species, thus completing the lifecycle. Although *C. ovis* is neither a flock health nor zoonotic issue, it is a food quality issue. Cysticerci, both viable and degenerate, are visible in the meat of infected animals rendering it aesthetically undesirable to the consumer. As a result, sheep heavily infected with *C. ovis* are frequently deemed unacceptable for human consumption and are condemned at slaughter by government meat inspectors. Thus, sheep industries in areas with a high prevalence of *C. ovis* can suffer serious monetary losses due to high numbers of condemned carcasses, and the loss of export markets (Arundel, 1972; Lawson, 1994).

The condemnation of sheep carcasses due to *C. ovis* infection is based on guidelines provided for meat inspection by the Food and Agricultural Organization of the United Nations (FAO). The guidelines recommend that a lamb carcass be condemned due

to *C. ovis* infection if it is heavily infected with the parasite; a carcass is considered heavily infected if "lesions are discovered in two of the usual inspection sites including the masseter muscles, tongue, oesophagus, heart, diaphragm or exposed musculature, and in two sites during incision into the shoulder and rounds" (Food and Agriculture Organization, 2000). However, if a carcass is lightly or moderately infected, the FAO recommendation is to trim the carcass by removing the cysts from the muscle, and hold the meat for 10 days at -10°C before approving for human consumption (Food and Agriculture Organization, 2000).

In Canada, there is currently no government system in place to inform producers if a sheep produced on their farm is subsequently condemned due to *C. ovis* infection. The Canadian sheep industry, with the exception of Quebec (which uses Agri-Traçabilité Québec), currently uses a national identification program, called the Canadian Sheep Identification Program (CSIP). The CSIP requires that all ovine animals in Canada be tagged with a unique CSIP number prior to leaving the farm of origin; and that the same tag remains with the animal until slaughter (Canadian Food Inspection Agency, 2008). Developed in 2004, the CSIP is used by the Canadian Food Inspection Agency (CFIA) for trace-back purposes if a reportable disease is detected in a sheep prior to, or during, slaughter. Reportable diseases are designated by the CFIA and include important foreign animal and zoonotic diseases – usually also listed by the World Organisation for Animal Health (World Organisation for Animal Health, 2011). Examples of reportable diseases include scrapie and foot-and-mouth disease. Currently, *C. ovis* infection is not a reportable disease in Canada (Canadian Food Inspection Agency, 2010), neither is it immediately or annually notifiable, meaning that there is no requirement for national

reporting of any kind. Accordingly, the CFIA does not trace-back sheep carcasses condemned due to *C. ovis* infection.

Currently, much of what is known about the epidemiology of *C. ovis* infection is based on research carried out in New Zealand and Australia, where it is considered endemic (Lawson, 1994). The epidemiology of *C. ovis* in Canada remains unknown, but is likely significantly different from New Zealand and Australia because of differences in flock management, and the presence of wild canids in Canada. Considering the struggles with predation faced by sheep producers in Canada (Canadian Sheep Federation, 2009), the potential for wild canids to serve as the definitive host of *T. ovis* and contribute to parasite transmission cannot be dismissed. Thus, it is essential that the distribution of, and risk factors for, *C. ovis* infection in Canadian sheep be identified. By understanding the role farm management practices have on the risk of *C. ovis* transmission in Canada, effective control and eradication programs for Canadian sheep farms can potentially be developed. The purpose of this study was therefore to: (1) determine the frequency with which sheep carcasses are condemned due to *C. ovis* infection in Canada, and (2) determine farm-level risk factors associated with carcass condemnations due to C. *ovis* infection in Canadian sheep.

2.2 Materials and Methods

2.2.1 Frequency of *Cysticercus ovis* condemnations in Canada

To estimate the frequency of sheep carcass condemnations due to infection with *C. ovis* in Canada, condemnation statistics were obtained from provincially inspected abattoirs within Ontario, as well as federally inspected abattoirs across the country. In Ontario, there are 121 licensed abattoirs that slaughter small ruminants, of which 114

(94%) are provincially inspected, with the remaining being federally inspected (Ontario Ministry of Agriculture, Food and Rural Affairs, 2011). Across Canada, there are 19 federally inspected abattoirs licensed to slaughter small ruminants; however, only 16 are slaughtering small ruminants on a regular basis. British Columbia (25), Saskatchewan (8) and Manitoba (14) also have federally inspected provincial abattoirs that slaughter small ruminants. Sheep condemnation statistics for Ontario provincially inspected abattoirs were obtained through the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA, Veterinary Science & Policy Unit, Elora, ON), while condemnation information for federally inspected abattoirs was collected through Agriculture and Agri-Food Canada (Red Meat and Poultry Sections, Ottawa, ON). The condemnation statistics indicate how many sheep are condemned each year, and the reason for each condemnation. These data do not include trimmed carcasses, with light *C. ovis* infection, which are subsequently passed.

2.2.2 Risk factors for carcass condemnations due to *Cysticercus ovis*

i) Study design

A retrospective case-control study was used to determine farm-level risk factors associated with sheep carcass condemnations due to infection with *C. ovis* in Ontario provincially inspected abattoirs. Data were collected over a two-year period between March 2009 and March 2011. The study was approved by the Research Ethics Board at the University of Guelph.

All Ontario provincially inspected abattoirs were informed of the study through a notice distributed by the Ontario Independent Meat Processors, and were asked to cooperate with the provincial and federal meat inspectors in obtaining tags from

condemned and control lambs. Beginning in March 2009, provincially inspected abattoirs in Ontario began collecting CSIP tags from sheep carcasses condemned due to infection with *C. ovis*.

ii) Case definition and selection

Carcasses were inspected based on the FAO guidelines for meat inspection at 114 provincially inspected abattoirs across Ontario. A case was defined as the farm of origin (usually birth-place) of a sheep condemned at slaughter in an Ontario provincially inspected abattoir due to infection with *C. ovis*. To obtain this information, the CSIP tags of condemned sheep were removed and forwarded to the Canadian Sheep Federation (CSF). Independent of the researcher carrying out this project, the CSF facilitated use of the national CSIP database, identified case farms from the CSIP tag of the condemned sheep, and passed the contact information to a veterinarian employed by the agriculture ministry of the province in which the farm was located. This provincial veterinarian was supplied with extension information, developed by the CSF, regarding the infection and its control. By telephone, the provincial veterinarian informed the producer that a sheep originating from their farm had been condemned at slaughter due to *C. ovis* infection. Both by telephone and mail, the producer was provided with information about *C. ovis*, including how it is transmitted, and possible control methods that could help reduce future infection risk. The provincial veterinarian was also able to answer questions about the infection in more detail if requested. Each producer was then asked if he/she would be willing to participate in a survey administered by the University of Guelph concerning farm management practices that may be associated with infection.

iii) Selection of controls

For every case farm identified, two control farms were selected by abattoir employees. Specifically, on a day when a sheep carcass was condemned due to *C. ovis*, the abattoir employees were asked to select the CSIP tags of five sheep carcasses that passed meat inspection and were approved for human consumption. From the five possible control CSIP tags selected for a particular case, two were chosen randomly by the CSF to serve as controls in the study. Using the same protocol described for the cases, the CSIP tags of the two controls were traced back to identify each animal's farm of origin. If either control tag was from the farm of the condemned sheep it was discarded and a new tag was randomly selected from the remaining tags. Potential control farms were also excluded from the study if they had ever previously had a sheep condemned due to *C. ovis* infection at an Ontario provincially inspected abattoir (according to CSF records), or if they had previously been selected as a control in the study. Thus, a control was defined as a farm that had not been identified as ever having had a sheep carcass condemned at an Ontario-provincially inspected abattoir due to *C. ovis* infection, and was matched to its case by abattoir and slaughter date.

Occasionally, farms selected as controls would, at a later date, have a sheep carcass condemnation due to *C. ovis* infection. In this situation, if more than one year had elapsed between the slaughter dates of the two animals, the control data were kept, but additionally the producer was re-interviewed as a case. If less than a year had elapsed, the control data were discarded and the producer was re-interviewed as a case. If available, alternative controls were selected from the three remaining potential control farms.

iv) Questionnaire administration

The questionnaire used in the study was pre-tested using the CSF board of directors. Producers that agreed to participate in the survey were mailed the questionnaire one week prior to being contacted by telephone, by the author, to complete the questionnaire. The author was blinded to the farm's classification as a case or control. The questionnaire (see Appendix 1) consisted of 17 closed-ended questions that asked producers about their farm and flock management practices in the year prior to condemnation of the lamb in question. Specifically, it collected information on farm location, flock size and management, farm dog anthelmintic treatments, flock predation, and deadstock disposal. The questionnaire did not ask the producer to identify if they were from a case or control farm. The case or control status of the farms selected and interviewed was not revealed by the CSF until data analysis began. Producers were assured that all responses would be kept confidential, and that no personal identifying factors would be disclosed.

2.2.3 Statistical analysis

After data collection was complete, the case-control status of each participating farm was made known to the researcher by the CSF, and served as the outcome of interest in the analyses. Questionnaire data were coded directly into the software program Stata 10.0 for Windows (College Station, TX, USA). The data were examined to identify missing observations. Any missing observations were excluded from analyses. The correlation between predictor variables irrespective of outcome status was assessed using a pair-wise Spearman's rank correlation coefficient. Variables with a correlation

coefficient greater than 0.8 were considered highly correlated, and only one was selected for multivariable analysis based on biological plausibility.

Using Stata 10.0, summary statistics were compiled for each predictor variable and Chi-square analyses were used to assess the association of each variable of interest with the outcome. Variables that were significantly associated with the outcome at $p \leq 0.2$ were included in a multivariable model as main effects. A multivariable logistic regression model was constructed using a manual backwards selection approach. All possible two-way interactions between variables considered significant ($p \leq 0.05$) in the main effects model were created and added to the model. Interaction terms that were not statistically significant ($p > 0.05$) were subsequently removed from the model. The contribution of each remaining variable in the model was evaluated using the likelihood ratio test. Variables remained in the model if they were associated with a significant likelihood ratio test ($p \leq 0.05$), were part of a significant interaction term ($p \leq 0.05$), or if they acted as a confounder. A confounder was defined as a variable that caused a greater than 25% change in the coefficient of another variable following the removal of the potential confounder from the model (Dohoo et al. 2009)

Residual analyses were undertaken through the assessment of standardized Pearson residuals, deviance residuals, leverage, and the delta-beta statistic. If residual inspection revealed an extreme observation, the model was reassessed with the observation removed from the data. If removal of the observation caused a change in the statistical significance of a variable at $p \leq 0.05$, or altered the direction of its association, the observation was permanently removed. However, if the model's interpretation did not significantly change following the observation's removal, it remained a part of the data.

2.3 Results

2.3.1 Frequency of *Cysticercus ovis* condemnations

i) Ontario provincially inspected abattoirs

Figure 2.1 shows the number of *C. ovis* condemnations at all 114 Ontario provincially inspected abattoirs each year from 2003 to 2011 as reported by OMAFRA. In 2008, the number of *C. ovis* condemnations increased considerably compared to previous years. The number of *C. ovis* condemnations at Ontario provincially inspected abattoirs was highest in 2008, with a total of 303 carcasses being condemned. Although the *C. ovis* condemnations in 2009 and 2010 decreased in comparison to 2008, the number of condemnations during these years, 144 and 123 respectively, were still markedly greater than the number of condemnations seen in any year prior to 2008 (Figure 2.1).

The number of *C. ovis* condemnations as a proportion of all sheep carcass condemnations at Ontario provincially inspected abattoirs, between 2003 and 2011, is shown in Figure 2.2. In 2008, a total of 626 of the 248,535 sheep slaughtered at Ontario provincially inspected abattoirs were condemned for various reasons; *C. ovis* represented 48.4% of all condemnations, making it the number one reason for carcass condemnations in sheep that year. By comparison, in 2003, *C. ovis* was not responsible for any of the 377 carcass condemnations reported. Thus, in a 5-year time period the proportion of condemnations in Ontario caused by *C. ovis* increased from 0% to nearly 50%.

ii) Federally inspected abattoirs across Canada

Figure 2.3 depicts the numbers of carcass condemnations due to *C. ovis* at 15 of 19 federally inspected abattoirs, and 34 of 47 federally inspected provincial abattoirs in

Canada, from 2002 to 2011, as reported by Agriculture and Agri-Food Canada (Red Meat and Poultry Sections, Ottawa, ON). Figure 2.4 depicts the proportion of all sheep carcass condemnations caused by *C. ovis* infection at federal, and federally inspected provincial abattoirs from 2002 to 2011. Noteworthy is the fact that the initial spike in the proportion of *C. ovis* condemnations at federally inspected abattoirs occurred one year prior to the initial spike in the proportion at Ontario provincially inspected abattoirs. Further, compared to 2009 and 2010 data, the available data on 2011 federal condemnations, currently only to March 31, suggest a much greater number of carcass condemnations due to *C. ovis* at federal abattoirs. Within a three month time period in 2011, the condemnation numbers at federally inspected abattoirs have already surpassed the total condemnations in each of the previous two years (Figure 2.3). Although there is variability each year, typically around 30% of the total carcass condemnations each year occur between January 1st and March 31st, suggesting that in 2011, the number of condemnations due to C. *ovis* at federally inspected abattoirs may surpass 400 carcasses. This elevated number of condemnations is noteworthy considering the total number of sheep slaughtered at federally inspected abattoirs from January to March 2011 decreased 14% during the same period in 2010 (Agriculture and Agri-Food Canada, 2011).

2.3.2 Distribution of *Cysticercus ovis* in Canada

Between March 31, 2009 and March 31, 2011 there were a total of 237 sheep carcasses condemned due to *C. ovis* at 114 Ontario provincially inspected abattoirs that originated from 133 different farms across Canada. Table 2.1 shows the province of origin of the condemned sheep, as well as the number and proportion of farms with condemnations, and the mean number and range of condemnations per farm by province.

In every province represented, there were individual farms that had multiple condemnations. Furthermore, despite constituting only 6.7% of the total Canadian sheep flock (Statistics Canada, 2011), Manitoba accounted for the highest number of sheep condemned in Ontario due to *C. ovis* infection over the study period (n=98), and had the greatest proportion of farms with at least one condemnation (19%; 37/195). These data do not account for condemnations that occurred in provinces other than Ontario.

2.3.3 Characteristics of farms in case-control study

Of the 133 farms with *C. ovis* condemnations, 131 could be traced-back to the farm of origin using CSIP; two could only be identified as Agri-Traçabilité Québec tags. Of those 131 farms that could be identified, 40 agreed to participate in the study and completed the questionnaire, resulting in a case farm response rate of 30.5% (40/131). The response rates for case farms differed between provinces and are shown in Table 2.2. Following the trace-back of controls, it was discovered that several potential control carcasses originated from the same farms as the condemned carcass to which they were matched. This is perhaps not surprising because animals from the same farm are frequently slaughtered in groups. As a result, several potential controls had to be discarded, causing the number of controls per case to be much less than 2:1. Overall, a total of 56 control farms participated in the study. Unfortunately, the response rate for control farms was not recorded, but it is believed to be slightly higher than that of cases (Jennifer MacTavish, CSF, personal communication, 2010).

The location of all participating farms by province is shown in Table 2.3. Spearman's rank correlation coefficients indicated that no two predictor variables were highly correlated with each other (>0.8). Therefore, all were included in univariable

analyses. A summary and univariable statistical comparison of characteristics and management practices of case and control farms is shown in Tables 2.4 and 2.5.

Regarding the primary method of marketing lambs, only a small proportion of interviewed cases and controls exclusively sold direct to slaughter (Table 2.4). The majority of producers mainly sold market lambs to either a middleman, defined as a sales barn or broker (65% (26/40) cases; 58.2% (32/55) controls), or direct to a feedlot (22.5% (9/40) cases; 32.7% (18/55) controls).

It was common for sheep farms to have domestic dogs: 78% (31/40) of case farms reported having dogs that have contact with the sheep flock, compared to 82% (45/55) of controls (χ^2=0.13; p=0.72). The roles that domestic dogs had on farms varied, but could generally be categorized into three groups: guardian, herding/companion, and stray. A guardian dog was classified as a dog that continuously lived with the sheep flock to provide protection from predators. Unlike guardian dogs that were in constant contact with the flock, herding or companion dogs were defined as dogs that only occasionally had contact with the sheep or pasture. Although there are differences between the role of herding and companion dogs on farms, these two groups of dogs were categorized together based on their presumed exposure level to sheep. Stray dogs were defined as domestic dogs that do not belong to the producer but wander onto farm property and have contact with the sheep or pasture. Stray dogs could be feral, or belong to hunters, hikers or neighbours and are not under direct supervision at all times.

Only 9.7% (3/31) of case farms with domestic dogs reported intentionally feeding sheep meat to their farm dogs, compared to 4.4% (2/45) of controls. On two of the case farms and both control farms, sheep meat was provided less frequently than once a

month; commercial dog food comprised the bulk of the diet. The remaining case farm fed sheep meat daily, using it as the primary component of their farm dogs' diet. Prior to offering sheep meat to dogs, two of the farms (one case, one control) either froze or cooked the sheep meat. However, the extent of freezing or cooking was not ascertained. Approximately 48% (15/31) of case farms and 24% (11/45) of control farms reported that farm dogs scavenged deadstock (χ^2=4.11; p=0.04).

Of the farms with dogs that contacted sheep, 74% (23/31) of case farms and 79% (35/45) of control farms reported treating their dogs with anthelmintics in the year prior to a sheep carcass condemnation (χ^2=0.16; p=0.69). However, the questionnaire did not collect information on the frequency of treatment in that year. When asked about the product used to treat farm dogs, 1 case used a product ineffective against cestodes (pyrantel) and 14 cases were unable to recall the product used. This was a similar problem in the control group, with 6 producers using products ineffective against cestodes (pyrantel or macrocyclic lactones) and 20 unable to remember the product name. The remaining producers reported treating farm dogs with praziquantel (2/31 (6.5%) cases; 2/45 (4.4%) controls), nitroscanate (5/31 (16.1%) cases; 6/45 (13.3%) controls) or fenbendazole (1/31 (3.2%) cases; 1/45 (2.2%) controls).

Predation was reported to be a significant problem on many farms; 50% (20/40) of case farms and 61.8% (34/55) of control farms (χ^2= 1.32; p=0.25) lost animals to predators in the year prior to the slaughter of the reference sheep. Coyotes were cited as the predator primarily responsible for sheep losses on the vast majority of those farms (90.7%; 49/54). Foxes and wolves were also reported as predators that would occasionally prey on sheep, being responsible for 5.6% (3/54) of predator attacks.

Various methods of deadstock disposal were reported by case and control farms, including burial in the ground (ground); burial in a manure pile (manure); composting of deadstock in a composting unit (compost); incineration (incinerate); and disposal or burial vessels which are leak-proof containers buried in the ground (vessel) (Table 2.6). Also reported was "no disposal", whereby producers either did nothing with fallen stock, or they actively discarded deadstock into an unoccupied field or forest, allowing it to be scavenged by wild or domestic canids.

2.3.4 Multivariable risk factor analysis

Farms located in British Columbia (n=2), and Nova Scotia (n=1), were removed from multivariable analyses because these provinces were not sufficiently represented in the data to contribute to the risk factor analysis.

Five predictor variables were significantly associated with the outcome at $p \leq 0.20$ in univariable analysis and were thus initially included in the main effects model. The final multivariable model is shown in Table 2.7; a Pearson goodness-of-fit test indicated that the final model fit the data well (Pearson $\chi^2=9.24$; $p=0.97$). Analysis of standardized Pearson and deviance residuals revealed no extreme observations in the data (Figures 2.5 and 2.6). One covariate pattern was found to have a large impact on leverage and delta-beta values (Figure 2.7). However, this covariate pattern was retained in the model as its removal did not change interpretation of the model significantly.

2.4 Discussion

The status of *C. ovis* infection in Canada has changed substantially in recent years. There was a large increase in sheep carcass condemnations due to the parasite in federally inspected abattoirs across Canada between 2006 and 2007, and in Ontario

provincially inspected abattoirs between 2007 and 2008. Also important to note is the sensitivity of detecting *C. ovis* using the standard meat inspection protocol (7 seconds per carcass) is 50% (McNab and Robertson, 1972). Hence, the true prevalence of infection in the Canadian sheep flock is likely higher than these study data suggest. Additionally, these statistics do not include infected carcasses that were trimmed and passed as these data are not routinely recorded at the abattoir level.

