

**IMPROVED POSTMORTEM DIAGNOSIS OF
TAENIA SAGINATA CYSTICERCOSIS**

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ABSTRACT

Bovine cysticercosis is a zoonotic disease for which cattle are the intermediate hosts of the human tapeworm *Taenia saginata*. Routine inspection measures are implemented in Canada by the Canadian Food Inspection Agency (CFIA), and similarly elsewhere, for the postmortem detection of larval parasite cysts (cysticerci) in beef destined for human consumption. Detection is based on the gross examination of traditional carcass predilection sites, although it is recognized that the parasite has no true predilection for a particular tissue or site. In order to evaluate the efficacy of the inspection protocol currently implemented in Canada, a study was undertaken to determine the distribution of *T. saginata* cysticerci in tissues of experimentally infected cattle. Forty-two cross-bred beef cattle were divided into five groups of 5-12 animals each and inoculated orally with either 10000, 5000, 1000, 100 or 10 *T. saginata* eggs obtained from cases of human taeniosis in Thailand. From 47 to 376 days post-inoculation (DPI), ten animals inoculated with 5000 eggs were killed and the carcasses partitioned into 31 tissue sites. These consisted of the traditionally inspected tissue sites of heart, masseter and pterygoid muscles, tongue, oesophagus, and diaphragm (membranous and crura); as well as non-traditional sites of lung, liver and 20 additional muscles or muscle groups. After the routine inspection for cysticerci of traditional tissue sites, tissues from all sites were each cut into approximately 0.5 cm thick slices and the total number of parasitic cysts and cyst density (cysts/g of tissue) were determined for each site. Traditional sites were similarly evaluated for the remaining 32 animals that were killed between 117 and 466 DPI. Sites were ranked based on cyst density. In the animals for which non-traditional sites were also evaluated, no sites had higher cyst densities than those traditionally inspected. When only traditional sites for all animals were compared, the heart ranked highest overall, although not significantly different from masseter, and was the most frequently affected site. The traditional site of oesophagus was among the poorest of all sites for detection of cysticerci. The heart was confirmed as the site of choice for detection of bovine cysticercosis based on high cyst density and frequency of infection. There was also enhanced visibility of parasite lesions in the heart due to the relatively early degeneration and resultant gross pathology that occurs in cardiac muscle. More thorough examination of the heart is recommended during post-mortem inspection for this parasite, particularly when examining animals from an infected herd.

Currently, confirmation by CFIA of suspect cysticerci recovered during meat inspection relies on gross, stereomicroscopic, or standard histological examination. Although degenerating cysticerci are more likely to be detected and submitted for diagnosis, they often cannot be definitively identified by these methods. A recently developed monoclonal antibody-based immunohistochemical (IHC) assay for post-mortem diagnosis of this parasite was optimized and standardized. The IHC method was compared to the currently used histological assay using 169 degenerated known-positive *T. saginata* cysticerci collected from the experimental infections in the first study and from field submissions, and known-negative specimens and lesions of various etiologies from non-infected cattle. The use of the IHC assay identified significantly more known-positive bovine cysticerci (91.7%) than the histological method (38.5%), and non-specifically reacted only with the other cestode species examined. Since *T. saginata* is the only larval cestode typically found in the muscle of cattle, this cross-reactivity is not significant and the IHC assay will be a useful tool for the identification of lesions caused by degenerated bovine cysticerci.

This research provided evidence to support changes to the current post-mortem inspection, detection and diagnostic procedures and will contribute to more effective and efficient control of bovine cysticercosis.

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LIST OF ABBREVIATIONS

ABC	avidin-biotin complex
AB-ELISA	antibody enzyme-linked immunosorbent assay
AG-ELISA	antigen enzyme-linked immunosorbent assay
DNA	deoxyribonucleic acid
DPI	days post-inoculation
HPS	hematoxylin-phloxine-safran
IHC	immunohistochemistry
Mab	monoclonal antibody
PBS	phosphate-buffered saline
PCR	polymerase chain reaction

1. INTRODUCTION

The intermediate stage (syn *Cysticercus bovis*) of the human tapeworm *Taenia saginata* is found primarily in cattle and has both aesthetic and food safety implications to consumers of beef worldwide. Thus, it is a disease notifiable to the World Organisation for Animal Health (OIE; former List B disease), an organization that provides health standards for the control of bovine cysticercosis, and is a reportable zoonotic disease in Canada (Government of Canada, Health of Animals Regulations, 1991). Although specific federal regulations exist for its control, diagnostic challenges occur at every stage of the control cycle, from recognition and confirmation of the parasite at slaughter to identification of the source of infection of the affected cattle (McAninch, 1974; Bundza et al., 1988; Borman-Eby et al., 1994, Lees et al., 2002). Confirmed or suspected (where another etiology cannot be demonstrated) cases of cysticercosis result in substantial financial costs both to affected cattle producers and to the Canadian Food Inspection Agency (CFIA), the agency of the federal government that is responsible for implementing regulatory measures including trace-back, quarantine, enhanced screening of suspect animals at slaughter, and compensation for condemned carcasses (CFIA Disease Control Manual of Procedures, Section 13, 2000). Although the apparent prevalence of bovine cysticercosis in Canada is amongst the world's lowest (Pawlowski and Murrel, 2001; Cabaret, 2002; OIE, 2004c), globalization, travel from endemic regions, and recycling of human sewage on livestock pasture (MacPherson, 1978) increase the risk of infection to Canadian cattle. Several Canadian outbreaks have been reported (McAninch, 1974; Bundza et al, 1988; Borman-Eby et al., 1994), and in recent years, there have been cases in Ontario, Quebec, and Alberta (Lees et al., 2002). The Alberta outbreak in a relatively small (3000 head) feedlot cost the federal government ca. \$1 million Cdn. in compensation alone (Tom Steele, personal communication). Outbreaks in single feedlots in the USA have resulted in bankruptcy for producers (Yoder et al., 1994).

Control of this parasite currently relies on visual detection of suspect parasites or lesions in infected animals at slaughter and laboratory confirmation of the etiological agent. Detection of

infected animals is by the routine inspection of various traditional or “predilection” sites in the carcass, and laboratory diagnosis is based on gross, stereomicroscopic, or histological examination of submitted specimens. The routine inspection procedure for screening of carcasses is well recognised to have low sensitivity of detection of animals with light infections (Saini et al, 1997); even when suspect lesions are detected, they are often so degenerated that definitive diagnosis by current methods is not possible. The objectives of this research, conducted at the CFIA’s Centre for Food-borne and Animal Parasitology (CFAP), were to improve and validate methods for the postmortem detection and diagnosis of bovine cysticercosis. One study entailed postmortem examination of various carcass sites of cattle experimentally infected with *Taenia saginata* to examine the efficacy of the current inspection procedure used in Canada, and to investigate the merit of examination of other, non-traditional, sites for parasite detection. A second study, using degenerated parasite material collected from chronic experimental infections, was conducted to examine the efficacy of a recently developed immunohistochemical assay (Ogunremi et al., 2004a) for identification of bovine cysticerci.

2. LITERATURE REVIEW

The zoonotic cestodes *Taenia saginata*, *Taenia saginata asiatica*, and *Taenia solium* infect humans as the normal definitive host and domestic cattle or swine as intermediate hosts. Human infection with the adult tapeworm is referred to as taeniosis, while infection of intermediate hosts with the larval stage, or cysticercus, is referred to as cysticercosis. Human infection with the adult tapeworm of these species, while unpleasant, is generally asymptomatic or manifests as mild non-specific gastrointestinal illness, and, once diagnosed, is easily treated with anthelmintics. Cysticercosis in naturally infected cattle does not generally cause clinical disease, although swine may occasionally manifest clinical signs. Humans also serve as intermediate hosts for *T. solium*, which can result in severe, sometimes fatal, neurological disease (neurocysticercosis). Neurocysticercosis is an emerging global disease with an estimated 50 million people affected, with 50,000 deaths annually (Aubry et al., 1995). Due to the public health implications of taeniosis and neurocysticercosis, and the negative aesthetic impact of infected meat, cysticercosis causes significant economic loss through condemnation of infected meat and offal, and trade restrictions for endemic regions. These diseases are therefore of international food safety and trade significance and fall under the mandate of the World Organisation for Animal Health (OIE), which provides disease control recommendations for cysticercosis in Chapter 2.10.1 of its *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE, 2004c). Although there are no published reports of porcine cysticercosis in Canada, bovine cysticercosis occurs sporadically and is a federally reportable disease necessitating immediate implementation of specific control measures.

2.1 Taxonomy and morphology of the zoonotic *Taenia*

Taenia tapeworms belong to the family Taeniidae, order Cyclophyllidea, class Cestoda. The scientific nomenclature for *Taenia* species is somewhat confusing, as within some species, the adult and larval stages have been assigned different scientific names. This is the result of an historical assumption that the larvae and adults, found in intermediate and definitive hosts,

respectively, were different species in different genera. Thus the larvae of *Taenia saginata* and *T. solium* were named *Cysticercus bovis* and *Cysticercus cellulosae*, respectively. It was not until the mid-nineteenth century that scientists such as Kutchenmeister and Leuckart demonstrated the link between larval and adult stages within species (Grove, 1990). However, the dual nomenclature persists to this day. The more recently described *T. saginata asiatica* has been correctly assigned one scientific name for all life stages. Infection of intermediate hosts with the larval stage of each of these species is most appropriately referred to as *Taenia saginata* cysticercosis or bovine cysticercosis, *T. solium* cysticercosis or porcine cysticercosis, or *T. saginata asiatica* cysticercosis.

The adult tapeworm can measure up to several metres in length and consists of an anterior scolex (≤ 2 mm in diameter) and a dorsoventrally flattened chain of progressively maturing hermaphroditic reproductive segments, or proglottids, which comprises the strobila. The scolex has four muscular suckers and, depending on species, may have an apical rostellum armed with two rows of hooks. The larval cysticercus is oval in shape, normally achieving a maximum dimension of approximately 2-10 mm when fully developed, depending on species. It consists of a fluid-filled bladder with an invaginated scolex-anlage, contained within a connective tissue membrane or capsule of host origin at the interface with the host tissue matrix. The bladder is presumed to protect the developing cysticercus from the pressure of the surrounding host tissue (Slais, 1970). Morphological differences in the scolex and proglottid anatomy can be used to distinguish tapeworm species, and host species and scolex morphology are used to identify cysticerci (Proctor, 1972; Soulsby, 1982). The thick walled eggs released from mature, or “gravid” proglottids are approximately 30-45 μm in size, and morphologically indistinguishable among species and genera within the Taeniidae.

2.1.1 *Taenia solium* (syn *Cysticercus cellulosae*)

Adult *Taenia solium* reach 3-5 m in length. The scolex has an armed rostellum with two rows of hooks, followed by a strobila consisting of up to 1000 proglottids each with 7-16 uterine branches and measuring up to 10 mm in breadth at maturity (Pawlowski and Murrel, 2001; Flisser et al., 2004; OIE, 2004c). The oval cysticerci can be the largest of the three zoonotic *Taenia* spp., reaching approximate dimensions of $\geq 0.5 - 1 \times 0.5$ cm and have a scolex bearing a rostellum armed with hooks similar to that of the adult tapeworm (Pawlowski and Murrel, 2001;

OIE, 2004c).

2.1.2 *Taenia saginata* (syn *Cysticercus bovis*)

Adult *Taenia saginata* reach 4-8 m in length. The scolex has no rostellum or hooks, and the strobila consists of approximately 2000 proglottids each with 14-32 uterine branches and measuring up to 14 mm in breadth at maturity (Pawłowski and Murrel, 2001; Flisser et al., 2004; OIE, 2004c). Cysticerci are oval, approximately 0.5 -1 x 0.5 cm in dimension and have a scolex with no rostellum or hooks (Pawłowski and Murrel, 2001; OIE, 2004c).

2.1.3 *Taenia saginata asiatica*

Taenia saginata asiatica is closely related to, but genetically and morphologically distinct from, *T. saginata* (Zarlenga et al., 1991). The adult tapeworm has a scolex with a hookless sunken rostellum, and proglottids with posterior protuberances and 11-32 uterine branches. The cysticercus is relatively small (approximately 2 mm) with a rostellum usually bearing two rows of rudimentary hooklets (Fan et al., 1995; Flisser et al., 2004; OIE, 2004c).

2.2 Life Cycle of the zoonotic *Taenia*

These tapeworms have an indirect life cycle and are relatively host specific for both larval and adult stages (Fig. 2.1). Humans are the only natural definitive hosts of the adult tapeworm. The adult tapeworm is fully developed and reproductively mature as early as 10-12 weeks (depending on species) after infection of the host (Lloyd, 1998a). Once mature, the tapeworm regularly sheds its most posterior segments, called gravid proglottids, which are discharged from infected humans spontaneously or with defecation. These proglottids contain thousands of immediately infective eggs that can remain in the proglottid or be expelled free into the surrounding fecal matrix or environment (Kyvsgaard et al., 1988). On average, a single *T. saginata* tapeworm releases six to nine proglottids daily (Pawłowski and Murrel, 2001). Although multiple and mixed species infections can occur, most taeniosis infections involve a single tapeworm. Upon ingestion by a suitable intermediate host, a hexacanth embryo, or oncosphere, hatches from the egg and uses its six hooklets to penetrate the intestinal mucosal within a few hours to enter the circulatory or lymphatic system. It eventually reaches the tissue site (such as the lymphatic space in skeletal muscle) where it eventually develops into a

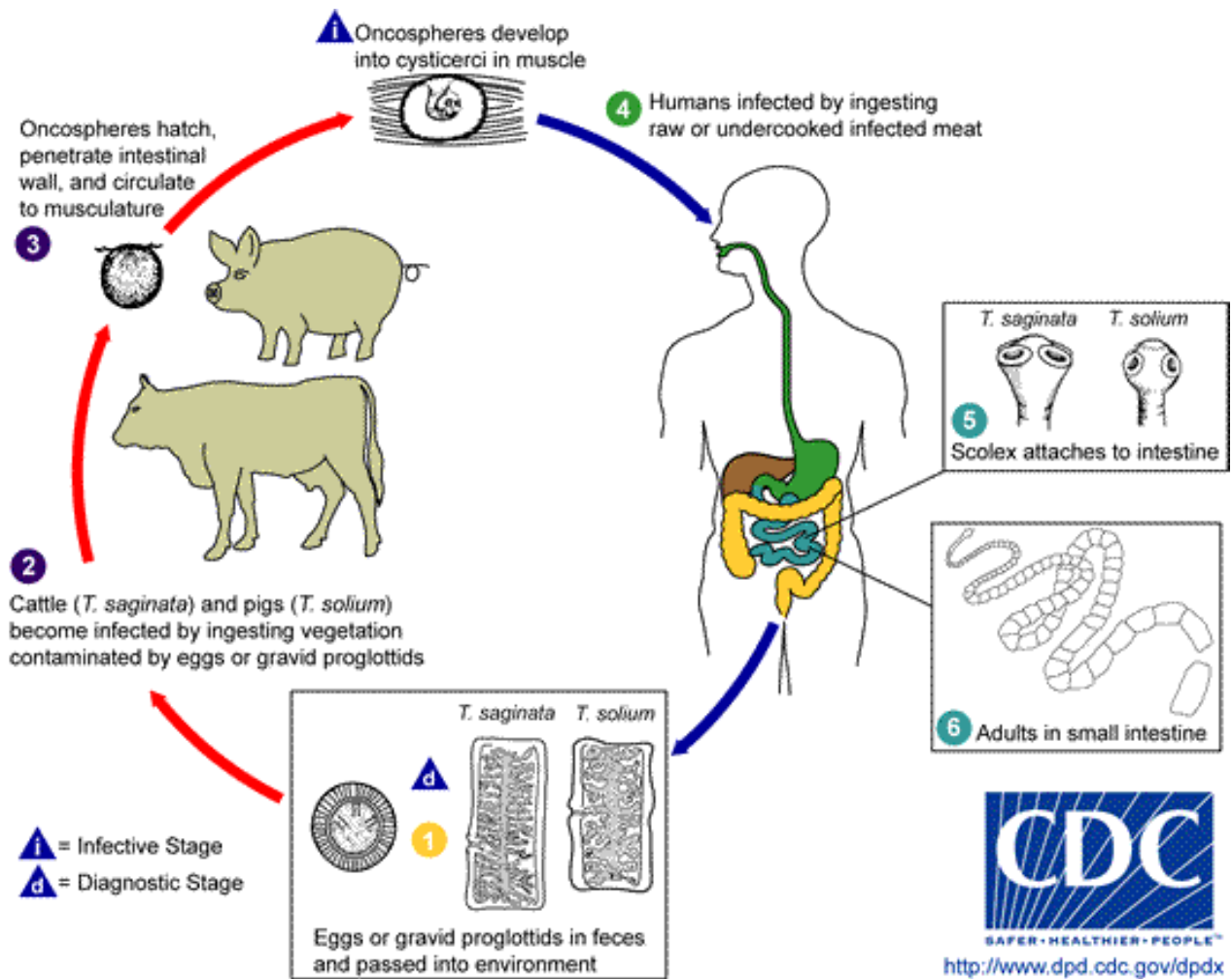


Figure 2.1. Life cycle of *Taenia saginata* and *T. solium*.

cysticercus which is infective to a human final host after about 10-12 weeks (McIntosh and Miller, 1960); or as early as 4 weeks for *T. saginata asiatica* (Fan et al., 1990). The intermediate hosts for *T. saginata* and *T. saginata asiatica* cysticerci are domestic cattle and swine, respectively. Reindeer (*Rangifer tarandus tarandus*) have also proven suitable intermediate hosts for *Cysticercus bovis* (Kirichek, 1985). In cattle, cysticerci are found predominantly in cardiac and skeletal musculature, and occasionally in other sites including liver, lung, kidneys and lymph nodes (Ginsberg and Grieve, 1959; Mitchell, 1973; Schillhorn van Veen, 1979). There is evidence that pre-natal infection of calves can occur (McManus, 1960; 1963). *Taenia saginata asiatica* cysticerci localize primarily on the serosal surface and within the parenchyma of the liver of pigs (Fan, 1988; Fan et al., 1989; Fan et al., 1992), and occasionally in extrahepatic peritoneal sites (Chung et al., 1996). Experimental infections have also been reported for cattle, goats and monkeys (Lloyd, 1998a). The normal intermediate host of *T. solium* is domestic swine, although a variety of other species, including humans and dogs, can serve as intermediate hosts (Lloyd, 1998a). The cysticerci localize in the tissues of the tongue, skeletal muscle (particularly thighs), subcutis, and central nervous system of pigs. Human consumption of infected pork or beef completes the cycle.

2.3 Disease and Occurrence

Human taeniosis, while unpleasant, is generally asymptomatic or manifests as mild non-specific gastrointestinal illness, including symptoms of pruritis ani, nausea, weight loss, abdominal pain, diarrhea, and anorexia (Thornton, 1979). More serious complications such as appendicitis have been reported (Pawlowski and Schultz, 1972). Cysticercosis in cattle does not typically cause detectable disease, but swine with heavy infections can be clinically affected (Lloyd, 1998a). Human cysticercosis with *T. solium* can result in severe, sometimes fatal neurological disease (neurocysticercosis), and is the most common cause of acquired epilepsy (Aubry et al., 1995; White, 2000).

Accurate global prevalence data are not available for these *Taenia* species. Both *T. saginata* and *T. solium* occur worldwide, with highest prevalence in developing regions where poor sanitary, animal husbandry and cultural practices facilitate parasite transmission between humans and domestic cattle and swine.

T. solium is endemic in Central and northern South America, west and southern Africa,

Southeast Asia, and to a lesser extent in parts of south and Eastern Europe (Pawlowski and Murrel, 2001). Sporadic cases of *T. solium* taeniosis and neurocysticercosis, primarily “imported” from endemic regions have been reported elsewhere, and endemic foci from immigration have been established in the USA., with approximately 1000 cases reported annually (Schantz et al., 1992; White, 2000; Sorvillo et al., 2007). Neurocysticercosis is an emerging global disease with an estimated 50,000 deaths annually (Roman et al., 2000).

Taenia saginata is most common in western and eastern central Africa, the Caucasus region and south-central Asia; less so in other parts of Africa, the Mediterranean, southeast Asia, Central America and South America and central Europe. Sporadic cases of *T. saginata* taeniosis and epizootic outbreaks of bovine cysticercosis occur in the US, Canada, and elsewhere (Pawlowski and Murrel, 2001).