The sudden increase in *C. ovis* condemnations in Ontario provincially inspected abattoirs may have been due to a change in purchasing of market lambs by federally inspected abattoirs located in western Canada. Specifically, the largest federally inspected abattoir in Canada, in terms of lamb slaughter numbers, is located in Alberta and reported large numbers of condemned sheep carcasses in 2007 due to *C. ovis* (Canadian Food Inspection Agency, personal communication 2011). The following year, in an attempt to reduce losses associated with the parasite, the abattoir decreased the number of lambs purchased from western Canada, and imported a greater number of lambs from the United States (Statistics Canada, 2010). Perhaps in 2008, instead of being killed in Alberta, many of the market lambs produced in western Canadian provinces were transported into Ontario for slaughter.

Overall, the reason for the sudden increase in the proportion of sheep carcass condemnations due to *C. ovis* infection in Canada, particularly when total sheep slaughter numbers are in decline (Statistics Canada, 2011), remains unclear. The movement of breeding sheep, guardian and working dogs between farms – sometimes across provincial boundaries – may have facilitated this increase by allowing previously naive sheep to contact the parasite (Soehl, 1984).

It is well documented that sheep develop and maintain a strong immunity to *C. ovis* provided they are continually exposed to the parasite's eggs (Rickard and Bell, 1971; Rothel et al. 1996). In Canada, because sheep are often housed indoors during the winter months, the protective immunity acquired by sheep while on pasture during the summer may wane at this time, predisposing them to infection when returning to pasture the following spring, and allowing cysticerci to accumulate in muscle. With this in mind, perhaps initially in Canada, a few naive flocks were exposed to *C. ovis* and it accumulated in the animals' muscle over several years prior to 2007. From these infected ewes, infection may have then spread to a larger number of naive flocks through the sale of infected dogs, or via infected sheep that were later consumed by dogs on other farms. Since introduction of the parasite into naive flocks tends to result in infections with more cysticerci per animal, due to a lack of immunity (Roberts et al. 1987; Cabrera et al. 1995), it would thus be easier to detect infected animals at slaughter and therefore would rapidly increase the condemnation statistics.

While still elevated compared to earlier years, the proportion of all condemnations that were due to *C. ovis* at federal and Ontario provincially inspected abattoirs has decreased since 2008. However, there is evidence that suggests a resurgence in 2011 (Figures 2.2 and 2.4). In many circumstances, the monetary losses associated with sheep carcass condemnations were felt by abattoirs (both federal and provincial), and occasionally feedlots, but rarely the farms of origin. Few producers sell animals directly to slaughter (Table 2.4) and, consequently, they would not have been held accountable if *C. ovis* was detected during meat inspection. Since 2007 and 2008, many large abattoirs and feedlots have introduced measures to determine the farm of origin of condemned

lambs and, when possible, have stopped buying lambs from those sources. While it is not known for certain how producers have responded to these actions, they have few options: either change on-farm practices to reduce the risk of *C. ovis* infection; or send lambs to another feedlot or abattoir and ignore the problem. Perhaps the pressure brought on producers by abattoirs and feedlots has resulted in a genuine decrease in the national risk of infection and thus a decline in carcass condemnations at federal and Ontario provincial abattoirs since 2008. It should also be noted that there has been a large effort by the CSF to educate producers on *C. ovis* transmission and control. This has been done through the distribution of a pamphlet, continuing education presentations at many producer and veterinary meetings, and by articles in nationally distributed newsletters (e.g. the CSF's *From the Flock*). Through changes in farm management, perhaps the effort to educate producers has contributed to the decreasing number of carcass condemnations from 2008 to 2010. Continuing to educate producers about the importance of control options is necessary to ensure high producer compliance and prevent a resurgence of carcass condemnations, which may already be occurring in 2011.

It is important to remember that condemnation data were only collected from federally inspected abattoirs across Canada and provincially inspected abattoirs in Ontario. It is possible, especially if large abattoirs and feedlots are being more selective about purchasing stock, that some of the provincial abattoirs outside Ontario are continuing to see high numbers of condemnations in sheep due to *C. ovis*. To obtain a more complete understanding of *C. ovis* in Canada at the present time, it is necessary to collect information from provincial abattoirs in provinces other than Ontario.

Data presented in Table 2.1 indicate that from March, 2009 to March, 2011, *C. ovis* infection in Ontario abattoirs was found in sheep originating from all Canadian provinces, other than Atlantic Canada. It should be noted that Atlantic Canadian provinces slaughter the majority of their lambs in Nova Scotia, instead of transporting them to Ontario. In effect, this decreases the probability of farms in these provinces being included in the study. Therefore, the possibility of *C. ovis* occurring in Atlantic Canada cannot be ruled out based on this study, particularly since it has been reported there in the past (Soehl, 1984).

Trace-back of condemned animals revealed that Alberta, representing 16% of the Canadian sheep flock (Statistics Canada, 2011), had the greatest number of farms with *C. ovis* condemnations (60 condemnations from 41 different farms). Interestingly, Manitoba, which contains only 7% of the total Canadian sheep flock, had a greater number of condemnations (n=98), albeit from only 37 farms. Meanwhile, Ontario and Saskatchewan, which represent 27% and 11% of the Canadian sheep flock, respectively, had slightly lower numbers of carcass condemnations, but these also originated from multiple farms. Compared to Alberta and Saskatchewan, which slaughter many animals locally, Manitoba tends to ship a large proportion of their lambs into Ontario for slaughter (West Hawk Lake Zoning Control Site, personal communication, 2011); possibly explaining the greater number of Manitoba condemnations during the study period. Quebec represents 30% of the Canadian sheep flock but only had two condemnations at Ontario plants during the study period. However, Quebec slaughters the vast majority of their animals locally, and rarely sends animals into Ontario (Statistics Canada, 2011). Additionally, Quebec does not use the CSIP system, instead relying on

another sheep identification program managed by Agri-Traçabilité Québec, whose database cannot be accessed by the CSF. Therefore, any Quebec sheep that were condemned in Ontario could not be traced back to their farm of origin and would have been excluded from the study. Thus, the prevalence of *C. ovis* in Quebec is likely greater than these data indicate. Likewise, British Columbia does not transport many lambs into Ontario for slaughter due to the great distance and, consequently, the data fail to accurately represent the true status of *C. ovis* in that province.

Prior to 2008, the sheep carcasses found infected with *C. ovis* at Ontario abattoirs had predominantly been associated with animals from British Columbia, Alberta, Saskatchewan and Manitoba (Paula Menzies, personal communication 2011) Ultimately, this led to the belief that *C. ovis* was a parasite found exclusively in western Canada. However, current data reveal that numerous carcass condemnations have originated from multiple farms across Canada; strongly suggesting that transmission of the parasite is occurring across western Canada, but also in Ontario.

The questionnaire response rate among case farms was relatively low at 30.5%. Numerous factors likely contributed to the low response rate among producers. Often the producer contact information listed in the national sheep database was not accurate; the telephone number listed would frequently be the wrong number, out of service, or absent. Additionally, many producers could not be reached after multiple attempts at contacting them, or the language barrier (besides English or French) was too great to successfully communicate. In other situations, the ear tags were traced back to feed companies that were either unable, or unwilling, to back trace the tags they sold to producers. Lastly,

several producers declined to participate, primarily because of concerns about maintaining confidentiality.

Importantly, many farm dogs in this study routinely consumed sheep meat, either through intentional feeding or scavenging deadstock. In New Zealand, using sheep meat as a food source for farm dogs has been reported on 61% (328/542) of farms (Singh et al. 2011), and has been described as a potential risk factor for *C. ovis* infection in lambs (Lawson, 1994). In Canada, it appears that most farm dogs are not intentionally fed sheep meat. However, many dogs on Canadian sheep farms scavenge deadstock, which may result in exposure to *C. ovis*. In fact, multivariable analysis revealed that farms that allowed scavenging of sheep carcasses by domestic dogs had 4-times greater odds of a carcass condemnation due to *C. ovis* than farms that did not.

Unlike in New Zealand, sheep production practices in Canada are greatly influenced by the presence of wild canids and concerns about predation. Thus, in addition to herding and companion dogs, it is common for producers to have one to several guardian dogs on their property. It seems reasonable to believe that these canids, which have continuous contact with the flock and pasture, would be associated with an increased risk of parasite transmission. However, regardless of their role, the presence of farm dogs alone, without considering the risk of scavenging, was not associated with *C. ovis* condemnations in sheep in this study.

The majority of sheep producers who owned farm dogs reported treating them with an anthelmintic at least once in the previous year (74% (23/31) cases; 79% (35/45) controls). To be effective against *T. ovis*, the product should contain one of the following compounds: praziquantel, nitroscanate, epsiprantel, or fenbendazole. In addition, it is

critical that dogs be dosed properly based on body weight for effective treatment and to reduce the risk of anthelmintic resistance developing. Treatment of farm dogs did not affect the odds of having a *C. ovis* carcass condemnation in this study. Unfortunately, most producers could not recall what kind of medication was used, thus, it remains unclear whether effective products were used to treat dogs. At least 7 producers (1 case, 6 controls) treated farm dogs with a product that was ineffective against cestode infection. Further, no data were collected on the frequency of anthelmintic treatment of farm dogs, or the dose administered. To prevent parasite transmission to lambs, dogs that have access to deadstock need to be regularly treated, preferably every five weeks (Heath and Lawrence, 1980; Lawson, 1994). All new dogs coming onto farm property should also be treated one week prior to arrival to avoid introducing *T. ovis* eggs on to farms. Failure to treat incoming farm dogs was considered responsible for at least one *C. ovis* outbreak in Canada in the past (Soehl, 1984).

Not all case farms owned domestic dogs, suggesting that sheep were infected by either wild canids or stray dogs, or that *T. ovis* eggs could have been transported to the farm by flies (Lawson and Gemmell, 1985; Lawson and Gemmell, 1990). It is also possible that sheep were infected after leaving the farm of origin, as the CSIP system does not allow complete traceability of animals.

Predation by wild canids, particularly coyotes, is an enormous challenge for Canadian sheep producers and, according to data obtained in this study, 50% (20/40) of cases and 61.8% (34/55) of controls reported losing animals to predation in the previous year (χ^2=1.32; p=0.25). Furthermore, 67.5% (27/40) and 63.6% (35/55) of cases and controls, respectively, reported that wild canids often scavenged deadstock on their farm.

The frequency of coyote predation and scavenging has led many to believe that they are involved in the transmission of *C. ovis* in Canada. However, the results of this study do not support that hypothesis. There was no association between sheep carcass condemnations and wild canid predation or scavenging. Both case and control farms were equally likely to suffer sheep loss to coyotes. Given the high incidence of coyote predation and scavenging in Canada, it is likely that coyotes are being exposed to viable *C. ovis* lesions through the consumption of sheep meat. However, the likelihood of coyotes transmitting the parasite back to sheep has yet to be determined. Considering the close proximity to humans, and the presence of guardian animals, it is speculated that livestock predation is a high risk activity for coyotes (Brent Patterson, Ministry of Natural Resources, personal communication 2010). Accordingly, it is unlikely coyotes defecate on pasture; especially at a level that contributes significantly to *C. ovis* transmission. Instead, it is believed that coyotes prefer to defecate in places with more cover – places unlikely to be grazed by sheep. However, various producers have reported that coyotes have been observed using round bales on pasture as perches from which to observe pastures and potential prey. In Texas, use of round bales by ranchers has been reported as a risk factor associated with *Neospora caninum* seropositivity in calves, perhaps due to wild canids defecating on them (Barling et al. 2001). If *N. caninum* transmission can occur via this route, it is possible for *T. ovis* to be transmitted as well. Thus, if coyotes use these locations for either defecation or sitting, there may be opportunity for contamination of forage with *T. ovis* eggs. More specific research needs to examine the locations of coyote feces in relation to sheep grazing, and presence of parasite eggs, specifically *T. ovis*.

The occurrence of stray dogs was significantly more common on case farms (50%) than control farms (20%). Stray dogs include neighbours' dogs, truly feral dogs, as well as those owned by hunters and hikers that may cross the farm in question. These values are likely an underestimate because stray dogs crossing remote pastures could go unnoticed. The model revealed a significant interaction between stray dogs on farms and Canadian province; meaning that the effect stray dogs have on the risk of sheep carcass condemnations is dependent on province. However, while statistically significant, the effect was miniscule, so the biological relevance of these findings is questionable. Nevertheless, it may still be prudent to include on-farm stray dog control in *C. ovis* control measures. There are several reports of stray dogs being associated with cestode cases in sheep in other countries (Buishi et al. 2005; Eichenberger et al. 2011; Mastin et al. 2011). For example, Mastin et al. (2011) investigated *Echinococcus granulosus* in Wales and listed roaming behaviour as the primary risk factor for farm dogs being coproantigen positive. Many times, stray dogs are not from other sheep farms, and are probably not routinely treated with a cestocide. Additionally, these dogs may be opportunists – scavenging deadstock and thus becoming infected. However, unlike coyotes, domestic dogs generally lack fear of humans, perhaps making them more likely to defecate on pasture or farm property.

Regarding provincial differences in *C. ovis* risk, it is believed that the current data lack sufficient power to accurately differentiate risk between provinces. Additionally, not all provinces slaughtered animals in Ontario, or did so in very small numbers, resulting in the exclusion of some provinces from analyses. When province was included as a fixed effect in the model to control for clustering, the province-specific odds ratio became

inaccurate because control selection was not weighted based on the number of animals from each province that are slaughtered in Ontario. The result is an inability to differentiate the odds of carcass condemnations between provinces.

Improperly disposing of deadstock may result in canids being exposed to carcasses infected with *C. ovis*. Compared to farms that bury, compost or incinerate deadstock, producers with no disposal method had nearly 12 times higher odds of sheep carcass condemnations in the multivariable analyses. Failure to dispose deadstock undoubtedly leads to scavenging by canids of all types. Canid exposure to sheep carcasses is essential for parasite transmission; therefore, to control *C. ovis*, deadstock must be disposed quickly and properly in a manner that minimizes the risk of scavenging by wild and domestic canids. Improperly disposed carcasses may attract stray dogs or coyotes to the farm and lead to scavenging, defecation and ultimately potential parasite transmission. By properly disposing of deadstock, the producer will reduce *C. ovis* exposure to canids of all types.

2.4.1 Limitations and future work

All participating farms in the case-control study were selected from cases identified in provincially inspected abattoirs in Ontario. Although 51% (1,385,000/2,717,000) of the sheep in Canada are slaughtered in Ontario, nearly half of Canadian sheep (49%) are slaughtered at federal and provincial abattoirs located in other provinces (Statistics Canada, 2010). To more accurately determine the prevalence and distribution of *C. ovis* in Canada, collection and trace-back of condemned carcasses is necessary from abattoirs in all provinces. In addition to being more representative,

involving more abattoirs in the collection process would increase sample size, statistical power, and the reliability of the results.

A significant limitation of the sampling method was the CSIP, which currently does not allow complete traceability of an individual animal. This is a potentially significant problem when responding to infections like *C. ovis*. When a sheep is condemned at slaughter due to infection with *C. ovis*, the CSIP only allows trace-back to the animal's farm of origin – usually its place of birth. There is currently no way of monitoring sheep movement after leaving the farm of origin, and before arriving for slaughter. Therefore, the possibility that a case farm might be free of *C. ovis*, and that the infection was acquired after the sheep left the farm, cannot be ruled out. For example 88% (35/40) of cases and 91% (50/55) of controls did not usually sell lambs direct to abattoirs. Therefore, prior to these lambs arriving at Ontario abattoirs for slaughter, animals typically resided on at least one other property. It is thus possible that infection with *C. ovis* could have occurred while the lamb was at this secondary location, provided it was there long enough prior to slaughter for cysticerci to develop (typically two weeks) (Ransom, 1913). In fact, lambs originally from farms free of *C. ovis*, and subsequently transported to a second property contaminated with *T. ovis* eggs, are more likely to acquire heavy infections because they lack immunity to the parasite (Roberts et al. 1987; Cabrera et al. 1995). It is these heavily infected animals that are more likely to be detected and condemned at slaughter. Unfortunately, the secondary locations could not be identified and included in the analysis. Additional concerns about the CSIP arise from the number of producers for which there was incomplete or incorrect contact information. To

be useful, the producer information provided for the CSIP must be correct, allowing for quick communication with the producer in the event of an animal health or food issue.

The results of the study may be influenced by interviewer bias when recruiting case farms from different provinces (Table 2.2). Following the identification of a case farm by the CSF, the provincial veterinarian from that province was asked to contact the producer and request participation in the study. Therefore, because multiple people were involved in the enrolment of cases, the possibility of differing case recruitment rates between provinces exists. Unlike the cases, all control farms were initially contacted and recruited by the CSF, lessening the effect of interviewer bias.

The time period from when the sheep was slaughtered until the questionnaire was administered to the producer was approximately four months. Although recall bias cannot be dismissed, it is believed to be a minimal issue because most of the predictor variables of interest tend to remain constant over time; even after several years, most producers rarely alter their flock management significantly. The only likely significant source of recall bias involved the dewormer used to treat farm dogs. It would have been valuable to know how many producers were using products effective against *T. ovis*. Unfortunately, most producers could not recall this information.

2.4.2 Conclusion

Cysticercus ovis has been reported sporadically in Canada for decades (Soehl, 1984), but the work described here represents the first investigation of the distribution of *C. ovis* on Canadian sheep farms. Although the sampling method used did not allow an estimation of the parasite's farm-level prevalence, it did provide strong evidence that *C. ovis* can be found on sheep farms across Canada. With the exception of Atlantic Canada,

since March 2009, all other provinces have had *C. ovis* condemnations associated with a farm(s) within them. Conclusions cannot be made for Atlantic Canada as they tend to slaughter animals locally, rather than in Ontario, and therefore were not recruited in this study.

Compared to the years from 2003 to 2006, condemnation data indicate that the prevalence of *C. ovis* in the Canadian sheep flock has increased in recent years. Elevated numbers of lamb carcass condemnations were observed at both Ontario provincially inspected abattoirs, and federally inspected abattoirs across the country. In an effort to better understand *C. ovis* epidemiology, a case-control study examining the characteristics and management practices of 40 case farms with *C. ovis* condemnations, and 56 control farms without, was undertaken. The results revealed plausible routes of parasite transmission on many farms, and the failure to dispose deadstock, carcass scavenging by farm dogs, and the presence of stray dogs, were all associated with increased odds of a farm having a sheep carcass condemned due to *C. ovis* infection. The results of this work will contribute to the development of effective control programs for *C. ovis* on Canadian sheep farms.

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Table 2.1: Number of *Cysticercus ovis* condemnations that occurred at 114 Ontario provincially inspected abattoirs between March 31, 2009 and March 31, 2011, by province of origin of the condemned animal.

Province	Number of *C. ovis* condemnations n=237	Number of farms with *C. ovis* condemnations n=133	Proportion of farms in province with *C. ovis* condemnations*	Mean *C. ovis* condemnations per infected farm	Range of *C. ovis* condemnations per infected farm
BC	2	1	0.2%	2.0	--
AB	60	41	6.7%	1.5	1-5
SK	50	30	11.5%	1.7	1-8
MB	98	37	19.0%	2.7	1-29
ON	24	22	1.45%	1.1	1-2
QB	3	2	0.2%	1.5	1-2

* Number of sheep farms by province provided by 2006 Agricultural Census, Statistics Canada.

Table 2.2: Questionnaire response rate of farms with *Cysticercus ovis* condemnations (case farms) from March 31, 2009 to March 31, 2011.

Province	Farms with *C. ovis* condemnations	Participating farms	Response rate (%)
BC	1	1	100.0
AB	41	11	26.8
SK	30	11	36.7
MB	37	6	16.2
ON	22	11	50.0

Table 2.3: Total number of sheep farms per participating Canadian province (Statistics Canada, 2006), number of farms with reported *Cysticercus ovis* condemnations ("case farms"), and number of farms without reported *C. ovis* condemnations ("control farms") participating in the study, by province.

Province	Number of sheep farms (%)	Number of Cases (%)	Number of Controls (%)
BC	590 (13.9)	1 (2.5)	1 (1.8)
AB	615 (14.4)	11 (27.5)	9 (16.1)
SK	260 (6.1)	11 (27.5)	21 (37.5)
MB	195 (4.6)	6 (15.0)	17 (30.4)
ON	1,515 (35.6)	11 (27.5)	7 (12.5)
Maritimes	80 (1.9)	0 (0)	1 (1.8)
Total	4,260 (100)	40 (100)	56 (100)

Table 2.4: Summary statistics and univariable associations between categorical predictor variables of interest and the *Cysticercus ovis* case status of Canadian sheep farms (n=40 case farms and n=56 control farms).

Variable		Cases (%)	Control (%)	χ^2 statistic	p-value
Province*	BC	1 (2.5)	1 (1.8)	7.63	0.05
	AB	11 (27.5)	9 (16.1)		
	SK	11 (27.5)	21(37.5)		
	MB	6 (15.0)	17 (30.4)		
	ON	11 (27.5)	7 (12.5)		
	NS	0 (0)	1 (1.8)		
Landcover type	Agriculture	33 (82.5)	48 (87.3)	1.0	0.32
	Forested	7 (17.5)	7 (12.7)		
New sheep on farm	Yes	23 (57.5)	36 (64.3)	0.27	0.61
	No	17 (42.5)	20 (35.7)		
Type of confinement	Pasture	15 (37.5)	18 (32.1)	0.72	0.84
	Confinement	5 (12.5)	7 (12.5)		
	Both	20 (50.0)	31 (55.4)		
Sell direct to slaughter	Yes	5 (12.5)	5 (9.1)	0.36	0.55
	No	35 (87.5)	50 (90.9)		
Guardian dogs	Yes	20 (50.0)	25 (45.4)	0.24	0.62
	No	20 (50.0)	30 (54.6)		
Herding/companion dogs	Yes	28 (70.0)	39 (70.9)	0.05	0.82
	No	12 (30.0)	16 (29.1)		

Table 2.4 continued,

Variable		Cases (%)	Control (%)	χ^2 statistic	ρ-value
Stray dogs*	Yes	20 (50.0)	11 (20.0)	9.0	0.003
	No	20 (50.0)	44 (80.0)		
New dogs	Yes	14 (35.0)	15 (27.8)	0.32	0.57
	No	26 (65.0)	39 (72.2)		
Deworm dogs [γ]	Yes	23 (74.2)	35 (77.8)	0.16	0.69
	No	8 (25.8)	10 (22.2)		
Canid predation	Yes	20 (50.0)	34 (61.8)	1.32	0.25
	No	20 (50.0)	21 (38.2)		
Scavenging by wild canids	Yes	27 (67.5)	35 (63.6)	0.68	0.41
	No	10 (25.0)	19 (34.6)		
	Unsure	3 (7.5)	1 (1.8)		
Farm dogs consuming carcasses*	Purposefully	3 (7.5)	2 (3.6)	4.11	0.043
	Scavenging	15 (37.5)	11 (20.0)		
	No	22 (55.0)	42 (76.4)		
Deadstock disposal*	Yes	26 (65.0)	48 (87.3)	6.64	0.01
	No	14 (35.0)	7 (12.7)		
Close proximity (<10 km) to another sheep farm	Yes	30 (75.0)	32 (59.3%)	2.23	0.11
	No	10 (25.0)	22 (40.7)		

[γ] as a proportion of producers who had domestic dogs
* indicates statistically significant univariable associations (p≤0.05)

Table 2.5: Summary statistics and the significance of univariable associations between continuous predictor variables of interest and the *Cysticercus ovis* case status of Canadian sheep farms.

Variable	Case (n=40)			Control (n=55)			χ^2	p-value
	mean	median	range	mean	median	range	statistic	
Flock size	176.6	90	15-1500	204.9	100	5-1850	1.24	0.21
Farm dogs	2.9	3	0-10	2.6	2	0-11	0.13	0.72

Table 2.6: Summary statistics of deadstock disposal methods used by Canadian farms with a sheep carcass condemnation due to *Cysticercus ovis* (case farms) and farms with no reported carcass condemnations (control farms).

Method	Cases (%) n=40	Controls (%) n=55	χ^2 statistic	p-value
Burial in ground	15 (37.5)	25 (45.5)	0.60	0.44
Burial in manure	12 (30)	12 (21.8)	0.81	0.37
Compost	3 (7.5)	4 (7.3)	0.00	0.97
Incineration	8 (20)	15 (27.3)	0.68	0.41
Vessel	1 (2.5)	5 (9.1)	1.90	0.17
Used as dog food	3 (7.5)	2 (3.6)	0.68	0.41
No disposal	14 (35)	7 (12.7)	6.64	0.01
Total*	56	70	--	--

* Totals exceed the number of farms as farms often use multiple methods depending on season and weather conditions.

Table 2.7: Final multivariable logistic regression model of factors associated with sheep farms being positive for condemnations due to *Cysticercus ovis* infection.

Variable	B (SE)	OR	95% CI	p-value
Dogs scavenging deadstock	1.40 (0.64)	4.04	1.16 – 14.04	0.028
No disposal of deadstock	2.47 (0.71)	11.78	2.93 – 47.40	0.001
Province of origin[1]				
Ontario	Referent	1	--	--
Manitoba	-2.30 (1.17)	0.1	0.01-1.0	0.05
Saskatchewan	-1.86 (1.00)	0.16	0.02-1.11	0.06
Alberta	0.47 (0.91)	1.6	0.27-9.48	0.6
Stray dogs[1]	19.01 (1.18)	1.82e+08	1.82e+07 –1.82e+09	<0.001
Manitoba*stray dogs	-15.88 (1.86)	1.26e-07	3.26e-09 – 4.87e-06	<0.001
Saskatchewan*stray dogs	-17.59 (1.55)	2.29e-08	1.10e-09 – 4.75e-07	<0.001

[1] Part of a significant interaction term

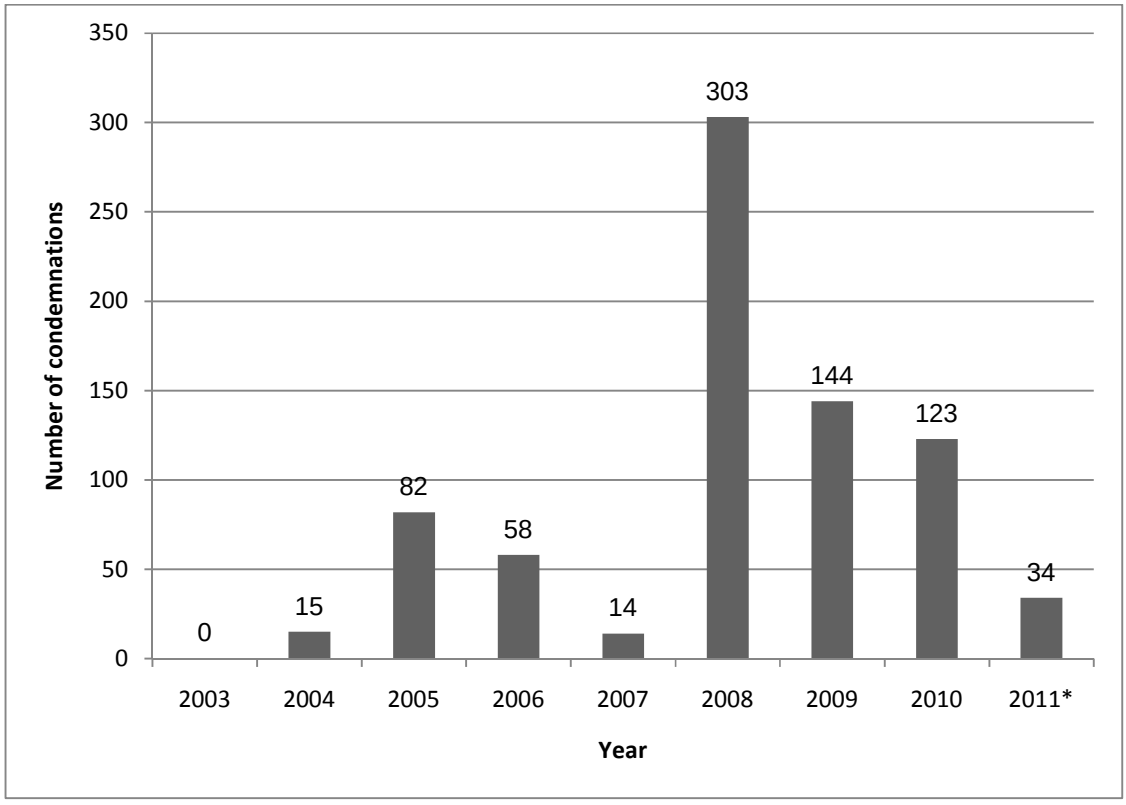

Figure 2.1: Total number of sheep carcass condemnations due to *Cysticercus ovis* infection that occurred at 114 Ontario provincially inspected abattoirs from 2003 to 2011. * Up to March 31st 2011 only.

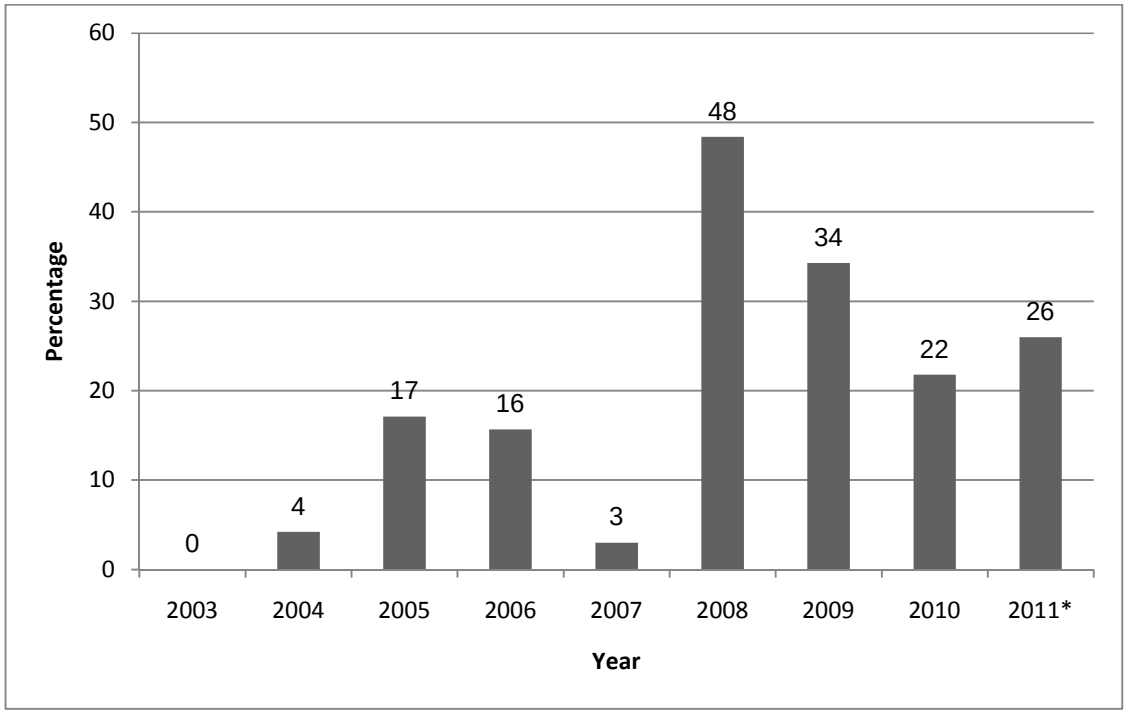

Figure 2.2: Percentage (%) of all sheep condemnations at 114 Ontario provincially inspected abattoirs due to *Cysticercus ovis*. * Up to March 31st 2011 only.

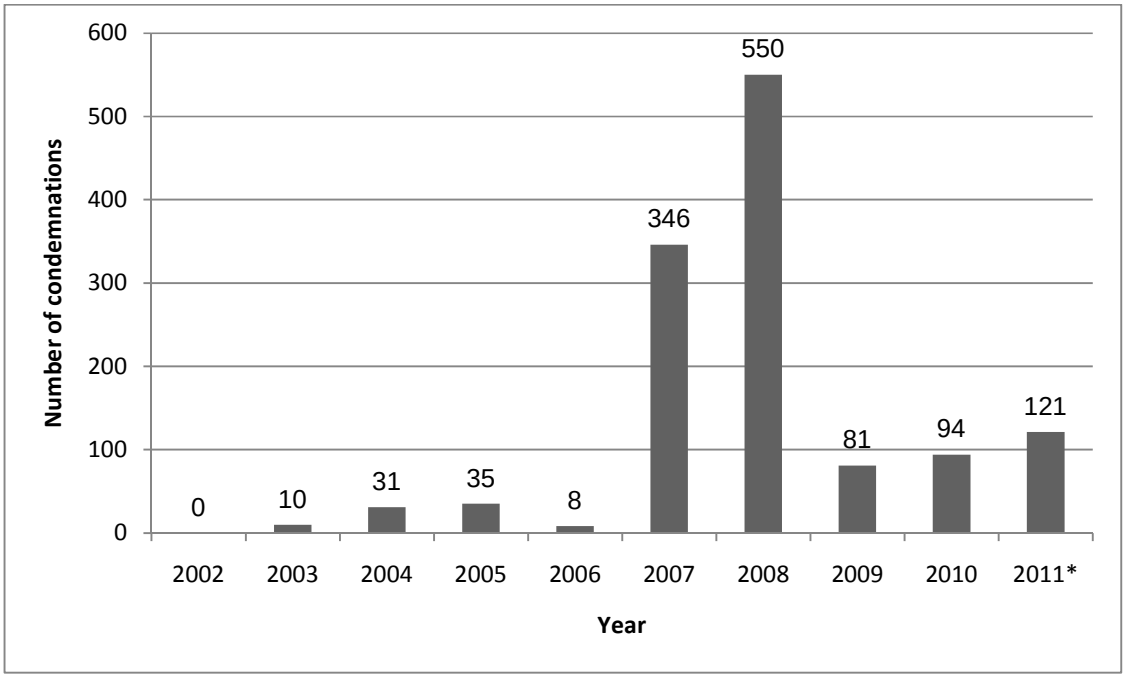

Figure 2.3: Total number of sheep carcass condemnations due to *Cysticercus ovis* infection that occurred at 15 of 19 (79%) federally inspected abattoirs across Canada, and 34 federally inspected provincial abattoirs (8 in British Columbia, 8 in Saskatchewan, 18 in Manitoba), from 2002 to 2011. * Up to March 31st 2011 only.

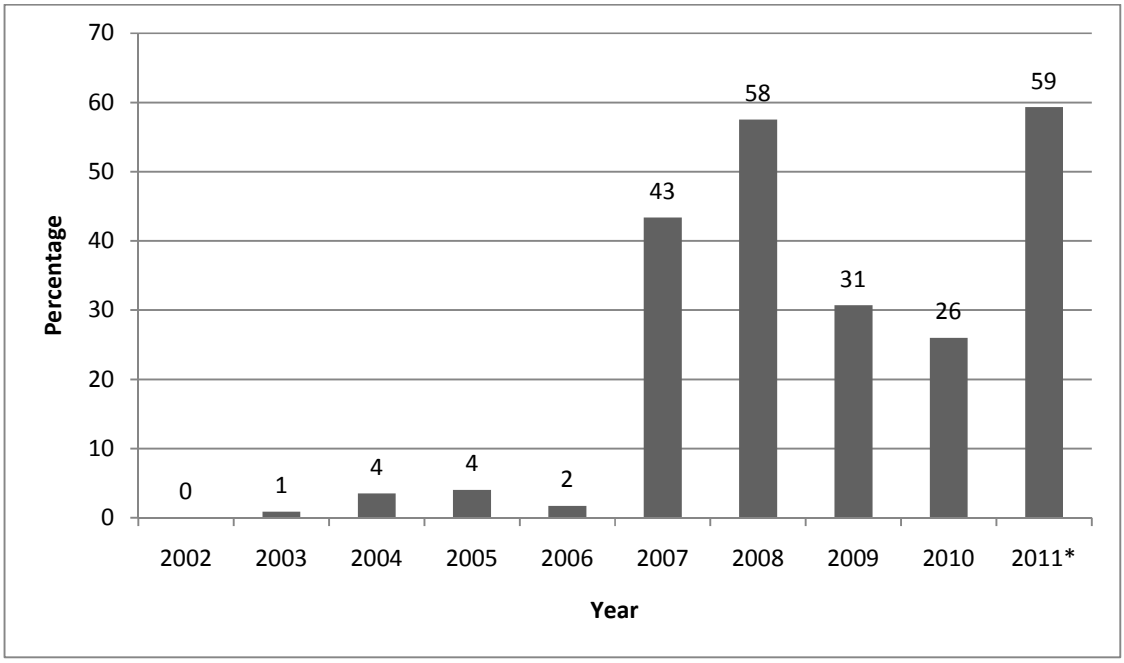

Figure 2.4: Percentage (%) of all sheep carcass condemnations due to *Cysticercus ovis* at 15 of 19 (79%) federally inspected abattoirs across Canada, and 34 federally inspected provincial abattoirs (8 in British Columbia, 8 in Saskatchewan, 18 in Manitoba), from 2002 to 2011. * Up to March 31st 2011 only.

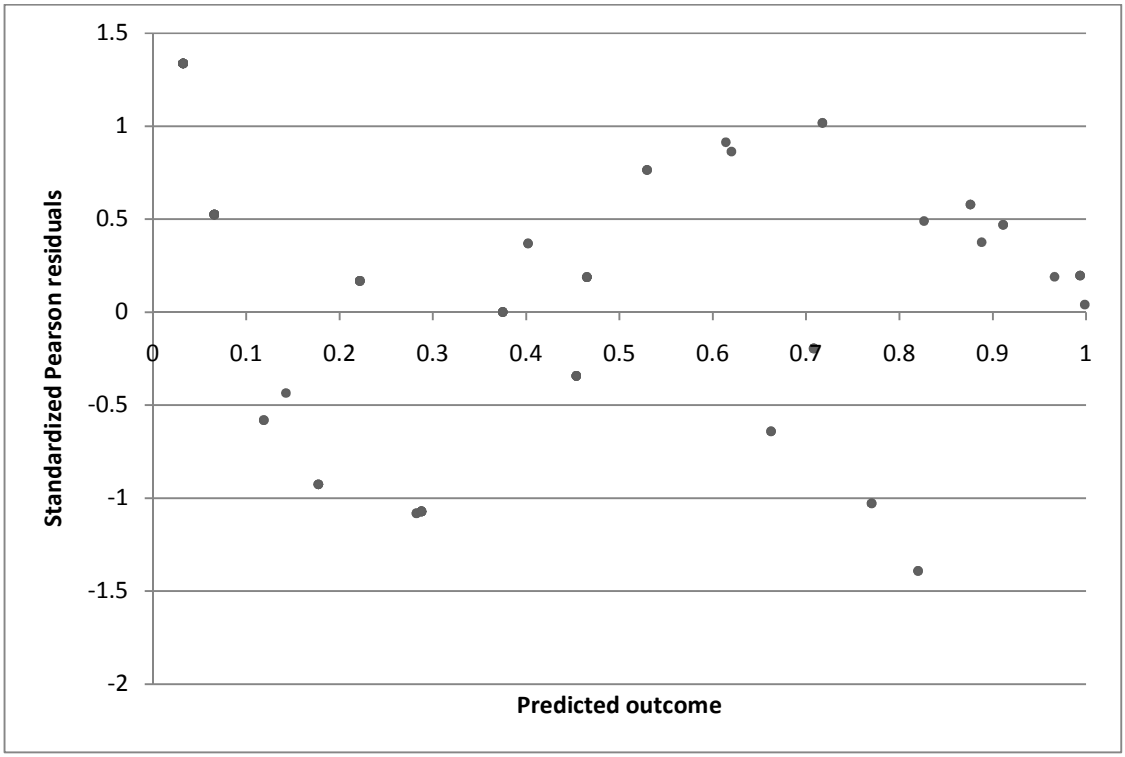

Figure 2.5: Standardized Pearson residuals for the multivariable model plotted against the predicted outcome (case-control status of farm).