Distribution of *T. saginata asiatica* is believed to be limited primarily to southeast Asia (China, Taiwan, Phillipines, Vietnam, Indonesia) and Korea (Ito et al., 2003). Prevalence estimates for this species must be interpreted with caution, as until recently, *T. saginata* and *T. saginata asiatica* were not distinguishable (Bowles and McManus, 1994).

2.4 Source and Transmission

A person infected with a single *T. saginata* tapeworm is capable of contaminating the environment with up to half a million eggs per day over the course of the infection, which, if left untreated, can persist for years (Pawlowski and Murrel, 2001). Since *T. saginata* proglottids have a tendency to spontaneously exit the anus independent of defecation, even when good hygienic facilities are available, inadvertent environmental contamination can occur.

Eggs contaminating the environment via defecation or spontaneous discharge of proglottids can be disseminated by water, wind, scavenging birds such as gulls feeding on raw sewage, oribatid mites, flies, earthworms, or fomites such as boots or farm machinery (Lawson and Gemmell, 1985; Kyvsgaard et al., 1988; Pawlowski and Murrel, 2001).

Infective *Taenia* eggs can persist under a variety of environmental conditions; as with most parasite environmental stages, cool and moist conditions favour long-term survival. *Taenia saginata* eggs in Europe have been demonstrated to remain infective after overwintering on pasture (Ilsoe et al., 1990), and in river water after a month (Pawlowski and Murrel, 2001). They can also survive in sewage and in sludge for up to several months, and are resistant to most

conventional chemical and disinfecting agents (Pawlowski and Murrel, 2001).

Transmission to livestock occurs via food or waterborne ingestion of infective eggs originating from human feces. A major source of infection to pigs is scavenged human feces containing *T. solium* gravid proglottids. This is facilitated by the common practice in many endemic rural regions of allowing pigs to roam free in areas without latrines, to feed on any available food that may or may not be supplemented by limited rations provided by the farmer. Such a concentrated source of eggs increases the risk of heavy infection, compared to those acquired via contaminated feed or water. Since cattle will not intentionally consume human feces, bovine cysticercosis occurs via inadvertent ingestion of directly or indirectly contaminated feedstuffs or water, including pasture fertilized with human sewage, which more likely results in lighter infections in infected animals.

2.5 Diagnosis and Control

Although cattle with cysticercosis are unlikely to exhibit clinical signs, pigs with heavy infections may be presumptively diagnosed antemortem by observation and/or palpation of cysts in the tongue (Gonzalez et al., 1990). In most cases, however, detection is made during postmortem carcass examination in both cattle and pigs. In most parts of the world where regulated postmortem screening for these parasites occurs, examination of so-called “predilection sites” is conducted during routine meat inspection. However, such procedures are insensitive, particularly for lightly infected carcasses (Saini et al., 1997). Viable cysticerci can be easily missed on meat inspection since the translucent cysts blend with the surrounding host tissue. Only upon death and degeneration of the parasite is there a sufficient host inflammatory response to create a more detectable lesion. Moreover, cysticercosis infections can consist of both viable and degenerate cysticerci (Juranek et al., 1976); thus detection of only degenerate cysts does not imply the absence of infective cysts elsewhere in the carcass. Depending on the extent of degeneration, the end result of which is a mineralized or fibrotic lesion, definitive parasite features may not be evident on gross examination or histology. The recent development of an immunohistochemical assay for parasite excretory-secretory antigen in degenerate *C. bovis* lesions will help in this regard (Ogunremi et al., 2004a). Molecular methods for characterizing adult *Taenia* (Gonzalez et al., 2000) have been applied to cysticerci, but require further validation before use in regulatory diagnosis can be recommended (Harrison et al., 2005). Once

identified as a cysticercus, host species and tissue location of the cysticercus can suggest a more specific etiology.

Immunity to taeniids is predominately antibody mediated (Ferrer et al., 2003). However, serological assays for bovine and porcine cysticercosis have not met with the same success as similar assays for human *T. solium* cysticercosis. Although commercially available enzyme-linked immunoassay (ELISA) and enzyme-linked immunotransfer blot assay (EITB) have high sensitivity and specificity when applied to human serum or CSF samples for *T. solium* cysticercosis, reliability has been low for naturally infected animals (Sloan et al., 1995; Sciutto et al., 1998). Similarly, in spite of ongoing research on the development of serological assays for bovine cysticercosis, using homologous or heterologous (eg. *T. solium*, *T. hydatigena*, *T. crassiceps*) antigens, or synthetic peptides (Ferrer et al., 2003) to detect circulating parasite antigen (AG-ELISA) or antibody (AB-ELISA), inadequate sensitivity and/or specificity has been reported (Geerts et al., 1981; Harrison and Sewell, 1981; Harrison et al., 1989; Hayunga et al., 1991; Smith et al., 1991; Bogh et al., 1996; Lloyd, 1998a; Van Kerckhoven et al., 1998; Dorny et al., 2000; Monteiro et al., 2006). Thus, there is no serological assay available commercially for use in animals (Lloyd, 1998b; OIE, 2004c). Currently, such an assay may have value as an epidemiological tool for screening herds for cysticercosis but would not be applicable for individual animal diagnosis (Wanzala et al., 2002; Monteiro et al., 2006).

Reliable methods for recovering *Taenia* eggs from various environmental matrices, including livestock feed and water, are not available. In most sporadic outbreaks of bovine cysticercosis in low prevalence regions such as North America, a definitive source is not identified. Even if a particular feed or water source is suspected, processing of relatively large volumes contaminated at low levels is problematic. Modified flotation methods have been attempted in such cases, but the high specific gravity of *Taenia* eggs, and confounding artefacts and adherent debris in the assayed matrix negatively impact sensitivity (Scandrett and Gajadhar, 2004). As well, since there is usually a minimum of several months after presumed exposure before the first index animal is detected at slaughter, the contaminated source may no longer remain, or if so, may have undergone degeneration, further confounding recovery of the agent and interpretation of results. Since taeniid eggs cannot be speciated based on morphology, and there is no baseline data available for levels of environmental contamination with other domestic and wildlife taeniid species, any positive findings must be interpreted with caution. Reliable

molecular methods for detecting low numbers of *Taenia* eggs are still being developed (Gonzalez et al., 2000; Nunes et al., 2005).

Globalization poses an increasing threat of incursions of cysticercosis and taeniosis via the increased international movement of people and importation of animals, their products, and potentially contaminated produce or other fomites from endemic regions. In theory, these infections can be eradicated (Flisser et al., 2003). Human taeniosis is the only source of cysticercosis and is easily and inexpensively treated with anthelmintics (such as niclosamide or praziquantel), pigs and cattle are the only significant reservoir of cysticercosis, and simple cooking or freezing measures render cysticerci non-infective. Most eradication efforts have been aimed at *T. solium* due to the severe consequences of human neurocysticercosis. Bovine cysticercosis, in addition to having a lesser public health concern, is less amenable to eradication due to the greater biological potential of the *T. saginata* tapeworm, greater difficulty in detecting animals that are often lightly infected, and a global propensity to consume raw or semi-cooked beef (Pawlowski and Murrel, 2001).

In spite of its low sensitivity, regulated postmortem inspection of cattle and pig carcasses at slaughter for cysticercosis helps to reduce transmission of these parasites. Affected carcasses are condemned, or treated by cooking or freezing to kill the parasite (Hird and Pullen, 1979; Saini et al., 1997). Disease control regulations often dictate that epidemiological investigation and quarantine of the suspect herd be conducted, with significant economic impact on both regulators and affected producers. Anthelmintic treatment of livestock is effective but does not reliably eliminate cysticerci, and is often not practical, particularly for cattle (Gallie and Sewell, 1983; Harrison et al., 1984; Gonzalez et al., 1998; Gonzalez et al., 2001). Vaccination of animals at risk holds more promise. Immunization of cattle with preparations of crude parasite antigen reduced infection in animals exposed to sewage on pasture (Rickard et al., 1981). More recently, recombinant subunit vaccines based on oncosphere antigens have proven highly effective in protecting cattle and pigs from experimental challenge with *T. saginata* and *T. solium* eggs, respectively (Lightowers et al., 1996; Gonzalez et al., 2005). A synthetic peptide vaccine against *T. solium* cysticercosis significantly reduced prevalence and intensity of infection in pigs exposed to natural challenge (Huerta et al., 2002).

Since *Taenia* eggs are inherently resistant to many environmental conditions and most practical and conventional chemical treatments, efforts should be aimed at preventing

environmental contamination with eggs. This will entail reducing the overall prevalence of human taeniasis, and preventing exposure of livestock to human feces and sewage. If sewage must be used as fertilizer, measures such as delayed grazing of cattle on treated pastures can be used to reduce the number of viable eggs in the applied sludge (Cabaret et al., 2002).

As humans are ultimately responsible for maintaining the parasite cycle, more prevalence data on human taeniosis is needed. This requires increased surveillance and mandatory reporting by public health agencies. Control programs for *T. solium* have included mass taeniocidal treatment (Allan et al., 1997). Education on the parasite cycle and on mitigating measures such as proper hygiene and latrine use, preventing access of livestock to human feces, and thorough cooking of meat, is an important aid in reducing overall parasite transmission.