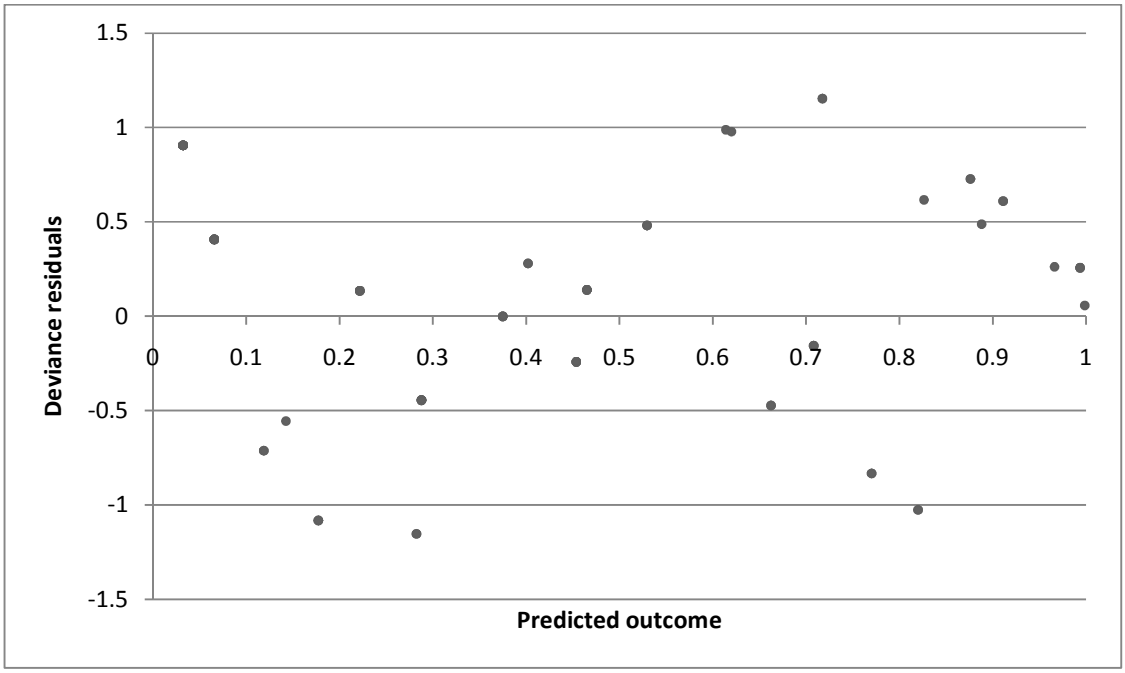

Figure 2.6: Deviance residuals for the multivariable model plotted against the predicted outcome (case-control status of farm).

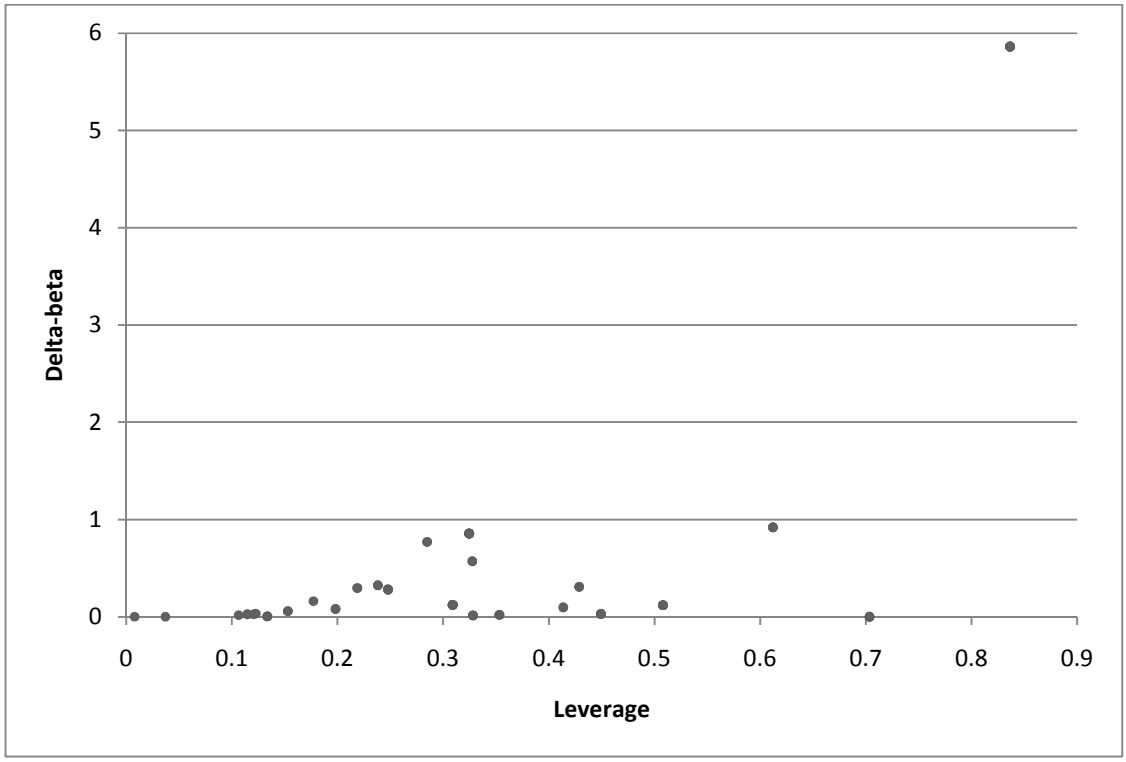

Figure 2.7: Calculated leverage values plotted against delta-beta, used to assess the influence of covariate patterns on the multivariable model.

Chapter Three

Development of a *Taenia ovis* transmission model and an assessment of control strategies

3.1 Introduction

In recent years the prevalence of *Cysticercus ovis* infection in the Canadian sheep flock has increased substantially (Chapter 2 –Figures 2.2 and 2.4), for reasons currently unknown. Potential financial losses caused by lamb carcass condemnations due to *C. ovis* are a major concern for the Canadian sheep industry.

Cysticercus ovis has an indirect lifecycle, meaning it involves two different host species (Gordon, 1939). The adult stage of the parasite, called *Taenia ovis*, is a tapeworm that inhabits the small intestine of canids, the definitive host (Ransom, 1913). The metacestode stage of the parasite is *C. ovis* which forms characteristic lesions within the skeletal and cardiac muscle of the intermediate hosts, sheep and goats (Ransom, 1913). Within the definitive host, *T. ovis* releases large quantities of eggs into the environment when the canid defecates. The eggs are immediately infective and may be inadvertently consumed by a sheep or goat. Following their consumption, *Taenia* eggs are activated and hatch within the digestive tract; the resultant oncosphere penetrates the intestinal wall and migrates via the hepatic portal circulation and bloodstream to the muscle where each larva forms a structure called a cysticercus (Ransom, 1913). After 2-3 months of development in the muscle of the intermediate host, the parasite becomes infective to the definitive host (Ransom, 1913). Transmission occurs when a canid consumes viable

lesions in the muscle of infected sheep or goats, allowing the larvae to subsequently mature in the intestine, completing the lifecycle (Sweatman and Henshall, 1962).

Currently, there is little published information describing the transmission dynamics of *C. ovis* between hosts. Furthermore, much of the information that does exist is based on research carried out in New Zealand and Australia several decades ago (Arundel, 1972; Johnson et al. 1989; Lawson, 1994; Rickard et al. 1995). Additionally, the available information about the epidemiology of *C. ovis* may not reflect what is currently occurring in Canadian sheep flocks because of differences in flock management and the effect wild canids (e.g. coyotes) have on sheep production practices in Canada.

Mathematical transmission models are becoming an increasingly accepted tool for the study of infectious disease processes. In general, disease models are a simplified representation of an infection and can be very useful for predicting pathogen transmission and suitable control options (Vynnycky and White, 2010). The earliest parasite transmission model was reported by Ross (1911) and was used to describe malaria transmission pathways. Since then, mathematical models have been used to describe numerous parasitic infections of both medical and veterinary importance. A mathematical transmission model for the rat tapeworm (*Hymenolepis diminuta*) was the first model for a cestode infection (Keymer, 1982). It used two coupled differential equations to imitate the indirect lifecycle of *H. diminuta*: a strategy that continues to be used to model other cestode species (Roberts et al. 1987; Roberts, 1994; Torgerson, 2003).

There are two mathematical models reported in the literature that describe cestode infections (including *T. ovis*) in New Zealand sheep flocks (Roberts et al. 1987; Roberts, 1994); however, these efforts were based on models developed from prevalence data for

Echinococcus granulosus and *Taenia hydatigena*. Despite having the same host species, there are differences in the biology of *E. granulosus*, *T. hydatigena*, and *T. ovis* which make it difficult to modify a model built for one species to use on another. To the best of the author's knowledge, no mathematical transmission model has been developed exclusively for *T. ovis*. A mathematical model of *T. ovis* transmission would allow for a better understanding of the dynamics of the infection, as well as the assessment of control strategies for the prevention of the spread of *T. ovis* infection in Canadian sheep flocks. Control measures to be instituted by the producer at the farm level are focussed on the prevention of infection burdens in the flock which may lead to lamb carcass condemnations, and prevention of the successful transmission of the parasite to farm dogs. The objective of this work was to develop and describe a mathematical model representing the transmission dynamics of *T. ovis* infection on Canadian sheep farms, including the effects of specific practical control measures.

3.2 Materials and Methods

3.2.1 The model

Using the modeling software AnyLogic® 6.5.0 (XJ Technologies, 2010), a basic infectious disease model was developed to represent the transmission dynamics of *C. ovis* infection on Canadian sheep farms. The complexity of the parasite's lifecycle, and the absence of sufficient data to extract transmission parameters, meant there were various assumptions in the model that had to be based solely on information obtained from the associated literature or expert opinion.

The transmission of *T. ovis* within a sheep flock is dependent upon both the definitive and intermediate host species, as well as the environment. As a result, three

separate compartmental models representing populations of guardian dogs (the definitive host), market lambs (the intermediate host), and the environment in which the transmission between the two hosts took place, were used to simulate the *T. ovis* lifecycle. After each individual model was complete, the three stages of the parasite lifecycle were linked to create a final compartmental, deterministic, density-dependent model which simulated *T. ovis* transmission on a weekly timescale. A representation of the final model is shown in Figure 3.1.

3.2.2 Canid component

The canid component of the model represented *T. ovis* infection in guardian dogs living with sheep. Guardian dogs are large breed dogs specifically bred and trained to live with the sheep flock and provide protection from predators. Besides guardian dogs, the definitive host of *T. ovis* can be other domestic canids, including herding and companion dogs, as well as wild canids. In Canada, the term wild canid refers to coyotes, foxes and wolves. It is speculated that the involvement of herding and companion dogs in transmission to sheep is less important than guardian dogs due to their shorter contact periods with the flock, and the smaller opportunity for defecation in areas where sheep feed. As mentioned, the transmission of *T. ovis* in Canada is further complicated by the potential role wild canids have in transmission. To date, there has not been research investigating whether wild canids play a significant role in transmitting *T. ovis*. It is clear, however, that wild canids are not required for the parasite to be successful, as clearly illustrated by previous outbreaks in New Zealand, a country without a wild canid population (Arundel, 1972; Lawson, 1994). In New Zealand, *T. ovis* is transmitted entirely by domestic dogs living on farms; although the potential role of stray dogs in *T.*

ovis transmission cannot be dismissed. Thus, until research suggests that wild canids are important in *T. ovis* transmission, it is more reasonable to focus control efforts on domestic canids because they are known to be involved in the lifecycle, and control options are significantly easier to implement with these animals. However, it is noteworthy that certain control options used to reduce infection risk in domestic dogs, particularly the prompt and thorough disposal of deadstock, would also effectively reduce the infection risk in wild and stray canids.

Thus, the only definitive host addressed in the model was the guardian dog. As illustrated by the model (Figure 3.1), in terms of infection status, each guardian dog was considered in one of the following mutually exclusive compartments: susceptible, exposed, or infective. The total number of guardian dogs on one farm was considered a population.

The susceptible population of guardian dogs was those animals not currently infected with *T. ovis* and had not been exposed. Following a sufficient exposure to the parasite through the consumption of infective sheep meat, susceptible animals progressed to the exposed population of dogs and ultimately developed a patent infection. The susceptible population of guardian dogs (represented as S_c) varies at time t according to the following equation:

$$dS_c(t)/dt = -\lambda S_c(t)D(t) + r_c I_c(t) \qquad \text{(Equation 1)}$$

where λ is the probability that, each week, susceptible guardian dogs consume infective deadstock at a frequency that produces sufficient exposure to viable cysticerci to result in infection. In the model, the only route by which susceptible dogs become exposed to the

parasite is through the consumption of deadstock containing viable *C. ovis*; either through intentional feeding by the producer, or scavenging (uncontrolled feeding of infective sheep carcasses, i.e. sheep that died or were slaughtered on farm). The quantity of deadstock, defined as the number of carcasses available to susceptible guardian dogs over time, *t*, is represented as *D(t)*. Thus, $\lambda S_c(t)D(t)$ represents the rate at which susceptible dogs consume infective deadstock. In Equation 1, r_c represents the rate at which infective canids recover per unit time, and become susceptible to future infection. Recovery results from death of the *T. ovis* parasite residing in the small intestine of a dog with a patent infection, likely through a host immune response. In Equation 1, the recovery rate (r_c) is multiplied by the number of infective guardian dogs (represented as I_c) to simulate the recovery of infective dogs, and the recommencement of their susceptibility to future infection. In this model, the recovery rate is:

r_c = 1/ (average duration of infection) (Equation 2)

Most commonly, only one adult cestode is found in an infected dog at any given point in time (Jackson and Arundel, 1971). Therefore, in the model, an infected animal contains a single tapeworm. It was estimated that nine months (36 weeks) post-infection, on average, the canine immune response eliminates *T. ovis* from the intestine (Sweatman and Henshall, 1962). Therefore, in the model, r_c = 1/36. The duration of canine immunity is short, possibly fading in less than two weeks following the elimination of the parasite (Andrews et al. 1983), and for the purposes of the model was considered nonexistent. After clearing the parasite, dogs were considered immediately susceptible to re-infection.

The exposed population of guardian dogs were animals that had ingested viable *C. ovis* lesions through deadstock consumption, but had not yet progressed to a patent *T.*

ovis infection. The duration that individuals remain in the exposed compartment is equivalent to the prepatent period of *T. ovis*, which is considered to be 6 weeks (Sweatman and Henshall, 1962). The exposed population of guardian dogs (represented as E_c) in the model varies according to the following equation:

$$dE_c(t)/dt = \lambda S_c(t) - f_c E_c(t) \quad \text{(Equation 3)}$$

where f_c is the rate at which individuals in the exposed compartment become infective per unit time. With a minor adjustment to Equation 2, f_c can be derived:

$$f_c = 1/ (T.\ ovis \text{ prepatent period}) \quad \text{(Equation 4)}$$

In the model, infective guardian dogs have a patent *T. ovis* infection and shed eggs into the environment during defecation. The absence of information regarding guardian dog defecation patterns led to the assumption that each dog has seven defecations per week which are evenly distributed across the pasture. The model also assumes that all defecations are equally infective, and there is no intermittent shedding of *T. ovis* eggs. As such, the infective population of guardian dogs varies according to the following equation:

$$dI_c(t)/dt = f_c E_c(t) - r_c I_c(t) \quad \text{(Equation 5)}$$

with individuals entering the infective compartment according to rate f_c, and leaving the infective compartment according to rate r_c.

There are two input parameters within the canid model that could be manipulated depending on the management practices on a particular farm (Table 3.1). The first parameter is the number of guardian dogs on a farm, and their distribution within each of the model compartments ($S_c(t)$, $E_c(t)$, and $I_c(t)$). In reality, the infection status of each guardian dog would not likely be known, unless treatment with an effective cestocide was

recently carried out to kill any parasites. The second input parameter (λ) is the probability of guardian dogs consuming deadstock on a weekly basis that has not been properly treated by freezing (Whitten, 1971) or cooking (Arundel, 1972). Increased frequency of deadstock consumption by guardian dogs is believed to be associated with increased risk of *T. ovis* infection.

3.2.3 Environmental component

Hot zones for *Taenia ovis* eggs

When an infective dog defecates, eggs from *T. ovis* are released into the environment. Research has shown that from the location where an infected dog defecates, a 25 m radius rapidly becomes contaminated with *Taenia* eggs (Gemmell and Johnstone, 1976; Gemmell et al. 1987). Although greater dispersion from the feces has been documented (Gemmell and Johnstone, 1976), the most contaminated areas of pasture were assumed to be within 25 m of where an infective dog defecates.

A sheep becomes infected by ingesting *T. ovis* eggs from contaminated pasture. To be classified as infective for model purposes, it was assumed the number of *Taenia* eggs ingested by a lamb was numerous enough to produce sufficient numbers of cysticerci that result in either the condemnation of the carcass at slaughter, or infection of canids that may scavenge the carcass. A critical component of the model was to properly estimate the rate at which lambs become infected through ingestion of *T. ovis* eggs on pasture (represented as φ). In the absence of field data, φ could not be accurately determined using standard techniques. Instead, to estimate φ, the total pasture area on which the lambs grazed was divided into a series of smaller, equal-sized, circular zones. When an infected guardian dog defecated in a particular zone, it became “hot”, meaning

the zone was heavily contaminated with *T. ovis* eggs. It was assumed that when a lamb grazed a hot zone (represented as Z_h), it was exposed to large numbers of eggs. The minimum infective dose for one cysticercus to develop has been estimated to be between 15 and 20 *T. ovis* eggs (Gemmel, 1965; Rickard and Bell, 1971). Therefore, to produce enough cysticerci for a carcass condemnation, or an infection in a dog, hundreds of eggs must be ingested by a sheep.

In light of the work by Gemmell et al. (1987), it was decided that a conservative 2.82 m radius around the feces (0.0062 acres) was suitable for defining a hot zone. The dynamics of hot zones on pasture are modeled according to the following equation:

$$dZ_h(t)/dt = \omega I_c(t) - \rho Z_h(t) \qquad \text{(Equation 6)}$$

where ω is the rate at which infected guardian dogs defecate on pasture creating hot zones, and ρ is the rate at which hot zones decay, and are no longer considered infective. The rate at which hot zones are created (ω) is a product of the number of infective guardian dogs on a farm (I_c), and the number of defecations per dog in one week on pasture (assumed to be 7). Defecation occurs randomly in the pasture, and was not dependent on the location of previous hot zones. It was also assumed that over time, *T. ovis* eggs contaminating the hot zone dispersed and decayed, eventually rendering the zone non-infective. Previous work has suggested that *Taenia* eggs can survive on pasture for six months under ideal circumstances (Lawson and Gemmell, 1983). However, to be conservative, especially considering the practical nature of the model, it was assumed that hot zones were no longer infective 12 weeks following their formation (Gemmell and Macnamara, 1976). Therefore, the hot zone decay rate (ρ) equals 1/12.

Deadstock

In the model, the only route of parasite transmission to guardian dogs was through consumption of infective deadstock through intentional feeding by the producer, or by scavenging. The opportunity for consumption of deadstock by guardian dogs varies greatly between farms and was represented using a probability, λ. The presence of infective deadstock depends on the mortality rate of infective sheep (μI_L), and the rate at which infective cysticerci within carcasses become non-infective (δ). There has been no research investigating the length of time *C. ovis* remains viable following the death of the host, but it has been suggested to be no longer than four weeks, particularly when deadstock are disposed properly (Ontario Ministry of Agriculture, Food and Rural Affairs, 2011a). Therefore, assuming cysticerci remain viable on average 4 weeks post-mortem, δ =1/4. The availability of infective deadstock (D) on a particular farm is modelled using the following equation:

$$dD(t)/dt = \mu I_L(t) - \delta D(t) \qquad \text{(Equation 7)}$$

3.2.4 Lamb component

The lamb component of the model was deterministic and density-dependent in nature, with the risk of *C. ovis* infection in lambs dependent upon the stocking density of a pasture. In terms of grazing management, the model assumes that the flock was set-stocked, meaning that animals are turned out on the same pasture for the entire grazing season, and that all sections of the pasture are grazed equally. The primary reason for this grazing assumption was to eliminate the effect pasture rotation has on *T. ovis* transmission.

Similar to the canid component, the lamb component of the model divided a lamb population into mutually exclusive compartments based on infection status. The susceptible population of the flock was assumed to be composed entirely of naive animals that had never been exposed to *T. ovis* eggs previously; additionally, any previous maternal passive immunity in these animals had waned. This ensured that all animals in the susceptible population were equally susceptible to subsequent *C. ovis* infection. The susceptible population of lambs (S_L) is modeled according to Equation 8.

$$dS_L(t)/dt = \varphi(t)S_L(t) - \mu S_L(t) \quad \text{(Equation 8)}$$

$\varphi(t)$ is the rate at which susceptible lambs contacted hot zones (Equation 9), and μ is the weekly lamb mortality rate from weaning until market.

$$\varphi(t) = C_e Z_h(t) / Z_t \quad \text{(Equation 9)}$$

It was assumed that, on average, lambs are weaned at 8 weeks of age, and then marketed at approximately six months old. During this approximate four month time period, the overall mortality is estimated to be 2.0% (Ontario Ministry of Agriculture, Food and Rural Affairs, 2011b). For simplicity, the model assumes that the mortality rate remains constant regardless of infection status of the lambs, and mortality is divided equally among the four months between weaning and market. Therefore, on a weekly time scale, the mortality risk was estimated to be 0.125% (μ = 0.00125) of the flock.

In Equation 9, C_e defines the proportion of pasture contacted by each susceptible lamb per week, given a particular stocking density and pasture quality. The term C_e is equivalent to the following equation:

$$C_e = \text{(grazing area per lamb) / (total pasture area)} \quad \text{(Equation 10)}$$

Additionally, in Equation 9, $Z_h(t)$ is the infective hot zone area at time, t, which is dependent on the number of infective dogs; Z_t is the total pasture area. Hence, the ratio $Z_h(t)/Z_t$ represents the probability of contacting a hot zone during a particular week. It was assumed that grazing a hot zone resulted in sufficient exposure to *T. ovis* eggs to produce a heavy *C. ovis* infection in susceptible lambs; meaning the infection was sufficiently substantive to result in carcass condemnation or transmission to canids. The exposed lamb population was not however, considered immediately infective because it takes time for infective cysticerci to develop within each animal. The number of exposed lambs (represented as E_L) is modeled using the following equation:

$$dE_L(t)/dt = \varphi(t)S_L(t) - \mu E_L(t) - f_L E_L(t) \quad \text{(Equation 11)}$$

where f_L is the rate at which exposed lambs develop infective *C. ovis* lesions. According to the literature, muscle lesions become infective 7 weeks post-exposure, on average (Ransom, 1913); therefore, $f_L=1/7$.

The model also assumes that following exposure, all such lambs progress into the infective population (represented as I_L). The transfer of lambs from the exposed population (E_L) to the infective population is described in Equation 12.

$$dI_L(t)/dt = f_L E_L(t) - \mu I_L(t) - \alpha I_L(t) \quad \text{(Equation 12)}$$

The expression $-\mu I_L(t)$ represents the movement of dead infective lambs into the deadstock pile where subsequent consumption by canids can occur. This movement is completely dependent on the lamb mortality rate (μ). Eventually, infective lambs develop an immune response which kills all viable lesions, and prevents future *C. ovis* infection (Rickard and Bell, 1971; Gemmell, 1972). The rate at which the immune response develops is defined as α. Following exposure to as few as 50 *Taenia* eggs, an immune

response is produced that destroys subsequent parasites while they are penetrating the lamb's intestinal mucosa, or shortly thereafter (Sweatman, 1957). Although the lamb immune response to *C. ovis* is dependent on multiple factors, on average, 12 weeks following parasite exposure should be sufficient for a protective immune response to develop (Ransom, 1913), therefore, $\alpha = 1/12$. Continual exposure to *T. ovis* eggs maintains a protective immune response against future infection (Rickard and Bell, 1971; Rothel et al. 1996). After the immune response is developed, lambs progress into the recovered population, $R_L(t)$ (Equation 13), and, assuming continued exposure to the parasite throughout the grazing season, are no longer an infection risk to canids.

$$dR_L(t)/dt = \alpha I_L(t) - \mu R_L(t) \qquad \text{(Equation 13)}$$

The lamb component of the model has several input parameters that can be adjusted to match a specific farming operation (Table 3.1). Estimates of total pasture area, and grazing area per lamb based on pasture quality, provided on a weekly basis, are necessary to estimate the proportion of pasture contacted per week (C_e), and ultimately the hot zone contact rate (φ). Additionally, the infection status of the flock is important and influences the distribution of lambs in each of the model compartments (e.g. $S_L(t)$, $E_L(t)$, $I_L(t)$, and $R_L(t)$).

3.2.5 Evaluating control strategies through differing input parameters

Once the model was complete, in order to evaluate the impact of different control options, it was necessary to determine baseline infection prevalences for both ovine and canine populations. The baseline prevalences serve as a control to which comparisons could be made once control strategies were implemented. The model's predicted baseline prevalences were calculated using the input parameters shown in Table 3.1.

The baseline model assumed that all lambs were in the susceptible population. Additional assumptions were needed about the stocking density and pasture quality on which the flock feeds. It was assumed that the quantity and quality of pasture would allow a 50 kg animal, requiring 3% body weight in dry matter intake per day, to consume 0.0025 acres every week (Jack Kyle, OMAFRA, personal communication).

The major assumption in the canine input parameters was the probability of consuming deadstock per week. The model assumes that each week, if deadstock is available (λ=1), guardian dogs on the farm have access to it, either through scavenging or intentional feeding. The input parameter values of the canid population in the baseline model are shown in Table 3.1.

A valuable aspect of creating a mathematical model is the ability to generate predictions regarding the efficacy of different control options. In this investigation, three common control options hypothesized to have efficacy in controlling *C. ovis* infection in lambs were analyzed and compared to the baseline infection prevalence to determine how they impacted parasite transmission on a farm. Namely, these were the routine cestocide treatment of guardian dogs, frequent pasture rotations, and the prevention of deadstock consumption by guardian dogs.

3.3 Results

3.3.1 Baseline *Taenia ovis* infection

When the model was run with the input parameters as given in Table 3.1, an output graph was generated illustrating how *T. ovis* infection spreads through the flock (Figure 3.2). The graph shows a clear increase in the infection risk of lambs as the dogs contaminate pasture with infective feces over time. Also apparent was that *T. ovis* eggs

were deposited on pasture at a greater rate than which they decayed during the first 12 weeks. This is shown in Figure 3.3, which illustrates how the cumulative hot zone area, which corresponds to eggs on pasture, increased with time. After 12 weeks, the number of hot zones stabilized on pasture because the older hot zones began to decay at a constant rate, while the infected dogs continued to defecate at a constant rate. Comparing Figures 3.2 and 3.3, the number of lambs infected with *C. ovis* directly increased as the area of pasture contaminated with *T. ovis* eggs increased.

3.3.2 Evaluating control strategies

i) Routine cestocide treatment of guardian dogs

Proper treatment of guardian dogs every five weeks with an effective cestocide is believed to significantly reduce the risk of *C. ovis* infection in lambs (Lawson, 1994). To imitate regular treatment of guardian dogs, an event in AnyLogic® 6.5 was created which occurred every five weeks to represent regular effective treatment with a cestocide. At the time it occurred, treatment resulted in all exposed ($E_c(t)$) and infective ($I_c(t)$) guardian dogs becoming susceptible to *T. ovis* infection. This represented the killing of both developing and adult tapeworms currently inhabiting the canid intestine, effectively stopping egg production. Importantly, there is no residual effect associated with treatment, and the guardian dogs were assumed to be immediately susceptible to re-infection (Andrews et al. 1983). Besides regularly dosing dogs with cestocide every fifth week, all other model parameters were identical to baseline values (Table 3.1).

Two deworming strategies were investigated, both involving routine treatment of guardian dogs every five weeks. One simulation examined how *T. ovis* transmission to lambs was affected if regular treatment (every five weeks) commenced six weeks

following the introduction of infective guardian dogs. The second simulation examined how routine treatment of dogs, beginning immediately upon arrival, influenced *T. ovis* transmission to lambs. The results of both simulations compared to baseline values are shown in Figure 3.4. When treatment started six weeks following the introduction of guardian dogs on pasture, there was a rapid decrease in the risk of *C. ovis* infection in lambs, as illustrated by the output's divergence from baseline following commencement of deworming. Treatment of dogs every five weeks eliminated parasite transmission to lambs because the dogs were never allowed to develop patent infections.

Regular treatment of guardian dogs also influenced the total pasture area contaminated with *T. ovis* eggs. The total hot zone area on a pasture following regular deworming of guardian dogs, beginning six weeks following their introduction to pasture, is compared to baseline values in Figure 3.5. As expected, the number of hot zones on pasture decreased following the treatment of dogs.

ii) Frequent pasture rotation

The set-stock assumption used in the baseline model was implemented as a means of simplifying the model, and to focus on examining the impact of other control strategies without the effect of pasture rotation. In reality, however, Canadian sheep production does not only rely on a set-stock approach to grazing; commonly the flock is rotated through a number of fenced pastures throughout the grazing season. By simulating rotation to a clean pasture every three weeks and not returning to that same pasture within the same grazing season, the influence pasture rotation had on the *C. ovis* infection risk in lambs was compared to baseline values. Though it fails to eliminate the risk of *C. ovis* in lambs, Figure 3.6 shows that pasture rotation every three weeks reduced infection risk

compared to set-stocking. As shown in Figure 3.7, pasture rotation lessens the egg burdens on pasture because hot zones cannot accumulate. A lower number of hot zones decreases the likelihood of lambs contacting them, ultimately reducing the risk of *C. ovis*. Importantly, however, the practice of rotating pasture did not eliminate the risk of *C. ovis*.

iii) Preventing consumption of deadstock by guardian dogs

In order for the transmission of *C. ovis* in a population of lambs to be successful, canids must have access to viable cysticerci in lamb muscle. Accordingly, it was believed that preventing the consumption of deadstock would serve as an effective preventive measure against *C. ovis* infection (Lawson, 1994).

The original baseline input parameters used (Table 3.1) meant there were no previously infected deadstock (*D(t)*) when the simulation began ($D(0)=0$). The naive flock of lambs also produced very low numbers of infective deadstock within the 24-week time period the lambs were on pasture with infective dogs. Therefore, the influence of deadstock consumption on *C. ovis* transmission could not be well addressed in the baseline model. To better examine the effect of proper deadstock disposal on *C. ovis* risk, the baseline number of deadstock available to dogs was increased to three infective sheep carcasses present in the environment when the dogs were introduced ($D(0)=3$). Additionally, when the simulation began, the baseline input status of the guardian dogs was changed to susceptible, rather than infected with *C. ovis*. These adjusted baseline input parameters for this simulation are shown in Table 3.2.

The adjusted baseline model, used as a control, assumed all dogs had access to deadstock during the weekly time interval ($\lambda=1$). By then running simulations with lesser probabilities of deadstock consumption (i.e. $\lambda=0.05$, $\lambda=0.00$), and comparing these output

graphs to the control, the influence of deadstock consumption on *C. ovis* transmission was evaluated. The results using the adjusted baseline model allowed comparison of how varying amounts of deadstock consumption by guardian dogs on a farm (λ) influence the number of infective guardian dogs present (Figure 3.8) and the infection risk for lambs (Figure 3.9). As expected, Figure 3.8 shows that as the probability of deadstock consumption increases, the risk of dogs becoming infected with *T. ovis* increases, leading to a greater number of dogs being infective over a 24-week period. Similarly, as shown in Figure 3.9, the model predicted that increased deadstock consumption by guardian dogs also increased the risk of lambs developing *C. ovis* infections.

3.4 Discussion

The baseline model performed as expected based on published information about *T. ovis*. Not surprisingly, when two infective guardian dogs were introduced onto pasture with sheep grazing, the model predicted an increase in the number of lambs infected with *C. ovis* during the next four months (Figure 3.2). The increase in lamb infections was consistent with the biology of the parasite and corresponds to the duration that infective dogs were maintained with the flock. The longer infective guardian dogs were on pasture, the more heavily contaminated with *Taenia* eggs the pasture became, which resulted in an increased probability of lambs contacting a pasture hot zone and becoming infected.

The model assumed that *Taenia* eggs are continually shed in dog feces at a constant rate throughout the nine month period that a dog is infective (Sweatman and Henshall, 1962). After dogs are introduced onto pasture, they were assumed to defecate at a constant rate, creating infective hot zones. *Taenia*-type eggs can remain viable on pasture for up to six months in ideal conditions (Froyd, 1962; Storey and Phillips, 1985).

Depending on where a farm is located, climatic conditions during the grazing season can vary greatly across the Canada. *Taenia* eggs can tolerate wide variations in temperature, but are highly susceptible to desiccation (Lawson and Gemmell, 1983); accordingly, eggs in drier climates would have shorter periods of infectivity. However, the developed model did not account for specific weather conditions. Instead, it assumed the Canadian grazing season allowed eggs to survive for twelve weeks before dispersal and decay reduced the number of viable eggs to a point where the hot zone was no longer considered infective. During most of the four-month grazing season, hot zones formed on pasture at a greater rate than they decayed (Figure 3.3), continually increasing the area of contaminated pasture. At twelve weeks, however, older hot zones began to decay; meanwhile, infective dogs continued to create new pasture hot zones at a constant rate. The result appears to be a stabilization of contaminated pasture area after 12 weeks.

The model predicted that naïve lambs, raised on contaminated pasture from weaning until market, would be exposed to high numbers of viable *T. ovis* eggs. The predicted results were consistent with the results of a study by Lawrence et al. (1996) which exposed naïve lambs to pasture known to be heavily contaminated with *T. ovis* eggs. After nine weeks on pasture, 98.6% (75/76) of lambs had *C. ovis* detected during meat inspection at slaughter. It is likely that such heavy infections, in such a large proportion of the flock, are rare. On most farms, the movement of lambs or dog defecation patterns, not accounted for in the model, probably result in a lesser exposure to *T. ovis* eggs. The defecation patterns of guardian dogs have not been described in the published literature, but could have a substantial influence on *T. ovis* transmission. For

instance, it is possible that guardian dogs routinely defecate in a specific location, as opposed to evenly across pasture as the model assumes.

Over the course of a patent infection, it remains unclear whether there is intermittent and variable shedding of eggs in the feces of canids. Intermittent shedding of eggs at different concentrations within the feces would influence the number, size and longevity of pasture hot zones. The more *Taenia* eggs released during defecation, the greater the infectivity of a particular hot zone. Additionally, greater numbers of eggs require more time to disperse, resulting in a potentially longer period of infectivity for lambs. Factors influencing the duration of viable eggs on pasture will greatly influence the transmission of *C. ovis* on farms.

In an effort to control *C. ovis* in a flock of sheep, the removal of dog feces from sheep feeding areas has occasionally been suggested. Aside from being too labour intensive to be practical on most farms, in order to be effective, feces would have to be removed immediately following defecation. *Taenia* eggs quickly disperse from the feces through proglottid movement, rainwater, wind and insect activity; unless feces were removed daily, the eggs would have likely already dispersed onto pasture, making the exercise futile.

3.4.1 Routine cestocide treatment of guardian dogs

There has been one documented instance in Canada where *C. ovis* outbreaks on five different sheep farms were directly attributed to the acquisition of working dogs (Soehl, 1984). In an effort to control *C. ovis* infection in lambs and reduce financial losses associated with the parasite, it is often recommended that producers regularly treat all dogs that have contact with their sheep flock with an effective cestocide at the proper

dose. Work done in New Zealand suggested that regularly treating working dogs with cestocides would reduce the risk of *C. ovis* infection in lambs (Arundel, 1972; Lawson, 1994). Incorporating regular cestocide treatment of guardian dogs in to the model, and comparing the predicted results to the baseline values, clearly illustrated the value of canine treatment in the prevention of *C. ovis* in lambs.

Based on the model output in Figure 3.4, treating guardian dogs prior to arrival on pasture, and retreating every five weeks, eliminated the risk of *C. ovis* infection in lambs from this source. Therefore, to maintain farm biosecurity, any new dogs coming onto a farm should be treated with a cestocide one week before arrival to ensure *T. ovis* eggs are not introduced. It should be noted that the model assumed that the cestocide used was effective against *T. ovis*, that each dog was correctly dosed for its weight, and that there were no drug-resistant tapeworms. Currently, there are no reports of cestocide resistance in canid tapeworms. To ensure protection against *T. ovis*, regular treatment of dogs with a cestocide must occur at intervals shorter than the reported six-week prepatent period. Therefore, treating guardian dogs every fifth week with cestocides assured that *T. ovis* eggs were never released during defecation. However, a New Zealand study suggested that a rigorous five-week cestocide treatment protocol resulted in the selection of parasites with a prepatent period less than five weeks (Heath and Lawrence, 1980). For this reason, regular cestocide treatment every four weeks is often recommended. However, because of the high costs associated with regular treatment of guardian dogs, a five-week interval was considered more realistic for many farms and was therefore used in the model, simply because it equates to less treatments.

3.4.2 Pasture rotation

During the course of the grazing season, Canadian sheep producers often rotate their flock through several paddocks or pastures, instead of using the set-stocking approach in the model. Importantly, guardian dogs always reside with the sheep as the flock moves. According to the model, rotation to clean pastures every three weeks, and not returning to a previously grazed pasture during the same grazing season, failed to eliminate the risk of *C. ovis* in lambs. However, it did reduce the infection risk compared to baseline values with set-stocking (Figure 3.6).

Following deposition on pasture, *T. ovis* eggs are immediately infective to sheep. Thus, rotating to clean pasture and not returning in the same grazing season does not eliminate the risk of *C. ovis* completely; rather, it reduces the overall incidence of infection within the flock by reducing the number of hot zones on pasture that sheep are grazing. Rapid rotation prevents the accumulation of high egg densities on pasture (Figure 3.7), which decreases the probability of lambs contacting a hot zone and becoming infected.

The model assumes that animals are continually being rotated to clean pasture every three weeks, and not returning to previously grazed pasture until the following season. However, on Canadian sheep farms, many producers will rotate back to previously grazed pasture after three weeks. An assumption in the model is that hot zones only begin to decay after 12 weeks. Therefore, because of the prolonged viability of *T. ovis* eggs in the environment (ρ= 12 weeks), rotating back to previously grazed pasture after three weeks simply slows the *C. ovis* infection rate, but does not reduce the area of contaminated pasture. Though the model assumed hot zones remain infective for 12

weeks, after a three week rotation, perhaps the number of hot zones on previously grazed pasture is slightly lower than it would be using set-stocking. Ultraviolet radiation, temperature, humidity, wind, rain, insect and microbial activity may all contribute to desiccation and dispersion of *T. ovis* eggs before the 12 week average used in the model (Lawson and Gemmell, 1983). Therefore, it is possible that returning to previously grazed pasture during the same season may slightly lower *C. ovis* infection burdens in lambs compared to a set-stocking approach.

3.4.3 Proper deadstock disposal

The model outputs in Figures 3.8 and 3.9 suggest that preventing canid access to deadstock reduced the number of infective guardian dogs and market lambs, respectively. Practically, it is often difficult to completely prevent guardian dog access to deadstock because of their close contact with the flock. However, the model indicated that significantly reducing canid contact time with deadstock, through timely and adequate disposal, substantially reduced the risk of developing *T. ovis* infection (Figure 3.8) and ultimately transmitting the parasite to lambs (Figure 3.9). Under the assumptions of this model, reducing farm dog exposure to deadstock appears to be a low-cost and clinically effective prevention strategy.

Regarding the transmission of *T. ovis* to lambs, the developed model assumed that the only definitive host of *T. ovis* is on-farm guardian dogs. Although they do not live with sheep as guardian dogs do, it is not uncommon for herding and companion dogs, living on farms, to have contact with pasture grazed by sheep. Thus, there is at least one documented instance of a *C. ovis* outbreak in Canada that was linked to the acquisition of herding dogs (Soehl, 1984). It should be noted that the transmission of the parasite in

Australia and New Zealand is likely due to these types of dogs. However, the contact time between companion/herding dogs and sheep is substantially lower than between sheep and guardian dogs; suggesting that guardian dogs are more likely to infect lambs in Canada, which is why they were used in this model. The role that wild canids have on the epidemiology of the infection in Canada remains unclear at this time. To understand the effect wild canids have on *T. ovis* transmission, more information is needed about their defecation patterns. If future work determines that other canids, besides guardian dogs, play a substantial role in pasture contamination with *Taenia* eggs, the model will need to be revised to accommodate these transmission pathways.