2.5.1 Detection of *Taenia saginata* cysticerci by postmortem carcass inspection

Control measures currently instituted for bovine cysticercosis by the CFIA rely on detection of cysts in affected carcasses during routine gross (organoleptic) postmortem inspection procedures. Cysts can be viewed grossly as early as 11 days post-infection, at which time they are about 2.5 mm in diameter (McIntosh and Miller, 1960). The inspection protocol involves incision and/or palpation of the tongue, internal and external masseters, esophagus, heart, and diaphragm, and observation of superficial and cut surfaces of the carcass exposed during routine dressing procedures (CFIA Meat Hygiene Manual of Procedures, Section 4.6.1, 2007). This is similar to inspection protocols implemented for this parasite in the USA (Snyder and Murrell, 1986; Saini et al., 1997) and Europe (Kyvsgaard et al., 1990). These “traditional” inspection sites are easily accessed during routine slaughter, result in minimal carcass damage, and are presumed to be “predilection” sites for the parasite. However, whether this parasite truly has a predilection for particular sites is controversial, and numerous studies have yielded inconsistent results (Mango and Mango, 1972; Juranek et al., 1976; Hammerberg et al., 1978; Sewell and Harrison, 1978a, b; 1978b; Pugh and Chambers, 1989; Oryan et al., 1995; Maeda et al., 1996). It has been proposed that a variety of factors, such as muscle activity, breed, age, and geographic area may affect localisation of cysts (Kearney, 1970). The heart is widely regarded to be an apparent predilection site for cysticerci; paradoxically, cysts in cardiac muscle degenerate earlier, and the resulting lesions may persist longer, than in other skeletal muscle sites (Soulsby, 1963; Gallie and Sewell, 1983; Harrison et al., 1984; Smith et al., 1991; Lloyd, 1998a). Although

viable mature cysts elicit minimal host reaction (Silverman and Hulland, 1961), degenerating *T. saginata* cysticerci incite a host inflammatory response (Sterba et al., 1979a) that makes them more obvious grossly than viable cysts. As well, since the heart is traditionally one of the more thoroughly examined inspection sites (CFIA Meat Hygiene Manual of Procedures, Section 4.6.1, 2007), degenerated cardiac lesions are among those most frequently detected by meat inspectors. Since cattle can harbor both viable (infective) and degenerate cysts concurrently, recovery of only degenerate cysts does not imply that no infective cysts remain in the carcass, or in herd mates. Complete resorption of degenerated cysts may take 3 years or longer (Penfold and Penfold, 1937) and viable cysts may persist for at least 2 to 3 years, and possibly for the life of the host (Penfold, 1937; Dewhirst et al., 1963; Froyd et al., 1964; Urquhart and Brocklesby, 1965; Van den Heever, 1967). Therefore, it is important to confirm cysticercosis even in cases where suspect lesions are obviously degenerated and non-infective.

It has been demonstrated that the current inspection protocol has a sensitivity of $\leq 50\%$ for detection of lightly-infected carcasses (Dewhirst et al., 1967; McCool, 1979; Walther, 1980), which is the degree of infection often associated with outbreaks in Canada. In addition to possible improvements to the inspection protocol by identifying more reliable target tissues in the carcass, provision to inspection staff of standardized training and reference material could improve the sensitivity of detection of this agent.

2.5.2 Confirmatory assays for *Taenia saginata* cysticerci

2.5.2.1 Gross and stereomicroscopic examination

A definitive diagnosis can be easily made by an experienced parasitologist, either by gross examination or by using a stereoscopic microscope, if the cysticerci are viable, or if an intact or partial metacestode can be seen in a recently degenerated cyst. A diagnosis of cysticercosis in cattle tissue samples implies that the organism is *T. saginata*, based on intermediate host specificity of this parasite. However, degeneration of cysticerci can begin within 20 days of infection (Soulsby, 1963) and differentiation of the resultant lesion from chronic inflammatory lesions of various other etiologies can be impossible to confirm by gross, stereomicroscopic, or even histological examination (Schandevyl and Vercruysse, 1982).

2.5.2.2 Histology

Histological methods are unreliable for differentiation of degenerated *T. saginata* cysticerci from chronic inflammatory lesions of various other etiologies (Schandevyl and Vercruysse, 1982). No standard criteria have been established for the histological evaluation of *C. bovis*-suspect lesions. Specimens are formalin-fixed, sectioned and stained, usually with hematoxylin and eosin. Other methods, including Gomori's technique for reticular fibers, have been advocated to demonstrate particular parasite features in lesions in advanced stages of degeneration (Slais, 1970); however, Geerts et al. (1980) reported that this stain was no better than hematoxylin and eosin in the identification of 32 degenerate cysticerci recovered from 25 bovine hearts. The application of criteria such as de-mineralization, staining technique, and number and region of sections viewed (Pampiglione et al., 1999) depend on the histopathologist conducting the assay (Yves Robinson, personal communication; Mary Sutton, personal communication). Several authors have described (and in some cases developed grading criteria for) the histological characteristics of viable and degenerating bovine and porcine cysticerci using a variety of staining techniques (Silverman and Hulland, 1961; Slais, 1970; Retzlaff, 1972; Sterba and Dykova, 1978; Sterba et al., 1979a, b; Safranov and Drogun, 1985; Aluga de and Vargas, 1988; Zivkovic, 1996). Silverman and Hulland (1961) were the first to describe in detail histological observations on bovine cysticerci obtained from several hundred naturally or experimentally infected cattle. Cysts were stained with haematoxylin and either eosin or van Gieson. Viable cysts were associated with minimal host inflammatory response with few eosinophils, and no giant cells, whereas degenerate cysts were associated with the formation of granulation tissue in the cyst space, the presence of giant cells around parasite fragments, the breakdown of the cyst wall, and an invasion of granulocytes, including eosinophils, typical of chronic inflammation. Identification of some lesions was complicated by the onset of degeneration of the cysts at variable stages of development. In general, for a mature cyst, degeneration was initiated via breakdown of the cyst wall and cuticle, followed by disappearance of the subcuticular layers, followed by the eventual dissolution of cestode remnants such as the suckers and rostellum of the scolex (Silverman and Hulland, 1961). Since hooklets are not present in *T. saginata*, the parasite remnants most often identified in advanced stages of degeneration are calcareous corpuscles (Silverman and Hulland, 1961). Calcareous corpuscles, found in larval cestodes, and located mostly in the scolex-anlage and neck region of cysticerci

(Slais, 1970) are microscopic ellipsoid mineral concretions of parenchymal cell origin (Rodrigues et al., 1997). Although the morphology and cellularity of the lesion can be used to differentiate it from some other commonly-encountered and grossly similar lesions such as those due to *Actinobacillus* sp., bacteriaemic myositis, or schwannoma, demonstration of a cysticercus-specific host structure (i.e. cyst wall), and/or larval parasite material (scolex, suckers, bladder, body wall, calcareous corpuscles) is necessary for a definitive diagnosis (Silverman and Hulland, 1961; Geerts et al., 1980; Bundza et al., 1986; Bundza et al., 1988; Marty and Chester, 1997). Since such features are often not evident in degenerated specimens, especially if the sample contains calcified tissues, an accurate identification cannot be made (Retzlaff, 1972; Schandevyl and Vercruysse, 1982; Safranov and Drogun, 1985).

2.5.2.3 Immunohistochemistry

Immunohistochemical (IHC) detection of infectious agents relies on the use of agent-specific antibody associated with a microscopically visible label to detect antigen in tissue sections. Assays incorporating enzyme conjugates for visualization of staining of formalin-fixed tissues sections under standard light microscopy have advantages over fluorescein dye conjugates which are traditionally used on fresh or frozen tissue sections and require an ultraviolet light microscope (Haines and Chelack, 1991). Monoclonal antibodies to infectious agents are less likely than polyclonal antisera to produce non-specific (background) or specific staining of non-target antigens via contaminating antibodies or antibodies with cross-reacting specificities, respectively. However, monoclonal antibodies can bind similar epitopes on either related or unrelated molecules, and ascites preparations from hybridomas implanted in mice contain contaminating antibodies as well as those to the target antigen (Haines and Chelack, 1991). Non-specific staining is minimized by using the highest possible dilution of primary monoclonal antibody or polyclonal antiserum which yields distinct specific staining, pre-treatment of tissue sections with normal serum from the same species from which the secondary antibody is derived, and incorporation of detergent to antibody diluents and washing buffers (Haines and Chelack, 1991). Immunohistochemistry has been used to identify antigens associated with viral, bacterial, and protozoal microorganisms, parasites, autoimmune disease, and neoplasia (Rickard et al., 1977; Laclette et al., 1987; Haines and Clark, 1991; Cornford et al., 2001; Pozio et al., 2006).

Diagnostic IHC methods are generally classed as either direct or indirect. Direct immunoenzyme methods rely on the addition of an enzyme substrate to the tissue section, which results in a visible reaction product where the labelled primary antibody has bound to the target antigen. Indirect methods rely on the same enzymatic reaction; however, the interaction of the target antigen with the primary antibody is detected by the binding of enzyme-labelled secondary (anti-immunoglobulin) antibodies. Indirect methods have advantages in that the labelled secondary antibodies are widely available commercially (ie. anti-rabbit or anti-goat immunoglobulins) and bind multiply to each primary antigen-specific antibody, thus amplifying the visible reaction (Haines and Chelack, 1991). Indirect methods are therefore more sensitive at detecting antigens present in relatively low amounts. Further amplification is achieved using the avidin-biotin complex (ABC) immunohistochemical method (Hsu et al., 1981) which has proven to be a sensitive, specific, and convenient assay for the detection of various viral antigens (Haines et al., 1992; 1993), and is being used increasingly to detect protozoal and helminth parasite antigens (Cruikshank et al., 1990; Dubey and Hamir, 2000; Sripa and Kaewkes, 2000; Crookshanks et al., 2007). The ABC immunostain relies on the high affinity of the B vitamin biotin for the egg white glycoprotein avidin. Biotinylated secondary antibodies are detected by binding to free biotin-binding sites on applied pre-formed avidin/ biotin complexes, which consist of 4 biotin molecules, and one avidin molecule. The biotin molecules are labelled with an enzyme, usually peroxidase (Fig. 2.2), which reacts with the enzyme substrate (Haines and Clark, 1991). This method can be performed on formalin-fixed, paraffin-embedded tissues, and is thus a natural adjunct to the analysis of specimens, such as *T. saginata* cysticercus-suspect lesions, traditionally examined histologically. Although the ABC method applied to formalin-fixed tissues is somewhat more labour intensive than direct or indirect immunofluorescence-based staining of fresh or frozen tissues (Haines and Chelack, 1991), it has a number of advantages, such as stability of stain, ability to view sections with a light microscope, and ability to counterstain the tissues to facilitate concurrent recognition and identification of morphological structures (Haines et al., 1993; Sripa and Kaewkes, 2000; Haines and West, 2005; Taylor, 2006). Loss of sensitivity due to formalin fixation can be minimized by limiting fixation to 24-48 hrs, and by using heat- or proteolytic enzyme-induced antigen retrieval (Haines and Chelack, 1991; Werner et al., 2000; Taylor, 2006). Although the ABC method is more complex and time consuming than other methods, it is appropriate for diagnosis of bovine cysticercosis in Canada,