3.4.4 Model limitations and future work

An infectious disease model is intended to be a simplified representation of a complex process that can be used to further the understanding of a particular infection (Vynnycky and White, 2010). The absence of available data on the infection rates in both host species meant that the true transmission parameters for *T. ovis* could not be extracted as is normally done through regression analysis; instead, transmission parameters were based entirely on information from the literature, and expert opinion. For example, it was not known how well hot zones, used to model the transmission of eggs to lambs, represent real world scenarios. Additional work is needed to determine the quantity and distribution of *Taenia* eggs on pasture as determined by defecation patterns of dogs and subsequent dispersion of eggs, and how frequently lambs contact the eggs. As knowledge about the epidemiology of *T. ovis* becomes available, model assumptions can be modified to better suit the actual transmission dynamics.

Although the model outputs could not generally be validated with field data, and therefore could not predict absolute infection risk on farm, the intent of the model was to assess the efficacy of various control options. In this sense, the absolute values of the model outputs were irrelevant. What mattered was how risk of *C. ovis* infection changed relative to baseline simulations when a control option was used. Unfortunately, the model could not be validated through sensitivity analysis due to the current lack of field data on the rate of parasite transmission in both definitive and intermediate hosts on Canadian sheep farms. Validation of the current model is the next step in the development of a more detailed, predictive model that could be used by both clinicians and producers to accurately predict infection risk and assess the impact of various control options.

Lastly, the use of agent-based, stochastic modeling should be considered for future models developed for *T. ovis* infection. For the model described, a deterministic approach was used for small populations of animals. This resulted in fractions of animals infected that are still contributing to infection in both dog and sheep populations. In general, deterministic models are better suited for large populations. Developing an agent-based model with actual counts of animals, as opposed to averages, may be more representative of parasite transmission. It would be interesting to compare the results of the current model with those of an agent-based model.

3.4.5 Conclusion

The work described here constitutes the first reported transmission model for *T. ovis* infection. The assumptions used to construct the model were all based on the current understanding of the parasite's lifecycle and likely transmission process in Canada. As

such, the developed model appears to be representative of the *T. ovis* transmission processes on Canadian sheep farms.

Combined with treatment upon arrival, regular treatment of guardian dogs every five weeks with an effective cestocide was predicted to successfully prevent *C. ovis* infections in a lamb flock when guardian dogs were the only definitive hosts. This therefore suggests that, as part of a good farm biosecurity program, all new dogs that are to have contact with the flock should be treated with a cestocide one week before arrival to avoid the introduction of parasites onto the farm. Additional control options, namely proper deadstock disposal and pasture rotation, were also shown to reduce the risk of *C. ovis* in lambs. In particular, denying guardian dog access to sheep carcasses through immediate and thorough disposal of deadstock appears to be a simple, inexpensive, way of effectively preventing *C. ovis* infection.

Ideally, this model will serve as the first step in the creation of a more detailed predictive model that can be validated through the collection of data describing the rate of transmission in both the definitive and intermediate hosts. The availability of such a model would be a useful tool for sheep producers and veterinarians to better understand, and control, *T. ovis* infection on farms.

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Table 3.1: Input parameters and values used in the baseline *Taenia ovis* transmission model when the simulation began (t=0).

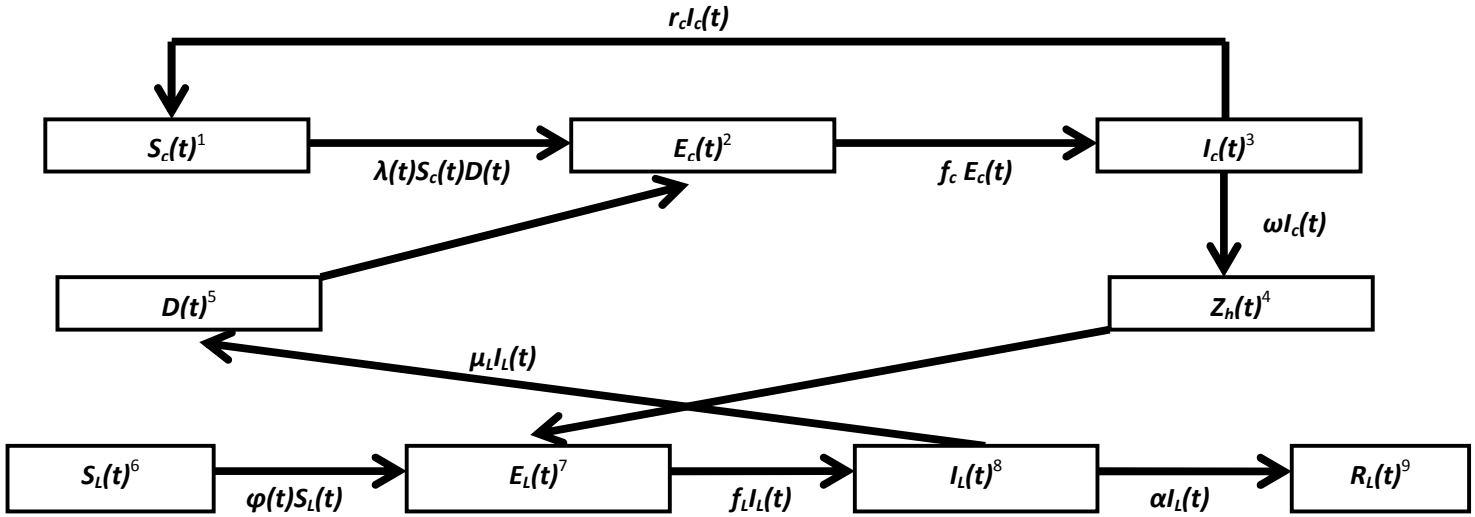
Parameter	Baseline Value
Sheep	
Stocking density	30 lambs per acre
Mean lamb weight	50 kilograms
Grazing area per lamb	0.0025 acres per week
Number susceptible (S_L)	30
Number exposed (E_L)	0
Number infectious (I_L)	0
Number recovered (R_L)	0
C. ovis prepatent period (f_L)	10 weeks
C. ovis immunity rate (α)	12 weeks per infection
Post-weaning mortality rate (μ)*	0.125% per week
Dog	
Number susceptible (S_c)	0
Number exposed (E_c)	0
Number infective (I_c)	2
Probability of consuming deadstock (λ)	100%
T. ovis prepatent period (f_c)	6 weeks
T. ovis recovery rate (r_c)	36 weeks per infection
Defecation rate (ω)	7 defecations per week
Environment	
Number of deadstock (D)	0
Deadstock decay rate (δ)	4 weeks per carcass
Feces hot zone decay rate (ρ)	12 weeks per zone

* The post-weaning mortality rate in market lambs is estimated to be 2.0% over the period from weaning (2 months) to market (6 months) (Ontario Ministry of Agriculture, Food and Rural Affairs, 2011b). On a weekly basis, this is approximately 0.125%.

Table 3.2: Input parameters and values used in the adjusted baseline model when time = 0 to determine the effect of proper deadstock disposal on *Taenia ovis* transmission.

Parameter	Baseline Value
Sheep	
Stocking density	30 lambs per acre
Mean lamb weight	50 kilograms
Grazing area per lamb	0.0025 acres per week
Number susceptible (S_L)	30
Number exposed (E_L)	0
Number infectious (I_L)	0
Number recovered (R_L)	0
C. ovis prepatent period (f_L)	10 weeks
C. ovis immunity rate (α)	12 weeks per infection
Post-weaning mortality rate (μ)	0.125% per week
Dog	
Number susceptible (S_c) **	2
Number exposed (E_c)	0
Number infective (I_c) **	0
Probability of consuming deadstock (λ)	100%
T. ovis prepatent period (f_c)	6 weeks
T. ovis recovery rate (r_c)	36 weeks per infection
Defecation rate (ω)	7 defecations/week
Environment	
Number of deadstock (D) **	3
Deadstock decay rate (δ)	4 weeks per carcass
Feces hot zone decay rate (ρ)	12 weeks per zone

**** indicates changes from the original baseline model shown in Table 3.1.**



Symbol	Parameter	Symbol	Parameter
$S_c(t)$	susceptible guardian dogs	$R_L(t)$	recovered lamb
$E_c(t)$	exposed guardian dogs	λ	deadstock consumption rate
$I_c(t)$	infective guardian dogs	f_c	canid infective rate
$D(t)$	number of deadstock	r_c	canid recovery rate
$Z_h(t)$	number of hot zones	μ_L	lamb mortality rate
$S_L(t)$	susceptible lamb	φ	lamb exposure rate
$E_L(t)$	exposed lamb	f_L	lamb infective rate
$I_L(t)$	infective lamb	α	lamb recovery rate
		ω	canid defecation rate

Figure 3.1: Simplified representation of the transmission model developed for *Taenia ovis* infection at a particular time (t).

1 $dS_c(t)/dt = -\lambda S_c(t)D(t) + r_cI_c(t)$
2 $dE_c(t)/dt = \lambda S_c(t) - f_cE_c(t)$
3 $dI_c(t)/dt = f_cE_c(t) - r_cI_c(t)$
4 $dZ_h(t)/dt = \omega I_c(t) - \rho Z_h(t)$
5 $dD(t)/dt = \mu I_L(t) - \delta D(t)$
6 $dS_L(t)/dt = \varphi(t)S_L(t) - \mu S_L(t)$
7 $dE_L(t)/dt = \varphi(t)S_L(t) - \mu E_L(t) - f_L E_L(t)$
8 $dI_L(t)/dt = f_L E_L(t) - \mu I_L(t) - \alpha I_L(t)$
9 $dR_L(t)/dt = \alpha I_L(t) - \mu R_L(t)$

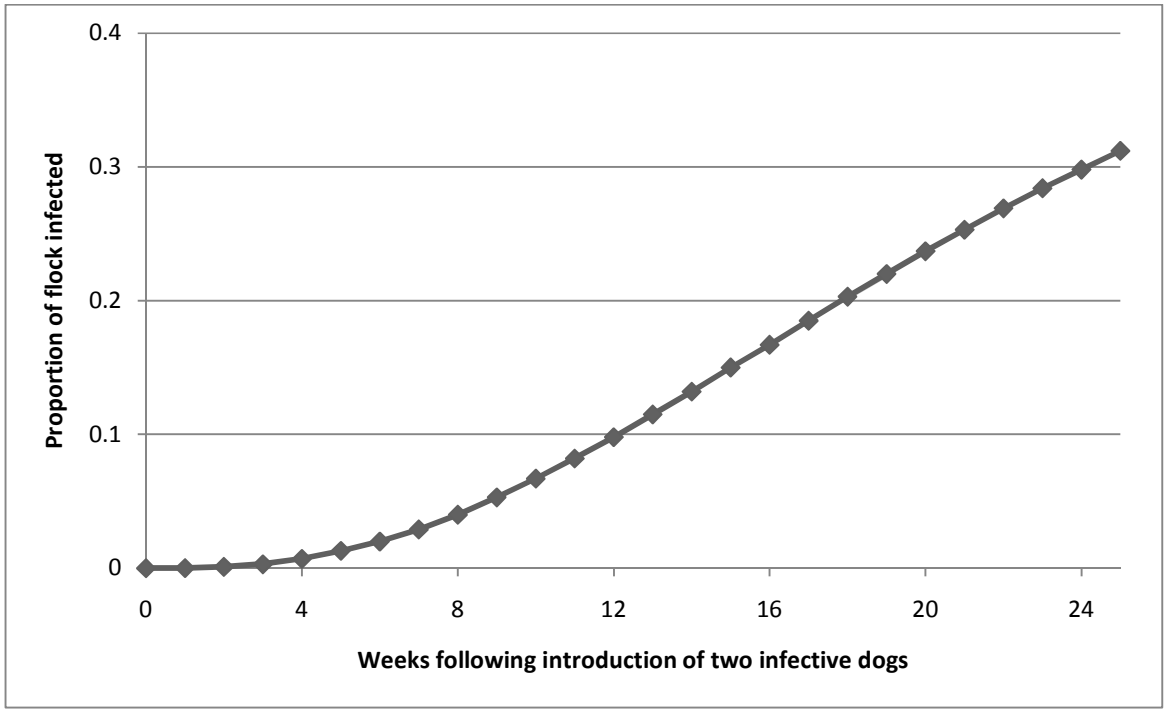


Figure 3.2: Output graph, using baseline input parameters, illustrating how a naive lamb flock would become infected following the introduction of two guardian dogs with patent *Taenia ovis* infections at t=0.

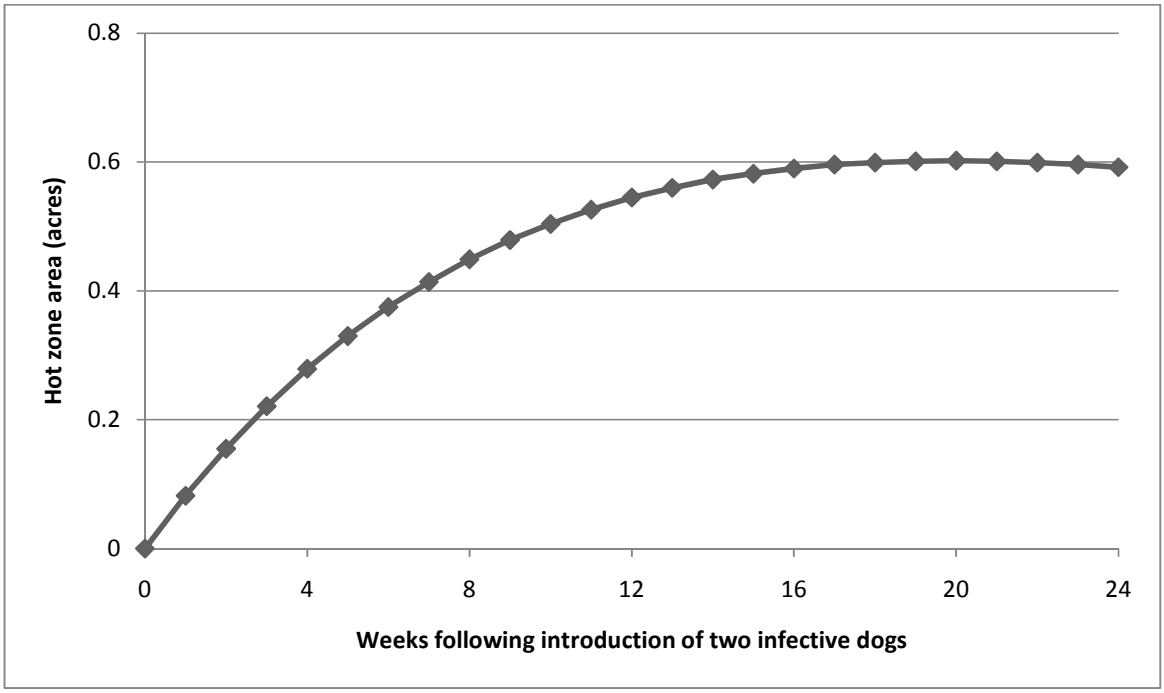


Figure 3.3: Output graph, using baseline input parameters, illustrating how the total area of pasture contaminated with *Taenia ovis* eggs (described as hot zones) increases following the introduction of two guardian dogs with patent infections at t=0.

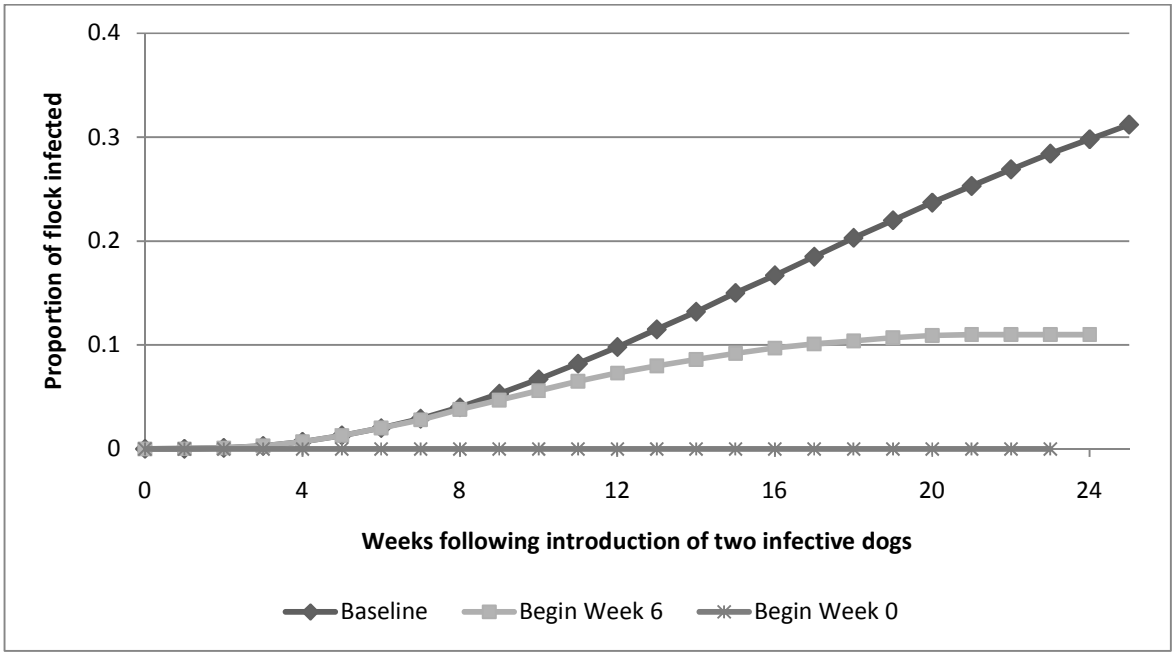


Figure 3.4: Output graph illustrating how routine treatment of guardian dogs with a cestocide every fifth week (commencement of routine treatment week 6 vs. week 0) influenced the risk of *Cysticercus ovis* infection in naive lambs. Input parameters used in this simulation are shown in Table 3.1.

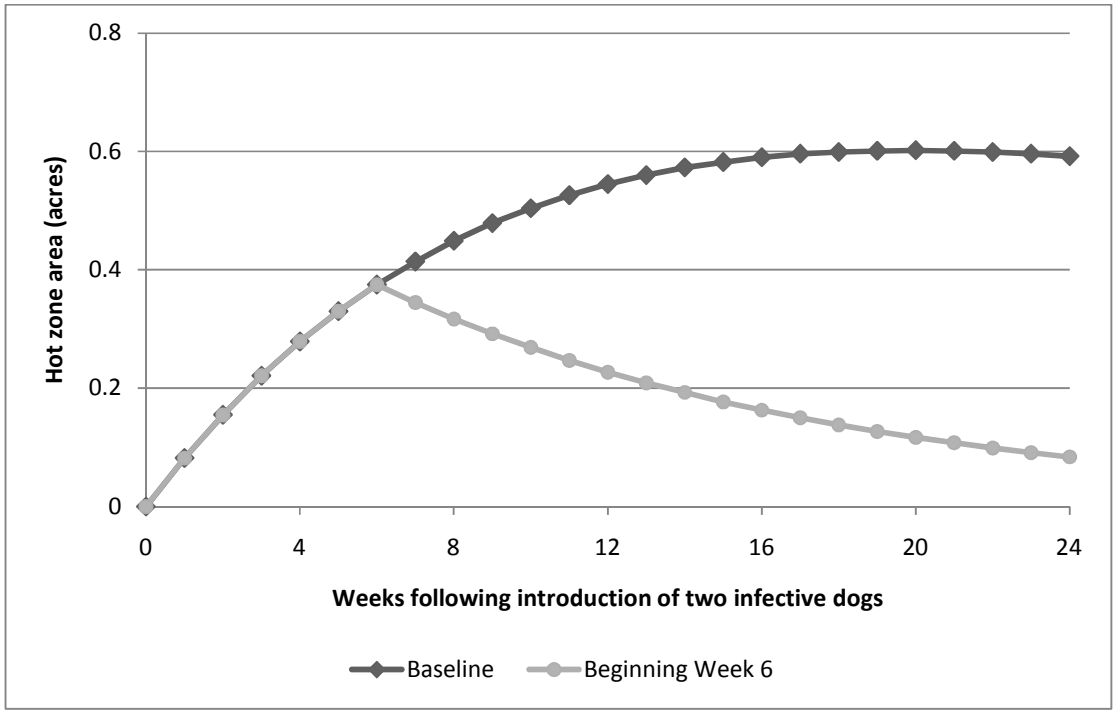


Figure 3.5: Output graph illustrating how routine treatment of guardian dogs with a cestocide every fifth week, beginning at week 6, influenced the pasture area contaminated with *Taenia ovis* eggs - described as hot zones. Input parameters used in this simulation are shown in Table 3.1.

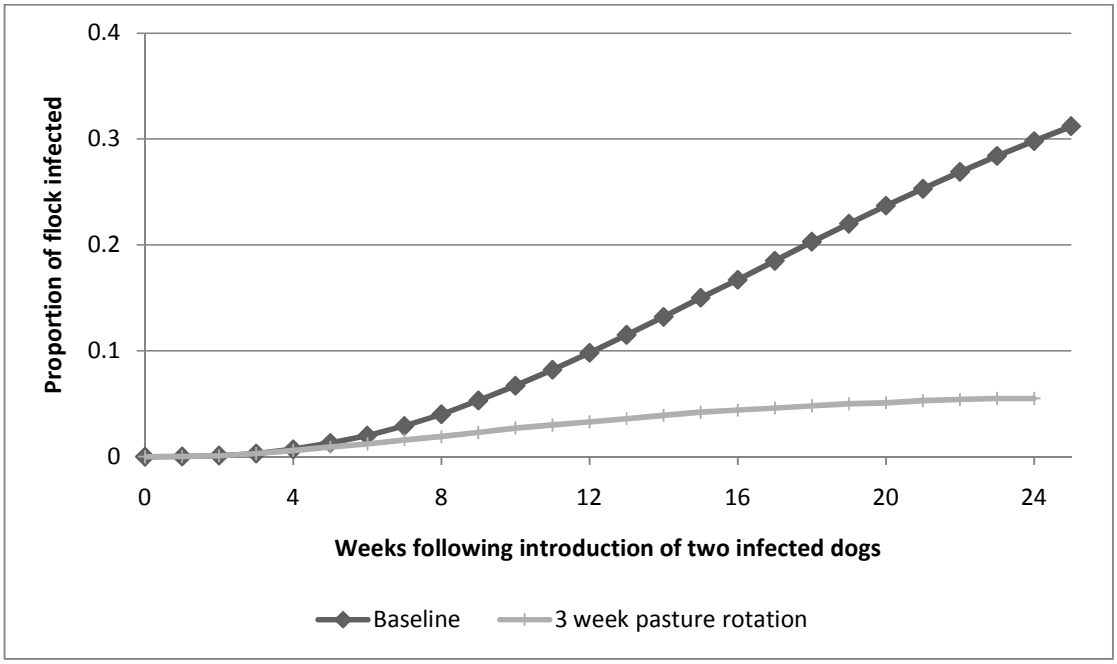


Figure 3.6: Output graph illustrating how rotating the flock to a clean pasture every three weeks, and not returning during the same grazing season, affects the within-flock prevalence of *Cysticercus ovis*. Baseline values assume a set-stock approach to flock management. Input parameters used in this simulation are shown in Table 3.1.

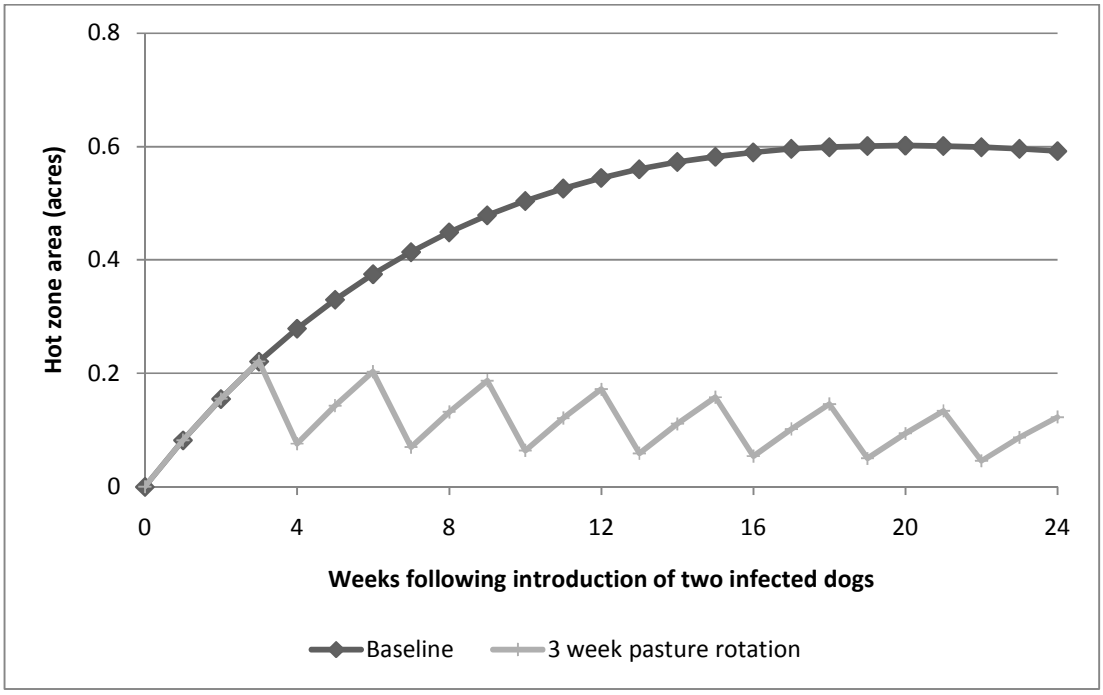


Figure 3.7: Output graph illustrating how rotating the flock to clean pasture every three weeks, and not returning during the same grazing season, affects the pasture area, available to sheep, contaminated with *Taenia ovis* eggs. Input parameters used in this simulation are shown in Table 3.1.

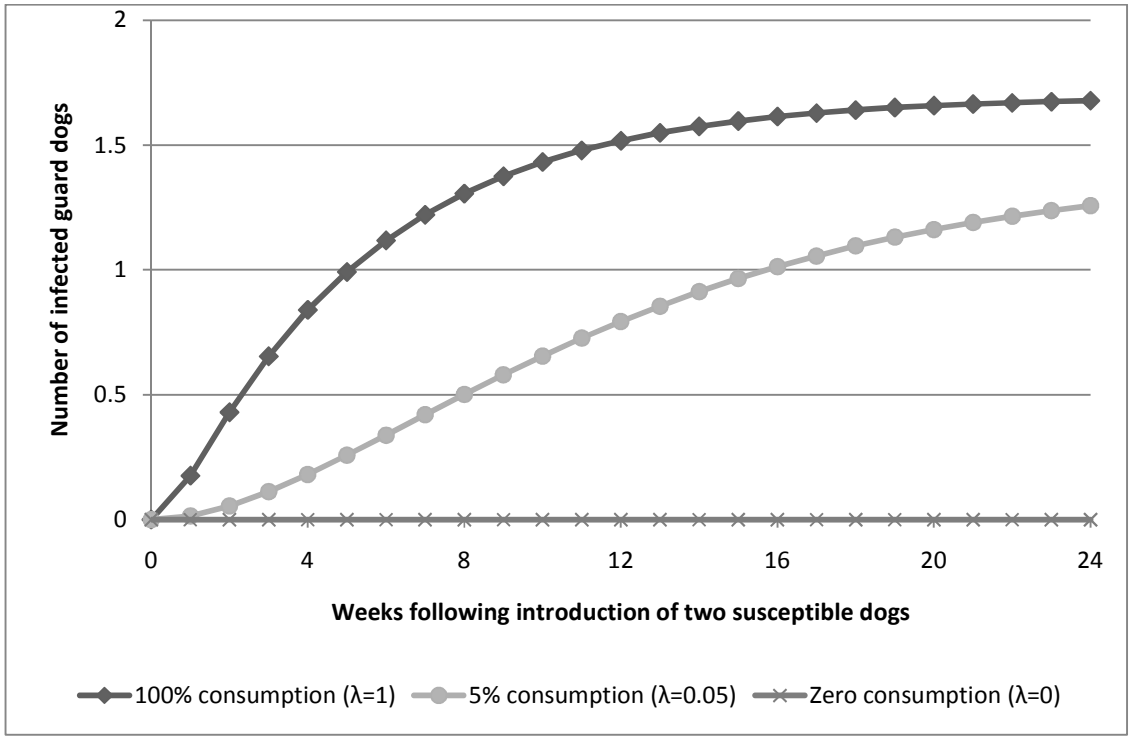


Figure 3.8: Output graph illustrating how varying amounts of deadstock consumption by guardian dogs influence the risk of *Taenia ovis* infection in susceptible dogs. Baseline refers to dogs that had access to infective deadstock throughout the week. Input parameters used in this simulation are shown in Table 3.2.

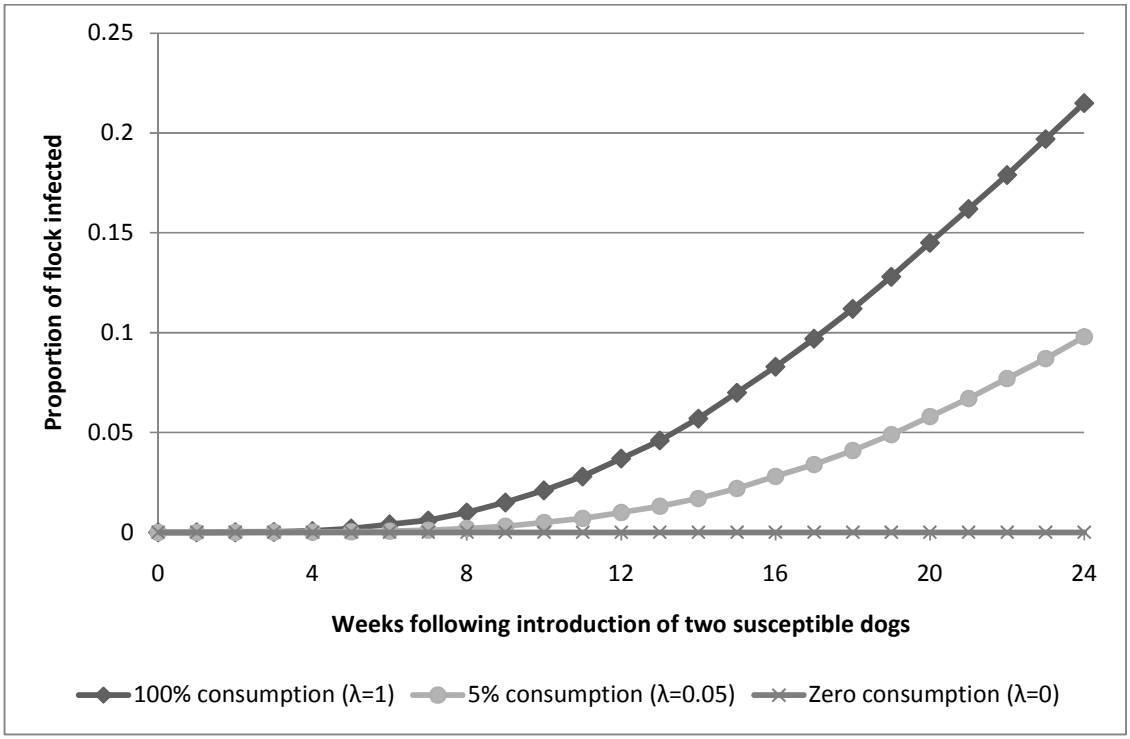


Figure 3.9: Output graph illustrating how varying amounts of deadstock consumption by two susceptible guardian dogs influence the risk of *Cysticercus ovis* infection in a flock of naive lambs. Baseline refers to dogs that had access to infective deadstock throughout the week. Input parameters used in this simulation are shown in Table 3.2

Chapter Four

Conclusions and recommendations

The overall goal of this research was to improve the understanding of the epidemiology of *Cysticercus ovis* in Canada so as to make suitable recommendations for its control. Much of the recent literature on *C. ovis* has focused on its immunology, with vaccine development being the primary goal (Johnson et al. 1989; Rickard et al. 1995; Lawrence et al. 1996; Rothel et al. 1996; Waterkeyn et al. 1997; Lightowlers et al. 2003; Jabbar et al. 2010). Very little research has focused on the parasite's epidemiology, or has tried to elucidate risk factors for infection. The epidemiological information that does exist is almost exclusively based on research carried out in New Zealand and Australia, where *C. ovis* infection is considered endemic (Arundel, 1972; Lawson, 1994). However, the epidemiology of the parasite on Canadian sheep farms may be significantly different from those countries, primarily due to differences in flock size, flock management practices and the presence of wild canids in Canada.

With this in mind, the primary objective of this study was to describe the distribution and frequency of sheep carcass condemnations due to *C. ovis* in Canada, and identify risk factors for infection on Canadian sheep farms. Additionally, a mathematical transmission model was developed for *Taenia ovis* infection using assumptions believed to be valid on Canadian farms. The model was intended to provide a better understanding of *T. ovis* transmission based on various input parameters, and to quantitatively assess the impact of various control options on parasite incidence. The following chapter highlights the main conclusions obtained from this research, discusses limitations, and proposes

areas of future research. Lastly, recommendations are made for Canadian producers to reduce the risk of *C. ovis* infection in their sheep.

4.1 Chapter two - summary and conclusions

The assimilation of condemnation statistics from both Ontario provincially inspected abattoirs, and federally inspected abattoirs across Canada, revealed a substantial increase in the proportion of sheep carcasses condemned due to *C. ovis* infection. Compared to data from earlier years, carcass condemnations due to *C. ovis* at federally inspected abattoirs increased dramatically in 2007, and continued to increase into 2008. These condemnations due to *C. ovis* represented a large proportion of the total sheep condemnations for all reasons. A similar event occurred at provincially inspected abattoirs in Ontario, but was delayed by one year (i.e. occurring in 2008). At abattoirs, sheep carcasses lightly infected with *C. ovis* may only be trimmed and passed, rather than condemned, and those data are not captured in the summary statistics. Therefore, the true prevalence of infection in the Canadian sheep flock is likely greater than these data suggest.

Using the Canadian Sheep Identification Program (CSIP), the trace-back of condemnations at Ontario provincially inspected abattoirs allowed the identification of each condemned animal's farm of origin. Alberta, Saskatchewan, Manitoba and Ontario all had multiple farms with at least one sheep condemned, and it was not uncommon for multiple condemnations to originate from the same farm. Sheep in British Columbia, Quebec, and Atlantic Canadian provinces tend to be slaughtered locally, as opposed to being transported to Ontario. Consequently, the status of *C. ovis* infection within these provinces could not be assessed in this study. Overall, the trace-back data strongly

suggested that transmission of *C. ovis* is occurring in multiple Canadian provinces. It remains unclear why the number of sheep carcass condemnations due to *C. ovis* has increased in recent years. Perhaps partially responsible is the constant transport of sheep from west to central Canada (West Hawk Lake Zoning Control Site, personal communication, 2011), the increased use of guardian dogs for predator protection, and the high proportion of flocks that were previously naive to the infection.

Comparing the farm and flock management practices between case and control farms was used to identify factors associated with a farm having a sheep carcass condemnation due to *C. ovis* infection. On Canadian sheep farms, the practice most highly associated with a condemned carcass was failing to dispose of deadstock. All producers that reported disposal through burial (either in the ground, or in a manure pile), composting, or incineration, were classified as having "deadstock disposal". Producers that reported doing nothing with fallen stock, or who actively discarded deadstock into an unoccupied field or forest to decompose and be scavenged, were classified as having "no disposal". On farms that failed to dispose of deadstock, the odds of having a sheep carcass condemnation due to *C. ovis* infection was 11.8 times greater (95% CI: 2.9 – 47.4; p=0.001) than farms that used deadstock disposal methods. The elevated risk of *C. ovis* infection on these farms is probably due to a greater likelihood of sheep carcasses being scavenged by canids. If available, both domestic canids (e.g. guardian dogs, herding dogs, companion dogs, stray dogs) and wild canids (e.g. coyotes, wolves and foxes) will readily scavenge sheep carcasses. In Canada, the regulations regarding deadstock disposal vary between provinces, but several options are available that

preclude any opportunity for scavenging by canids (Ontario Ministry of Agriculture, Food and Rural Affairs, 2011).

Independent of how deadstock were disposed, the scavenging of deadstock by farm dogs was found to be significantly associated with carcass condemnations due to *C. ovis*. On sheep farms that reported scavenging of deadstock by farm dogs, the odds of having a carcass condemnation due to *C. ovis* infection was 4.0 times greater (95% CI: 1.2–14.0; p=0.028) than farms that did not report scavenging by farm dogs. Interestingly, feeding farm dogs sheep meat is thought to be the factor primarily responsible for the *C. ovis* epidemic in New Zealand several decades ago (Lawson, 1994). Unfortunately, in this study, we were not able to assess purposefully feeding deadstock to farm dogs in multivariable analyses, as not enough participating farms reported this feeding practice. However, the scavenging of sheep meat by farm dogs in Canada could be having a similar effect. These results suggest that domestic dogs are important in transmitting the parasite to sheep, regardless of the role wild canids play through scavenging or predation.

There was no statistical difference ($p>0.05$) between the occurrence of predation or scavenging by wild canids on case and control farms. However, the possible effect of wild canids in parasite transmission could be hidden in the deadstock disposal method. Failure to dispose of deadstock, which was associated with sheep carcass condemnations, could result in *C. ovis* exposure to wild canids not noticed by producers. Therefore, the possible involvement of wild canids in parasite transmission to sheep should not be ignored. Understandably, preventing predation and scavenging by wild canids is a challenge for producers; however, efforts should be made to reduce their presence on farm.

Multivariable analysis revealed a significant interaction term between province and the presence of stray dogs on farm property (p=0.007), meaning that the effect of stray dogs on carcass condemnations due to *C. ovis* is dependent on province. The size of the effect was very small, however, and the biological relevance of the interaction is questionable. However, stray dogs were significantly more likely (p=0.003) to be reported on case farms (50%), compared to controls (25%). Therefore, it is crucial that stray dogs are considered when devising a *C. ovis* control strategy. These results are consistent with a recently published study that investigated the source of a severe *C. ovis* outbreak in England (Eichenberger et al. 2011). Another recent study, that investigated risk factors for *Echinococcus granulosus* seropositivity in farm dogs in Wales, found that stray dogs were at greatest risk of being seropositive for the parasite compared to working or companion dogs (Mastin et al. 2011). Stray dogs are difficult to control, but possible options to reduce their presence on a farm include: ensuring secured fencing around pasture areas; using guardian animals; and properly disposing of carcasses which may attract dogs to farm property.

In New Zealand, the *C. ovis* control program has largely focused on monthly treatment of farm dogs with cestocides to prevent parasite transmission (Ovis Management Ltd., 2011). This study found no association between sheep carcass condemnations and treating farm dogs with anthelmintics. However, importantly, the questionnaire only asked if treatment was done within the year prior to having a carcass condemned. Perhaps producers who treated dogs more regularly would have had lower odds of a condemnation, but this could not be ascertained in this study. Furthermore, many producers could not recall the deworming product they used. Treatment with an

effective cestocide is essential for controlling *Taenia ovis*, and if producers were not treating dogs properly it could mask the benefit of deworming dogs on *C. ovis* control.

Prior to this study, *C. ovis* condemnations were not traced-back to their farm of origin. As a consequence, the infection remained unknown and of little personal consequence to a large percentage of Canadian producers. Although articles on *C. ovis* and its control have been written for sheep producers over the last decade (Paula Menzies, personal communication 2011), changes to farm and flock management to address this issue have been slow to occur, possibly because producers did not recognize that their flock was at risk. By informing producers of carcass condemnations in this study, perhaps they became aware of this previously rare parasite and learned how to control it.

4.1.1 Limitations and future work

Using the guidelines provided by the Food and Agriculture Organization of the United Nations (Food and Agriculture Organization, 2000), the specificity of detecting *C. ovis* during meat inspection is believed to be good. There are few conditions in sheep that present similarly to *C. ovis*. In contrast, the sensitivity of meat inspection at detecting *C. ovis* is considered poor at approximately 50% (McNab and Robertson, 1972). Consequently, the potential for misclassification of a case farm existed if the farm had a low prevalence of infection, or had strong flock immunity to *C. ovis*, resulting in a low number of cysticerci per animal. This could potentially result in an infected carcass being missed or trimmed at meat inspection, and not condemned. If it occurred, this type of misclassification would bias the results towards the null, so the reported odds would be an underestimate of the true association.