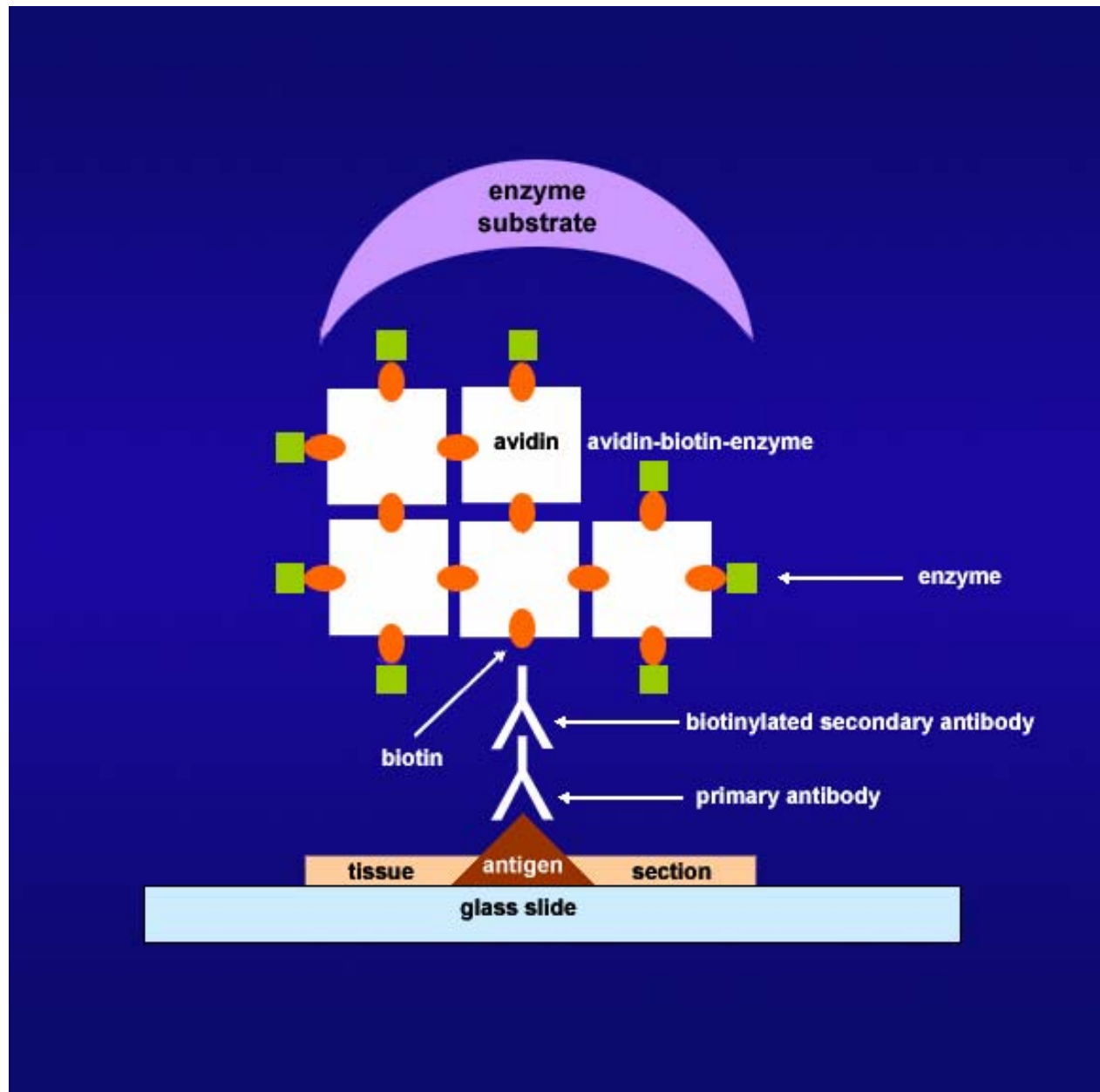


Figure 2.2. Schematic representation of avidin-biotin complex (ABC) immunohistochemical staining method (adapted from Haines and Chelack, 1991)

since the parasite is encountered relatively infrequently, only index cases require diagnostic confirmation, and suspect-specimens are often submitted already formalin-fixed (instead of fresh-chilled as preferred for initial gross examination).

An ABC immunostaining method, based on that of Haines and Chelack (1991), has been developed at the CFIA Centre for Food-borne and Animal Parasitology for formalin-fixed *T. saginata* cysticerci (Ogunremi et al., 2004a, b). This assay uses a monoclonal (Mab) IgG1 antibody (158C₁₁A₁₀) to an excretory/secretory (ES) tegmental protein of cultured *T. saginata* cysticerci (Brandt et al., 1992) developed by Draelants et al. (1995). Known-positive viable and degenerated cysticerci from experimentally infected cattle killed at 62-376 days post-inoculation provided the test material. Preliminary evaluation demonstrated this assay to be 100% sensitive in the identification of 87 viable and 115 degenerated cysts evaluated from a variety of tissue sites of the experimentally-infected cattle; 100% specificity was obtained on a total of 46 known-negative specimens, composed of *Sarcocystis* (n = 5), *Actinobacillus* (n = 5) and a variety of normal bovine tissues (hemal lymph nodes, lymph nodes, adipose tissue, skeletal and cardiac muscle) from the experimental infections and uninfected animals. In viable cysts, the cuticle, bladder parenchyma, and sucker and rostellar epithelium were consistently stained. In the most degenerate cysts, in which few or no recognizable parasite features were evident, the staining reaction was confined to an area in the centre of the lesion (Ogunremi et al., 2004a).

IgM monoclonal antibodies generated against bovine cysticercus ES antigen have demonstrated cross-reactivity with serum antigens of *T. solium*, *T. ovis*, and *Echinococcus granulosus* (Brandt et al., 1992). Because *T. ovis* is not found in cattle, and *T. solium* and *E. granulosus* only rarely so (Geerts et al., 1980), this cross-reactivity is not diagnostically relevant. Although the IgG1 Mab has not yet been evaluated in IHC tests for the identification of these, or other taeniids, it has been demonstrated to be 98.7% specific when used in a serological ELISA to test cattle infected with a variety of common helminths and hemoparasites (Van Kerckhoven et al., 1998).

2.5.2.4 Molecular methods

Diagnostic polymerase chain reaction (PCR) is a molecular method used to amplify and thus optimize detection of specific nucleotide sequences. It relies on the availability of appropriate target nucleic acid sequences that flank regions of interest (“primers”), the synthesis

of these oligonucleotide primers and a suitable DNA isolation (extraction) technique for test samples (Gottstein, 1994). Genomic libraries are being increasingly generated for more and more parasite species, facilitating development of species-specific DNA sequences for use as primers in diagnostic PCR assays (McManus, 1990; Gottstein, 1994; Singh, 1997).

Restriction fragment length polymorphism (RFLP) on the PCR-amplified internal transcribed spacer 1 (ITS1) and 2 (ITS2) regions of the 28S ribosomal DNA (rDNA), and mitochondrial cytochrome *c* oxidase 1 (CO1) genes, has been used to identify adult *T. saginata* and other taeniids (Bowles and McManus, 1993;1994; Gasser and Chilton, 1995). Bowles and McManus (1994) genetically characterized adult *Taenia saginata* from the closely related human taeniid *T. asiatica* (Asian *Taenia*), by first amplifying a CO1 gene fragment followed by restriction endonuclease *Msp* 1 digestion to differentiate *T. saginata* from *T. asiatica*. When incubated with *Msp*, *T. saginata* CO1 remained intact, while that of *T. asiatica* was cleaved. More recently, protocols utilising multiplex PCR and RFLP for the molecular differentiation of genomic (gDNA) and rDNA from adult *T. saginata*, *T. saginata asiatica*, *T. solium* and *Echinococcus granulosus* have been developed, and have been successfully applied to the identification of cysticerci of *T. saginata* and *T. solium* (Gonzalez et al., 2000; Gonzalez et al., 2002; Gonzalez et al., 2004). However, use by other researchers of the same primers (Abuseir et al., 2006), or of different PCR assays (Van der Logt and Gottstein, 2000; Geysen et al., 2007) has yielded unreliable results on both viable and degenerated bovine cysticerci from naturally infected cattle. Although most of the current methods have been applied to fresh or frozen samples, they could presumably be adapted for formalin-fixed tissues; however, fixation can result in further degradation of DNA (Mygind et al., 2001), and there are no diagnostic advantages to doing so, unlike those already stated for the *in situ* immunohistochemical assay. Use of both IHC and PCR assays for the diagnosis of a variety of other pathogens indicated that PCR was more sensitive than IHC (Brunnert et al., 1994; Bazler et al., 1999; Held et al., 2000; Tegtmeier et al., 2000). The suggestion that false negative results for degenerated *T.saginata* cysticerci may be due to insufficient DNA remaining in the specimen (Van der Logt and Gottstein, 2000; Geysen et al., 2007) invites a future comparison between the performance of molecular assays which target residual cysticercal DNA with the antigen-based IHC assay.

2.6 Test method validation

Valid test results are essential for reliable diagnosis, and are the goal of any quality assured testing system. Validation evaluates the fitness for an intended use of a test method and is performed on an optimized, standardized, and documented procedure (Dubey, 1997; Heckeroth and Tenter, 1999; Hoos et al., 2001). In general, the five stages of validation are 1) developing or choosing a method for a particular use; 2) optimizing and standardizing the method (including reagents); 3) determination of the method's performance characteristics, including sensitivity and specificity (accuracy), and repeatability and reproducibility (precision); 4) continued monitoring of assay performance; and 5) maintenance and enhancement of validation criteria during routine use of the method. Assays having acceptable accuracy (sensitivity and specificity), and precision (repeatability/reproducibility) for their intended use are considered reliable and are essential for confidence in diagnostic results used to ensure domestic food safety and disease control, and increasingly to meet international standards for export of animals and their products (ISO, 1999; Greiner and Gardner, 2000; Gajadhar and Forbes, 2002; OIE, 2004a, b).

2.7 Brief overview of thesis

Two integrated experiments were designed to evaluate the distribution and degree of infection of *Taenia saginata* cysticerci in the carcasses of experimentally infected calves and to validate an immunohistochemical assay for the identification of degenerated cysticerci in lesions detected on postmortem carcass examination.