Misclassification bias could also occur by incorrectly classifying control farms as cases due to limitations with the current CSIP system. Unfortunately, the CSIP does not allow the traceability of sheep from the time they leave their original farm until the time they arrive at slaughter; a sheep can only be traced to the farm of origin. As demonstrated in this study, most Canadian producers do not sell sheep direct to slaughter. The majority of sheep go from the farm of origin to a sales barn, where they reside for likely less than three days prior to slaughter; however, the opportunity exists for infection at a secondary location provided sheep were there for at least two weeks prior to slaughter (e.g. at a feedlot). Thus, if a sheep was infected with *C. ovis* after leaving its original farm, and subsequently condemned at slaughter, the original farm would be incorrectly classified as a case farm. As before, this type of misclassification would bias the results towards the null, so the reported odds would be an underestimate of the true association.

A mandatory traceability system would allow a better understanding of the risk factors for *C. ovis* infection in Canada. It would also aid in generating individual accountability for infection. Currently, producers are not held responsible for *C. ovis* condemnations unless marketed directly to slaughter. This is because the burden of the condemnation is put on the current owner (e.g. purchaser at the sales barn, or feedlot owner). While the financial burden would not change, a traceability system would allow every location at which the sheep resided prior to slaughter to be targeted for education and control efforts. Ironically, an effective vaccine exists against *C. ovis* infection in sheep (Johnson et al. 1989), but has never been produced commercially. Until individual producers can be identified and held accountable for a condemnation that originated on their farm, there is likely no value in commercialization of this vaccine. Producers are

unlikely to pay for even a moderately priced vaccine if the monetary losses associated with carcass condemnations continue to fall only on abattoirs and large feedlots.

The initial steps towards a traceability system have already been initiated by the Canadian Food Inspection Agency. By December 31, 2012, all sheep in Canada must be tagged with a radiofrequency identification (RFID) tag (Canadian Sheep Federation, 2011). The implementation of RFID tags makes it easier for farms and feedlots to identify incoming animals, allowing for better records and greater traceability.

The current case-control study selected participants exclusively from farms that slaughter sheep at Ontario provincially inspected abattoirs. To increase the confidence of the risk factor analysis at the national level, it would have been beneficial to select cases and controls from all abattoirs with condemned sheep in Canada. Through cooperation with abattoirs outside of Ontario, a greater proportion of Canadian sheep producers could be captured in the sampling frame, which would increase the national scope of the study as well as the sample size. A larger sample would improve the accuracy of the results and the external validity of the study.

Despite the financial losses caused by *C. ovis*, the parasite is not a reportable, immediately notifiable or annually notifiable infection in Canada (Canadian Food Inspection Agency, 2010). Thus, there is no formal reporting of the parasite's occurrence other than as a cause of condemnations, and then only as summary numbers. As a result, this research represented the first effort to obtain information on the frequency and distribution of *C. ovis* condemnations in Canada. Furthermore, the study facilitated discussion between producers, veterinarians and industry workers across the country about an infection that has the potential to threaten the Canadian sheep industry. Through

numerous presentations, and the distribution of brochures and national newsletters containing information about *C. ovis* (e.g. Canadian Sheep Federations's *From the Flock*, and *Points of View*), the importance of this parasite has been made clear to producers across the country.

Efforts to control *C. ovis* infection in Canada would be greatly assisted by a national program, similar to Ovis Management Ltd. in New Zealand. Unfortunately, the dichotomy between provinces, and provincial and federal meat inspection, makes the initiation of such a program very difficult. However, it is crucial, especially given the widespread transport of sheep and dogs across Canada, that in the coming years dialogue remains open between provinces, including veterinarians (government and practitioners) and producers about *C. ovis* condemnations and control efforts.

4.2 Chapter three - summary and conclusions

A mathematical transmission model was developed to predict the dynamics of *T. ovis* infection on Canadian sheep farms. It was created using a compartmental, deterministic model-building approach. Assumptions were made about farm management practices and parasite transmission based on published information in the literature, and expert opinion. Using the risk of *C. ovis* infection in lambs as the output measure, various control options were quantitatively assessed through comparison with a control. Many of the predictions obtained from the model were expected based on the biology of *T. ovis* and previous studies (Arundel, 1972; Lawson 1994); however, the ability to quantify these predictions has not been previously described. Additionally, the model simultaneously considers multiple farm characteristics that may influence parasite transmission.

Treating all incoming farm dogs with an anthelmintic effective against *T. ovis* was shown to have a major impact on reducing the risk of *C. ovis* infection in lambs. Moreover, if farm dogs have any access to deadstock, whether it is through intentional feeding or scavenging, the model indicated that treatment, when repeated within five weeks following exposure, sufficiently prevented pasture contamination with *T. ovis* eggs such that lamb infection did not occur. Similarly, producers unsure of their dog's eating habits (e.g. if there was opportunity to scavenge deadstock) should repeat treatment every five weeks to avoid the possibility of pasture contamination, and sheep exposure to parasite eggs. This recommendation is also consistent with recommendations from Ovis Management Ltd. in New Zealand. Although monthly treatment of farm dogs appears effective at preventing *C. ovis*, the current cost of cestocides in Canada, combined with the sometimes malicious disposition of guardian dogs, often results in low producer compliance.

Given that guardian dogs were the only definitive host included in the model, quick and thorough disposal of deadstock was predicted to eliminate the risk of *C. ovis* infection in lambs. However, it is important to note that this prediction was based on the premise that guardian dogs did not harbour *T. ovis* when first brought on farm. "Thorough disposal" was considered to be any method that prevented canids, of any type, from having access to carcasses. Practically, this can be a challenge for producers because canids will often scavenge carcasses before the producer has an opportunity to dispose of them. Burying carcasses in the ground was the most commonly reported method of disposal in this study; however, during the winter months, the frozen ground often resulted in carcasses being left indisposed for longer periods of time, possibly

increasing the risk of scavenging. Even when carcasses are disposed, persistent or hungry canids will often gain access to them through digging. Despite its practical limitations, when properly implemented, thorough deadstock disposal provides a seemingly low cost method of *C. ovis* control.

Rotating the flock (and its guardian dogs) to clean pasture every three weeks, and not returning to previously grazed pasture in the same season, was simulated to assess its impact on *C. ovis* infection risk in lambs. As expected, because *Taenia* eggs are immediately infective in canine feces (Taylor et al. 2007), rotating pastures failed to eliminate the risk of *C. ovis* infection in lambs. However, it did decrease the area of pasture contaminated with parasite eggs, and therefore slightly reduced the infection risk in lambs.

4.2.1 Limitations and future work

During model development, many assumptions were required about flock management practices, as well as parasite biology and transmission. The model was designed to incorporate what were believed to be significant risk factors for *C. ovis* transmission. However, in its current state, the model should not be considered a definitive tool for predicting the risk of *C. ovis* infection in lambs on Canadian farms. For example, except for guardian dogs, the model does not consider the role of other potential definitive hosts, such as working or companion dogs, stray dogs, or wild canids. With the exception of guardian dogs, which remain with sheep 100% of the time, it is difficult to quantify the contact time of other canids with the flock. As more information about the parasite in Canada becomes available, modifications to the model can be made to improve its predictive ability. Specifically, information on the scatting behaviour of

canids (domestic and wild) is needed to generate more accurate results. For instance, if domestic dogs tend to defecate in the same location, rather than randomly on pasture, the pasture area contaminated with *Taenia* eggs would not increase daily, thus lowering the infection risk in sheep. Additionally, to further increase the accuracy of the model, information is needed about the contact rate between *Taenia* eggs and grazing sheep, the infectivity half life of eggs, and egg dispersion on pasture.

A crucial component of any mathematical transmission model is establishment of the transmission parameters that govern animal movement between compartments. In most circumstances, transmission parameters are extracted from data sets that describe the rate of infection in a naive population (Vynnycky and White, 2010). Unfortunately, no research has been carried out to investigate the rate of *C. ovis* infection in a naive flock following introduction of a canid with a patent *T. ovis* infection. Accordingly, the parasite transmission rate between dogs and sheep had to be estimated using the idea of "pasture hot zones" and the probability of lambs contacting them. In the absence of such rate data, it is impossible to verify the accuracy of the transmission parameters that we used. If these data become available, the model validity should be confirmed through a sensitivity analysis. In doing so, the model would generate more accurate predictions.

4.3 Overall recommendations

Based on this study, several conclusions can be made about the epidemiology of *C. ovis* in Canada and recommendations can be made for its control on Canadian farms. The following are a list of important recommendations:

- The farm-level prevalence of *C. ovis* on Canadian sheep farms is increasing. Regardless of the cause, it is important to limit further spread of the parasite through the implementation of control strategies.
- Failure to dispose of deadstock was highly associated with *C. ovis* condemnations on farms, likely because it leads to carcass scavenging by canids. In terms of *C. ovis* transmission, the disposal method used is irrelevant, so long as it ensures that canids cannot access deadstock.
- Scavenging of deadstock by farm dogs was found to be associated with lamb carcass condemnations caused by *C. ovis*. Thus, if farm dogs are discovered eating sheep meat, or producers are unsure of their dogs' eating habits, treatment with a cestocide is necessary within five weeks following exposure to prevent the release of parasite eggs. With less frequent treatment, the risk of parasite transmission to sheep likely increases.
- In this study, the presence of stray dogs on farms was significantly more common on case farms compared to controls. It is therefore critical that all stray dogs are kept off farm property through effective fencing, the use of guardian animals, and proper carcass disposal – which may attract stray dogs.
- This study revealed no association between the presence of wild canids on farms (predation or scavenging) and the risk of *C. ovis* condemnations, suggesting that domestic canids act as the primary definitive host for *T. ovis* on Canadian sheep farms. However, because the possibility of parasite transmission through wild canids exists, efforts should still be made to reduce their occurrence on farms, particularly with respect to scavenging deadstock in areas where sheep are found.

- As evidenced by findings from the transmission model, all dogs entering farm property should be properly treated with an anthelmintic effective against *T. ovis* one week prior to arrival. This is consistent with the recommendations made by Ovis Management Ltd.
- Continued trace-back of carcasses condemned due to *C. ovis* is a crucial component for successful, long-term, control of the parasite in Canada. Also, informing producers of carcasses that were trimmed but passed, might improve surveillance and lead to better control. Unfortunately this would necessitate a major change in meat inspection reporting.
- The implementation of a traceability system is necessary to hold everyone involved in Canadian sheep production accountable for sheep health, food safety and quality. The use of RFID tags in the near future will improve animal traceability, and will potentially be an important step in educating producers that may have been responsible for infection of sheep with *C. ovis*.

The cause of the sudden and dramatic increase in sheep carcass condemnations due to *C. ovis* in Canada in 2007-2008 will likely never be known for certain. As a result, it is important to continue to educate sheep producers regarding this parasite, and how to control it. Avoidance of risk factors, and continued communication between abattoirs and producers will be essential for preventing further spread of the parasite.

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APPENDIX 1

Administered Questionnaire

CSIP Tag #:	Contact Name:
Month Slaughtered:	Contact Telephone #:
Date Interviewed:	Interviewer:

Collaborators:

Dr. Paula Menzies, Department of Population Medicine, University of Guelph
Dr. Andrew Peregrine, Department of Pathobiology, University of Guelph
Dr. Andria Jones, Department of Population Medicine, University of Guelph
Dr. Jocelyn Jansen, Ontario Ministry of Agriculture Food and Rural Affairs
Mr. Brad De Wolf, Department of Population Medicine, University of Guelph
Ms. Jennifer MacTavish, Executive Director, Canadian Sheep Federation.
Dr. Chris Clark, Western College of Veterinary Medicine, University of Saskatchewan

Introduction:

Good day. I am calling regarding a project studying the current situation regarding carcass condemnations in lambs due to infection with *Cysticercus ovis*, a parasite of dogs that causes cysts in the muscle of sheep. My name is Brad De Wolf, from the University of Guelph in Guelph, Ontario. Your contact information has been passed on to me by Jennifer MacTavish, Executive Director of the Canadian Sheep Federation. Have you received a copy of the questionnaire? There is no need for you to write anything down, I will record your responses.

I would first like to say that I do not know if you have a sheep or lamb condemned due to *C. ovis,* or if you have been selected as a control farm - and you will not be asked to tell me at any time. Thank you for agreeing to be interviewed for this research project examining the problem of *C. ovis* infection in sheep and lamb carcasses. This project is a collaborative project involving the University of Guelph, and the University of Saskatchewan, as well as the Canadian Sheep Federation (CSF) and the Ontario Ministry of Agriculture, Food and Rural Affairs. The project is funded by the CSF and Animal Health Strategic Investment monies from the Ontario government.

Across Canada there has been an increasing number of carcass condemnations due to infection with *C. ovis,* the sheep stage of the dog tapeworm *Taenia ovis*. This disease is often called “sheep measles” because of the appearance of the parasite cysts in the skeletal muscle, heart and diaphragm of the sheep. The carcass is condemned if on inspection there are numerous cysts in the muscle, making it unfit for human consumption. Carcasses with only a few cysts are trimmed and passed. This study is only looking at cases in which the infection is severe enough so that the carcass has been condemned. Because of the economic impact of this infection, our research team is investigating factors possibly associated with lambs being infected with *C ovis* and then condemned at slaughter. This information will help the sheep industry to control or possibly eradicate this disease.

You have been selected for an interview because either you have had a lamb or sheep recently condemned due to *C ovis* infection, i.e. are a “case” farm, or because you had lambs slaughtered on the same day at the same plant that had an animal condemned, and based on the national ID tag in your sheep, you have been randomly selected as a “control” farm. It is necessary to compare both types of farms to determine if there are any important differences which might point to ways that we can recommend preventing this infection.

We would greatly appreciate you taking a few minutes to answer some questions regarding your farm management. All results will be kept strictly confidential and no personal identifying factors will be used in any reports. Once the project is completed, you will be provided with a summary of all questionnaire responses, along with a copy of your own. We are very appreciative of your cooperation in studying this disease problem.

If you agree, we will enter the CSIP number of the lamb in a draw for a cash prize of $250 as a way of showing our appreciation for your cooperation. There will be one prize each awarded to a participant from the “case” group and the “control” group. Odds of winning will be better than 1 in 200. All questions can be answered on the telephone. The survey should take only 15 minutes to complete. You are not obligated to answer any question you don’t feel comfortable with; however, we encourage you to answer all questions openly and honestly. You are also welcome to withdraw from the survey at any time without consequences. Do you have any questions before we begin

Questions
1. Please describe the general location of your farm (e.g. south-west Ontario) using the map in Figure 1 at the back of this questionnaire _____
2. Please indicate the type of land cover type where your sheep-raising operation is located: (☑ most appropriate) ☐ Forested ☐ Agricultural cropland/rangeland
3. Please indicate how large your flock was on average over the last year: a) # of breeding ewes in flock: _____ b) # of lambs marketed / year: _____
4. Have you brought new sheep or lambs onto your farm (including rams)? (☑ Yes or No) ☐ Yes ☐ No If yes, where did these animals come from? Please ☑ all that apply: ☐ From within your province ☐ From outside the province (indicate which province) _____ ☐ Source unknown (e.g. purchased from a middleman or sales barn) ☐ From another country, (specify)_____
5. Are your market lambs? (☑ most appropriate answer) ☐ Raised primarily on pasture ☐ Raised entirely in confinement (e.g. barn and / or dry-lot) ☐ Raised both on pasture and in confinement

6. Please indicate how your market lambs are sold? (☑ most appropriate answer). If more than one way, please rank most to least common method; 1 = most common

☐ ____ Direct to slaughter

☐ ____ Freezer trade

☐ ____ Direct sales to a feedlot

☐ ____ To a middle-man (e.g. salesbarn or broker)

☐ ____ Other _____

7. Do you have domestic dogs on your farm property? (☑ Yes or No)

☐ Yes ☐ No, I do not own any dogs on my farm and no dogs visit the farm.

If No, please skip to Question # 12

8. If you do have domestic dogs on your property, please ☑ all that apply:

☐ I have dogs but they <u>never</u> have contact with the sheep (i.e. never in barn or pasture)

☐ I have guard dogs and/or guard puppies that live with the sheep

If ☑, # of dogs _____ and breed(s) _____

☐ I have herding &/or companion dogs and/or puppies that have contact with the sheep

If ☑, # of dogs _____ and breed(s) _____

☐ Stray dogs (e.g. neighbour's dog) do wander onto farm property.

If ☑, does this occurs at least once/month?

☐ Yes ☐ No, less frequently than once/month.

9. Have you brought any dogs &/or puppies onto your farm that came from another sheep farm? (☑ Yes or No)

☐ Yes ☐ No

If yes, where did this dog / these dogs come from? Please ☑ all that apply:

☐ Within the province

☐ From outside the province (indicate which province) _____

☐ From middleman (source unknown)

☐ From another country (specify)_____

10. Have your dog(s) been de-wormed? (☑ one)

☐ Yes ☐ No

If yes, do you know what product(s) was used? _____

If yes, do you know if the product(s) was effective against tapeworms? (☑ one)

☐ Yes ☐ No ☐ Don't know

If yes, where did you obtain the deworming product(s)?

☐ Local veterinarian ☐ Farm supply store, pet supply store or grocery store

☐ Internet order ☐ Other (specify) _____

11. Do you feed sheep carcasses (butchered or on-farm deads) to dogs on your farm? (☑ Yes or No)

☐ Yes ☐ No

If yes: Daily Weekly Monthly Less Frequently (circle most correct response).

If yes: Do you always cook the carcasses to well-done prior to feeding?

☐ Yes ☐ No

If yes: Do you always freeze the carcasses for a period of time prior to feeding?

☐ Yes ☐ No

If yes: How are the carcasses kept frozen? ☑ all that apply

☐ Chest freezer – whole carcass

☐ Chest freezer – cut-up or ground meat

☐ Freeze outside in winter

12. Describe your dead-stock management system? Please ☑ all that apply:

☐ Use dead-stock pick-up service.

☐ Bury carcasses in compost pile (not containing manure) used for dead-stock only.

☐ Bury carcasses in ground.

☐ Bury carcasses in manure.

☐ Use a disposal vessel, buried in the ground.

☐ Local landfill site.

☐ Other (please describe) _____

13. Have predators killed or maimed sheep on your property? ☑ one response.

☐ Yes ☐ No

If yes, please indicate how many separate attacks have occurred in the 12 month time-frame: _____

If yes, circle which predator or predators you believe were responsible for the attack(s).

Coyotes Wolves Foxes Domestic dogs (yours)

Bears Cougars Domestic dogs (stray)

Carrion birds (crows, magpies, vultures, eagles) Other :

14. Have your sheep carcasses been scavenged by:

Wild canids (coyotes, wolves, foxes)	☐ Yes	☐ No	☐ Not sure
Farm dogs (own, stray)	☐ Yes	☐ No	☐ Not sure

Comments:

15. Which of the following practices do you use to reduce sheep losses to predators? (Check all that apply)

☐ Guard dogs

☐ Guard llamas or alpacas

☐ Guard donkeys or horses

☐ Yard or bring into barn at night

☐ Keep flock in total confinement

☐ Electric fence

☐ Trap or hunt coyotes / wolves

☐ Noise deterrent

☐ Other: _____

☐ Nothing

16. As far as you are aware, are there any farms with sheep or goats within a 10 kilometre radius (as the crow flies) of where your sheep are kept?

(☑ Yes or No)

☐ Yes ☐ No

If yes, please circle the distance to the closest farm (as the crow flies):

Adjacent property < 1 km 1 to 2 km 2 to 5 km > 5 km

17. Have you instituted any changes to your management to reduce the risk of *C. ovis* infection?

☐ Yes ☐ No

If yes, please check all that apply:

☐ Changed handling of dead-stock so cannot be scavenged by canids.

If yes, please indicate how you now handle dead-stock:

_____

☐ Routinely de-worm dogs to kill tapeworms:

If yes, please indicate how dogs are now de-wormed:

_____

☐ Changed how dogs are fed sheep or lamb carcasses.

If yes, please indicate how dogs are fed sheep or lamb carcasses:

_____

☐ Other

_____

Figure 1. Regions of Canada