In Chapter 3, the experimental infection of calves with *T. saginata*, collection of cysticerci, and distribution, density, and frequency with which various tissue sites (both those examined for bovine cysticerci during regulated meat inspection in Canada and considered to be predilection sites for the parasite, and those not traditionally inspected) were infected with cysticerci are described and discussed.

In Chapter 4, optimization, standardization and validation of the immunohistochemical assay for bovine cysticercosis developed at the CFIA's Centre for Food-borne and Animal Parasitology (Ogunremi et al., 2004a), and of the currently implemented histological assay, using cysticerci recovered from the experimental infections and a variety of known-negative specimens, are described and discussed.

In Chapter 5, a summary of the significance of the research findings, and final

conclusions regarding the contribution of this work to improved postmortem detection and confirmatory diagnosis of bovine cysticercosis are provided.

3. DISTRIBUTION OF *TAENIA SAGINATA* CYSTICERCI IN TISSUES OF EXPERIMENTALLY INFECTED CATTLE

3.1 Abstract

Bovine cysticercosis is a zoonotic disease warranting routine inspection measures for the postmortem detection of parasite cysts (cysticerci) in beef destined for human consumption. Detection is based on gross examination of traditional carcass predilection sites, although there is evidence to suggest that examination of other sites may offer improvements in sensitivity. In order to evaluate the efficacy of current inspection protocols, this study determined the distribution and number of *Taenia saginata* cysticerci in the tissues of experimentally infected cattle. Forty-two commercial beef cattle were divided into five groups of 5-12 animals each and inoculated with either 10000, 5000, 1000, 100 or 10 *T. saginata* eggs. From 47 to 376 days post-inoculation (DPI), ten animals inoculated with 5000 eggs were killed and the carcasses partitioned into 31 tissue sites. These consisted of the traditionally inspected tissue sites of heart, masseter and pterygoid muscles, tongue, oesophagus, and diaphragm (membranous and crura); as well as non-traditional sites of lung, liver and an additional 20 individual muscles or muscle groups. After performing the Canadian Food inspection Agency's (CFIA) routine inspection protocol for cysticerci on traditional tissue sites, tissues from all sites were cut into approximately 0.5 cm thick slices and the total number of parasitic cysts and cyst density (number of cysts/g of tissue) determined for each site. Traditional sites were similarly evaluated for the remaining 32 animals killed between 117 and 466 DPI. Sites were ranked based on cyst density. In the animals for which non-traditional sites were also evaluated, none yielded higher cyst densities than those traditionally inspected. When only traditional sites (for all animals) were compared, the heart ranked highest overall, and although it was not significantly different from the masseter muscle, was the most frequently affected site. The traditional site of oesophagus was among the poorest of all sites for detection of cysticerci. The heart was confirmed as the site of choice for detection of bovine cysticercosis based on high cyst density and frequency of infection, and greater visibility of gross lesions due to the early inflammatory

response in cardiac muscle. More extensive examination of the heart is recommended to improve detection of infected animals.

3.2 Introduction

The larval stage (syn *Cysticercus bovis*) of the human tapeworm *Taenia saginata* occurs in the tissues of cattle. Humans are the obligate final host and become infected by ingesting infected beef that has been inadequately cooked (Hird and Pullen, 1979) or frozen (Hilwig, 1978). The adult tapeworm in the human intestine sheds large numbers of eggs into the environment via feces and cattle become infected through ingestion of contaminated feed or water. The infective hexacanth embryo, or oncosphere, hatches from the egg in the bovine small intestine and migrates via submucosal blood or lymphatic vessels to cardiac or skeletal muscle, and occasionally to other tissue sites including liver, lung, kidneys and lymph nodes (Ginsberg and Grieve, 1959; Mitchell, 1973; Schillhorn van Veen, 1979). There it develops into the larval cestode known as a cysticercus, or cyst, which is infectious to humans after about 10-12 weeks (McIntosh and Miller, 1960). Cysticerci are fully developed four to five months after infection, and are oval, approximately 0.5 -1.0 cm long, and consist of a translucent fluid-filled fibrous capsule containing a larval tapeworm which is essentially a fluid filled bladder with an inverted scolex. Cysts that have died and are undergoing degeneration can vary in appearance depending on the degree of inflammation, necrosis, and mineralization in the resulting lesion (Geerts et al., 1980).

Although the apparent prevalence of this parasite in Canada is among the world's lowest (Pawlowski and Murrel, 2001), global trade, travel from endemic regions, and recycling of human sewage (MacPherson, 1978) on livestock pasture increase the risk of infection to Canadian cattle. Sporadic outbreaks in Canadian cattle have been reported over the years (McAninch, 1974; Bundza et al., 1988, Borman-Eby et al., 1994), and there is concern that the incidence of bovine cysticercosis is increasing, with recent confirmed outbreaks in Alberta (Lees et al., 2002), Ontario, and Quebec (unpublished observation, Canadian Food Inspection Agency). Bovine cysticercosis in Canada results in herd quarantine and increased carcass demerits and condemnations; thus it has significant economic impact on both affected producers and the regulatory agency responsible for monitoring, control and compensation.

Clinical disease is not usually a feature of bovine cysticercosis, and control measures

currently rely on the detection of cysts in affected carcasses during routine gross postmortem inspection procedures. Cysts can be visualized grossly as early as 11 days after cattle consume *T. saginata* eggs, at which time they are about 2.5 mm in diameter (McIntosh and Miller, 1960). The Canadian Food Inspection Agency (CFIA) mandates organoleptic visualization and/or palpation and/or incision of the heart, masseter and pterygoid muscles, tongue, oesophagus, and diaphragm, and observation of superficial and cut surfaces of the carcass exposed during routine dressing procedures. This is similar to inspection protocols implemented for this parasite in the USA (Snyder and Murrell, 1986; Saini et al., 1997), Europe (Kyvsgaard et al., 1990), Africa (Pugh and Chambers, 1989) and elsewhere, and is consistent with meat inspection procedures for this parasite recommended by the World Organisation for Animal Health (OIE) (2004c). These traditional inspection sites are easily accessed during routine slaughter, result in minimal carcass damage, and are presumed to be predilection sites for the parasite. However, whether this parasite truly has a predilection for any particular site(s) is debatable (Sewell and Harrison, 1978 a, b; McCool, 1979). Numerous studies of naturally or experimentally infected cattle have yielded disparate results (Dewhirst et al., 1967; Mango and Mango, 1972; McAninch, 1974; Babalola, 1976; Juranek et al., 1976; Hammerberg, B., 1978; Karim, 1979; McCool, 1979; Nyaga and Gathuma, 1979; Walther, 1980; Pugh and Chambers, 1989; Kyvsgaard et al., 1990; Oryan et al., 1995; Maeda et al., 1996; Pramanik et al., 1999) although heart and masticatory muscles are consistently identified as amongst the best sites for detecting infection. It has been proposed that a variety of factors including breed, muscle activity, age, and geographic area may be responsible for the observed differences (Kearney, 1970). Pawlowski and Schulz (1972) proposed that the choice of sites inspected should be based on studies within a particular country or region.

Studies in the USA and elsewhere have demonstrated that postmortem inspection procedures for bovine cysticercosis have a sensitivity of $\leq 50\%$ for the detection of lightly-infected carcasses (Dewhirst et al., 1967; McCool, 1979; Walther, 1980). This study was conducted to evaluate carcass distribution of *T. saginata* cysticerci in Canadian cattle maintained under representative field conditions, to determine the sensitivity of the current inspection protocol used in Canada, and to generate possible recommendations for improved parasite detection.

3.3 Materials and Methods

3.3.1 Preparation of inoculum

The *T. saginata* eggs used in this study were kindly provided by Jitra Waikagul and Paron Dekumyoy of the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Gravid proglottids of *T. saginata*, freshly discharged from patients in Bangkok, were identified, washed in physiological buffered saline (PBS), and shipped refrigerated in PBS to the Centre for Food-borne and Animal Parasitology in Saskatoon, Canada. Confirmation of parasite species identity was based on proglottid anatomy and molecular characterization of eggs and proglottids (Bowles and McManus, 1994). Proglottids were gently manipulated with forceps in a petri dish filled with PBS to expel developed eggs. The eggs were then processed according to the method of Takemoto et al. (1995), and stored in PBS at 4°C for up to six weeks until use. Viability of eggs was assessed by an in-vitro hatchability/viability assay derived by combining the sodium hypochlorite and enzyme methods (followed in that order) reported by Wang et al. (1997) for *T. solium*, and substituting 1 ml bovine bile for pig bile. A few days before inoculation of calves, the stored egg suspension was vortexed and a number of representative aliquots were pipetted and counted to determine the concentration and total number of eggs available. On each of the two consecutive days prior to inoculation a viability assay as described above was conducted on a representative sample of eggs. Based on an average viability of approximately 70%, the total number of eggs per dose was adjusted to approximate the administration of 10, 100, 1000, 5000, or 10000 viable eggs.

3.3.2 Experimental inoculations

Forty-two cross-bred beef calves, of both sexes, approximately 8 -11 months of age, and in a research herd (Goodale Farm, University of Saskatchewan) with no history of cysticercosis, were experimentally inoculated with *T. saginata* eggs. An initial lot of ten calves were inoculated with 5000 eggs each, and approximately 14 months later, using a fresh batch of eggs, a second lot of 32 calves received 10000 (5 calves), 5000 (2 calves), 1000 (5 calves), 100 (10 calves) or 10 (10 calves) eggs each. Immediately prior to inoculation at the research farm, 500 μ l of suspension containing the estimated number of eggs was placed in a small gelatine capsule, which was inserted within a slightly larger capsule to contain any leakage. Each animal was

restrained, intubated and the encapsulated egg suspension placed in the tube and flushed into the rumen with warm water administered by hand pump. Several 500 μ l aliquots of egg suspension were collected throughout this process for subsequent verification of egg counts. All animals were group penned for several days after inoculation to reduce the possibility of cross contamination of uninfected herdmates with *T. saginata* eggs, and then released outdoors into the general research herd. All animal use was approved by the University of Saskatchewan Committee on Animal Care and Supply in accordance with guidelines of the Canadian Council on Animal Care.

3.3.3 Postmortem examinations

3.3.3.1 Traditional and non-traditional inspection sites (complete carcass examination)

The animals within the initial lot of cattle that received 5000 viable eggs were killed at varying intervals from 47–376 days post inoculation (DPI). Each carcass was partitioned into 31 pre-determined boneless muscle or visceral sites, including those sites traditionally inspected. Traditional sites consisted of: heart, masseters, pterygoids, membranous diaphragm, diaphragmatic pillars, tongue, and esophagus. Non-traditional sites were based on whole or partial primal cuts of beef carcasses (Juranek et al., 1976) and consisted of dorsal and ventral chuck, forelimb, front shank, brisket, plate, longissimus dorsi, thoracic muscles, flank, abdominal muscles, psoas, gluteals, biceps femoris, semimembranosis, semitendinosus, medial thigh muscles, quadriceps, gastrocnemius, and hindshank, as well as lesser head muscles, lungs and liver. The “lesser head muscles” were a non-traditional site consisting of the cumulative muscle tissue dissected from the skull, excluding masseter and pterygoid. Examination of bilateral non-traditional carcass sites alternated between left and right sides for successive carcasses. The tissue comprising each site was weighed (g) and cut into approximately 0.5 cm slices using a commercial meat slicer (Hobart Corporation, Troy, Ohio, USA). Each slice was examined grossly on both sides for viable or degenerated cysts using an illuminated X 1.75 magnifying lens. Any cyst that had been inadvertently sectioned was counted if greater than one half of the cyst remained in the tissue slice. Total cyst counts were recorded for each site. The number of cysts detected via routine inspection of traditional sites was determined prior to comprehensive evaluation by the following procedure: visual examination of the heart surface,

and of all surfaces revealed by incision and eversion with approximately six evenly-spaced deep incisions made into the myocardium from the endocardial surface; visual examination of incisions parallel to the mandible of masseter and pterygoid muscles; palpation of the freed tongue and oesophagus and visual examination (following any required incision) of any palpated masses; visual examination of the freed diaphragm and its crura. This differed from the current CFIA inspection procedure that requires only three shallow incisions into the heart (CFIA Meat Hygiene Manual of Procedures, Section 4.6.1, 2007).

3.3.3.2 Traditional inspection sites only

The remaining 32 cattle that received 10000, 5000, 1000, 100 or 10 eggs were killed 117 – 466 DPI. Traditional sites were evaluated using the routine inspection procedure followed by comprehensive slicing, as described above. If no cysts were detected in traditional sites further examination of the carcass was conducted using the slicing procedure until a positive infection status could be determined or all tissues were examined and the carcass considered negative.

3.3.3.3 Enhanced inspection

The current CFIA inspection procedure requires that more thorough examination of the traditional sites and the remainder of the carcass be performed when suspect lesions are recovered on routine inspection, or when animals originate from a known infected herd. This “enhanced” inspection includes incisions made into the forequarters and rounds of the carcass and was performed on the experimentally infected cattle by making a single full thickness incision into each triceps brachii muscle of the forelimbs, midway between the muscle’s origin and insertion, and similarly incising the semitendinosus muscles of the hindlimbs.

3.3.3.4 Identification of cysticerci

Taenia saginata cysticerci were identified as such based on gross and/or stereomicroscopic examination. A viable cysticercus consisted of a larval tapeworm (with fluid-filled bladder and invaginated scolex) contained within a typically translucent cyst wall. Cysts containing caseous to mineralized contents with or without recognizable parasite material were considered degenerated cysticerci unless another etiology was evident.

3.3.4 Statistical analysis

Statistical analysis was performed by Sarah Parker of the Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, using a commercial statistical software package (Statistix, Version 8).

3.3.4.1 Traditional and non-traditional inspection sites (complete carcass examination)

For the initial lot of ten animals given 5000 eggs each, cyst density was calculated for each site by dividing the number of cysts recovered from each site by the mass (g) of the tissue comprising the site. Cyst density data were non-normally distributed so non-parametric statistical tests were used to evaluate differences. Overall median cyst densities of all examined anatomical sites were compared using the Kruskal-Wallis analysis of variance (Norman and Streiner, 2000). Where *a priori* pairwise comparisons were made of median cyst densities for anatomical sites, the Wilcoxon Rank Sum test was used (Norman and Streiner, 2000).

To determine which sites typically had a higher cyst density within each animal, sites for each calf were ranked based on cyst density. Ranking was done by ordering the sites within each calf based on the observed cyst density and applying a number rank to each site, starting at 1 for the site with the highest cyst density. Where there were ties in cyst density the successive ranks that would have applied to the tied sites were added together and divided by the number of tied sites. Each tied site was then assigned this average rank value. For example, if three tissues had the 3rd highest cyst density, the values 3, 4 and 5 were added together (12) and divided by 3. Each of these tissues would then have been assigned the rank of 4. Rank data are ordinal data so non-parametric tests were used to evaluate observed differences. Ranks were compared using the Kruskal-Wallis analysis of variance. The Wilcoxon Rank Sum test was used to make *a priori* pairwise comparisons.

3.3.4.2 Traditional inspection sites only

Since a variety of inoculation doses were given, traditional sites were compared for all animals based on rankings of cyst density within animals. Ranking and statistical analysis of the traditional sites was done in the same manner as that described above for ranking of all anatomical sites. The Wilcoxon Rank Sum test was used for pairwise comparisons with the overall α -level kept at 0.05. A chi-square test (Norman and Streiner, 2000) was used to compare

the frequency with which traditional sites were infected or uninfected with cysticerci.

3.3.4.3 Routine inspection vs comprehensive heart examination

The number of positive animals detected by routine inspection was compared to those detected by comprehensive heart examination. Since these represented paired observations on the same animal the McNemar's chi-square test (Norman and Streiner, 2000) was used to assess significance of the observed difference in detection rates.

3.4 Results

3.4.1 General findings

Thirty-seven of the 42 animals which received a minimum comprehensive examination of all traditional sites were infected with one or more cysticerci. Seven of these confirmed-positive animals had no cysts in any traditional sites. Further examination of the carcasses of the remaining five animals failed to detect cysts. Four of the five negative animals had been inoculated with ten eggs each, and one with 100 eggs (Table 3.1).

For the initial lot of ten animals inoculated with 5000 eggs, following comprehensive inspection of all traditional and non-traditional sites, including lungs and liver, five animals were found to have 5–65 cysticerci in the lungs (some of which were fully developed and viable), and four of these same five animals had 20–55 degenerated cysticerci in the liver (Table 3.2). Degenerated cysticerci were also observed on the serosal surface of the forestomachs in eight animals, in the parotid salivary gland of one animal, and in the submaxillary and submandibular lymph nodes of another animal. There was a trend towards decreasing total numbers of cysts recovered per animal over time (Fig. 3.1). Both viable (Fig. 3.2) and degenerated (Fig. 3.3) cysts were present in the eight animals killed by 154 DPI. Degenerated cysts were much more obvious grossly than their viable counterparts. Most cysts in the heart were degenerated (Fig. 3.3), including those in the first animal killed at 47 DPI. Viable cysts recovered from the head and neck occasionally had a diffusely hemorrhagic or congested appearance to the cyst wall, and could not be easily distinguished from regional hemal lymph nodes without incision (Fig. 3.4). Only degenerated cysts were recovered from the final two animals in this lot, killed at 285 and 376 DPI.

Table 3.1. Organoleptic detection of cysticerci (cysts) via routine and comprehensive inspection of all traditional carcass sites, and of heart alone, in cattle administered various doses (10-10000) of *Taenia saginata* eggs.

Dose of <i>T. saginata</i> eggs	Number of animals inoculated	Number of animals confirmed infected	Number of infected animals in which cysts were detected			
			Routine inspection ^a		Comprehensive inspection ^b	
			All traditional sites	Heart	All traditional sites	Heart
10000	5	5	3	2	5	5
5000	12	12	11	11	11	11
1000	5	5	5	4	5	5
100	10	9	1 ^c	1	7 ^c	7
10	10	6	0	0	2 ^d	1
Total	42	37	20	18	30	29

^a routine inspection of traditional sites entailed: incision and eversion of the heart with approximately 6 evenly-spaced deep incisions made into the myocardium from the endocardial surface, incision parallel to the mandible of masseter and pterygoid muscles, palpation of the freed tongue and oesophagus, and visualization only of the freed diaphragm and its crura

^b each traditional site was cut into approximately 0.5 cm thick slices using a commercial meat slicer, and each slice examined grossly on both sides for cysts using an illuminated X 1.75 magnifying lens

^c heart only traditional site affected

^d heart only traditional site affected in 1 animal; left masseter only traditional site affected in other animal

Table 3.2. Number of cysts recovered, cyst density, and site rank (based on cyst density within animal) for traditional and non-traditional carcass inspection sites in cattle inoculated with 5000 *Taenia saginata* eggs.

Site ^{a b}	Cyst count (n=10)		Cyst density (per 100 g) (n=10)		Site rank (n=9)	
	Median	(Range)	Median	(Range)	Median	(Range)
Pterygoid - Right	7.0	(0 - 14)	5.42	(0.00 - 13.00)	3	(1 - 23.5)
Heart	72.0	(17 - 451)	4.85	(0.82 - 23.74)	4	(1 - 5)
Masseter - Left	16.0	(1 - 44)	4.54	(0.30 - 12.22)	3	(1 - 8)
Masseter - Right	18.0	(0 - 54)	4.39	(0.00 - 15.43)	3	(1 - 23.5)
Pterygoid - Left	4.0	(0 - 13)^c	3.20	(0.00 - 7.65)^c	5	(1 - 23.5)^d
Diaphragm - pillars	14.0	(2 - 55)	2.15	(0.24 - 7.86)	5	(1 - 14)
Lesser Head Muscles ^e	10.5	(0 - 36)	1.66	(0.00 - 6.00)	8	(5 - 26)
Tongue	15.0	(0 - 51)	1.56	(0.00 - 4.86)	9	(5 - 30)
Diaphragm - membranous	11.0	(1 - 54)	1.36	(0.12 - 5.18)	7	(6 - 17)
Psoas ^f	12.0	(0 - 36)	0.89	(0.00 - 2.77)	19	(9 - 29)
Quadriceps ^f	32.0	(3.5 - 142)	0.77	(0.11 - 3.29)	11	(7 - 20)
Forelimb ^f	44.0	(6 - 214) ^c	0.62	(0.11 - 3.09) ^c	12	(7 - 22)
Biceps Femoris ^f	22.0	(0 - 72)	0.62	(0.00 - 2.42)	21	(11 - 28)
Semimembranosis ^f	24.0	(3 - 64)	0.58	(0.10 - 1.92)	18	(10 - 23)
Longissimus Dorsi ^f	24.0	(1 - 57)	0.54	(0.03 - 1.52)	20	(15 - 23)
Gluteals ^f	14.0	(1 - 52)	0.53	(0.06 - 2.20)	13	(9 - 27)
Medial Thigh ^f	8.0	(0 - 31)	0.49	(0.00 - 1.82)	19	(13 - 26)
Hindshank ^f	4.0	(0 - 25)	0.43	(0.00 - 2.58)	18	(11 - 30)
Front Shank ^f	7.0	(0 - 20) ^c	0.43	(0.00 - 1.31) ^c	22	(10 - 28)
Semitendinosus ^f	5.0	(0 - 39)	0.42	(0.00 - 2.95)	24	(12 - 30)
Dorsal Chuck ^f	20.0	(1 - 84) ^c	0.39	(0.03 - 2.00) ^c	16	(10 - 26)
Gastrocnemius ^f	5.0	(0 - 37)	0.36	(0.00 - 3.36)	23.5	(9 - 28)
Abdominal ^f	7.0	(0 - 52) ^c	0.35	(0.00 - 2.45) ^c	21	(12 - 27)
Ventral Chuck ^f	13.0	(1 - 71) ^c	0.34	(0.03 - 1.84) ^c	17	(9 - 28)
Eosophagus	1.0	(0 - 8)^c	0.33	(0.00 - 3.81)^c	25	(8 - 30)^d
Thoracic ^f	7.0	(0 - 41) ^c	0.32	(0.00 - 1.63) ^c	21	(16 - 28)
Plate ^f	8.0	(0.5 - 32) ^c	0.18	(0.01 - 1.51) ^c	21	(11 - 28)
Brisket ^f	4.0	(1 - 33) ^d	0.15	(0.05 - 1.4) ^d	20	(12 - 31) ^d
Flank ^f	2.0	(0 - 8) ^c	0.13	(0.00 - 1.11) ^c	26	(18 - 30)
Lungs	3.0	(0 - 65)	0.04	(0.00 - 2.65)	24	(4 - 30.5)
Liver	0.0	(0 - 55)	0	(0.00 - 1.79)	29.5	(1 - 30.5)

^a Each site cut into 0.5 cm slices which were examined visually for cysts; sites sorted based on median cyst density

^b Traditional sites and values in bold

^c Values for only 9 animals available for this tissue site

^d Values for only 8 animals available for this tissue site

^e Lesser head muscles consisted of the cumulative muscle tissue dissected from the skull, excluding masseter and pterygoid

^f Unilateral non-traditional sites from left or right carcass

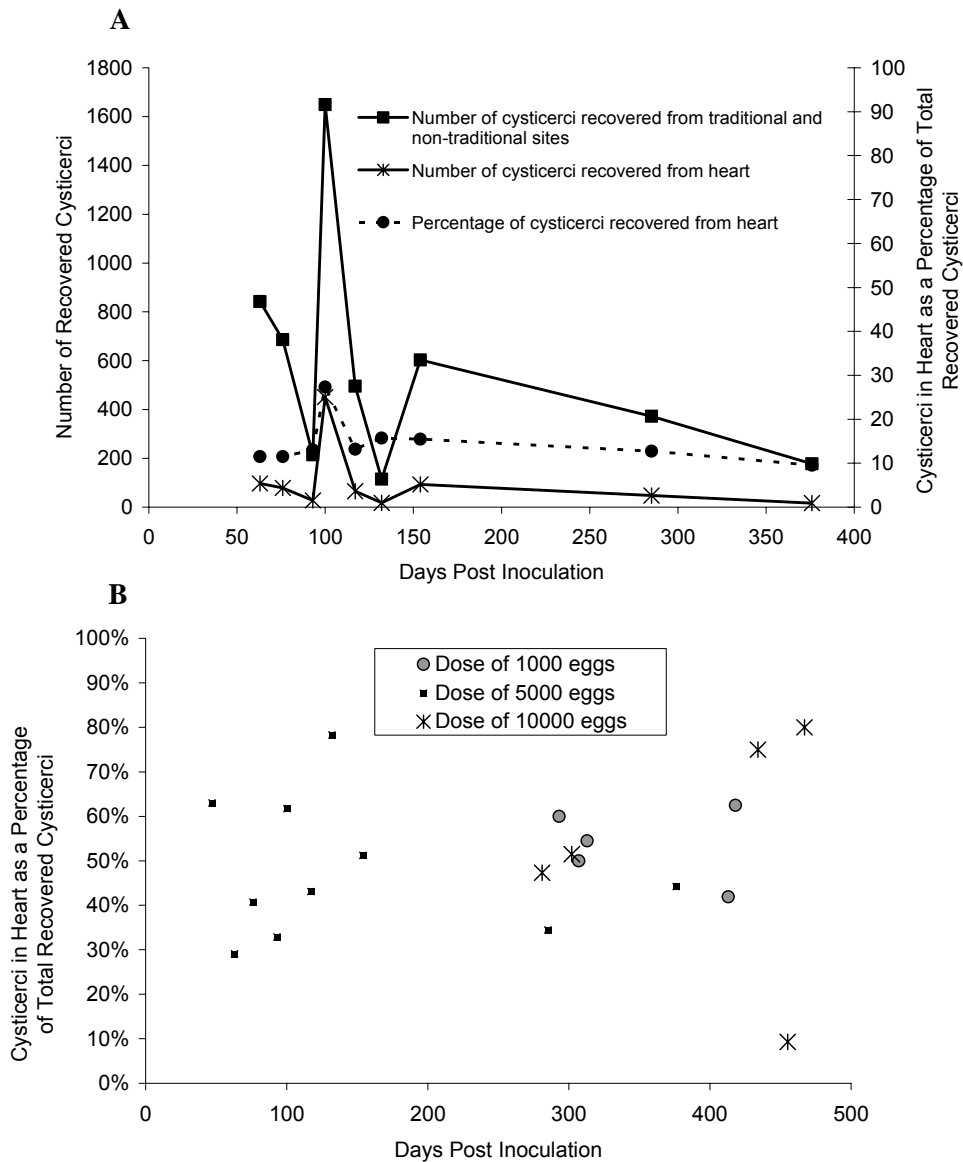


Figure 3.1. Number of cysticerci recovered, and percentage found in the heart, in carcasses of cattle experimentally infected with *Taenia saginata*. A) Total number of cysticerci recovered from combined traditional and non-traditional carcass sites, from heart only, and percentage in the heart, for 9 cattle inoculated with 5000 *T. saginata* eggs (counts for bilateral non-traditional sites determined for half carcass only). B) Percentage of cysticerci recovered from traditional carcass sites found in the heart for 20 cattle inoculated with 1000, 5000, or 10000 *T. saginata* eggs.

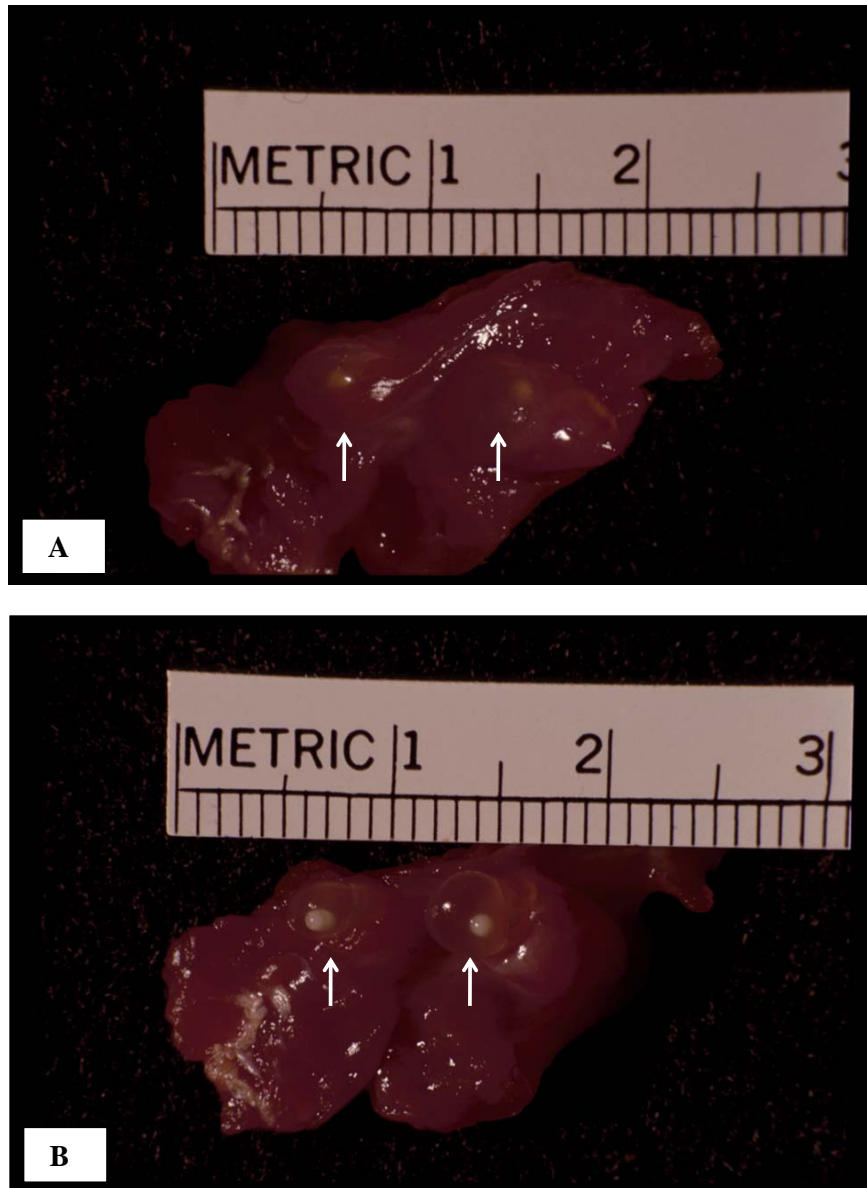


Figure 3.2. Viable cysticerci *in situ* (**A**), and with outer capsule incised (**B**), in masseter muscle from an experimentally infected calf killed 117 days post-inoculation with 5000 *Taenia saginata* eggs. Arrows indicate cysticerci.

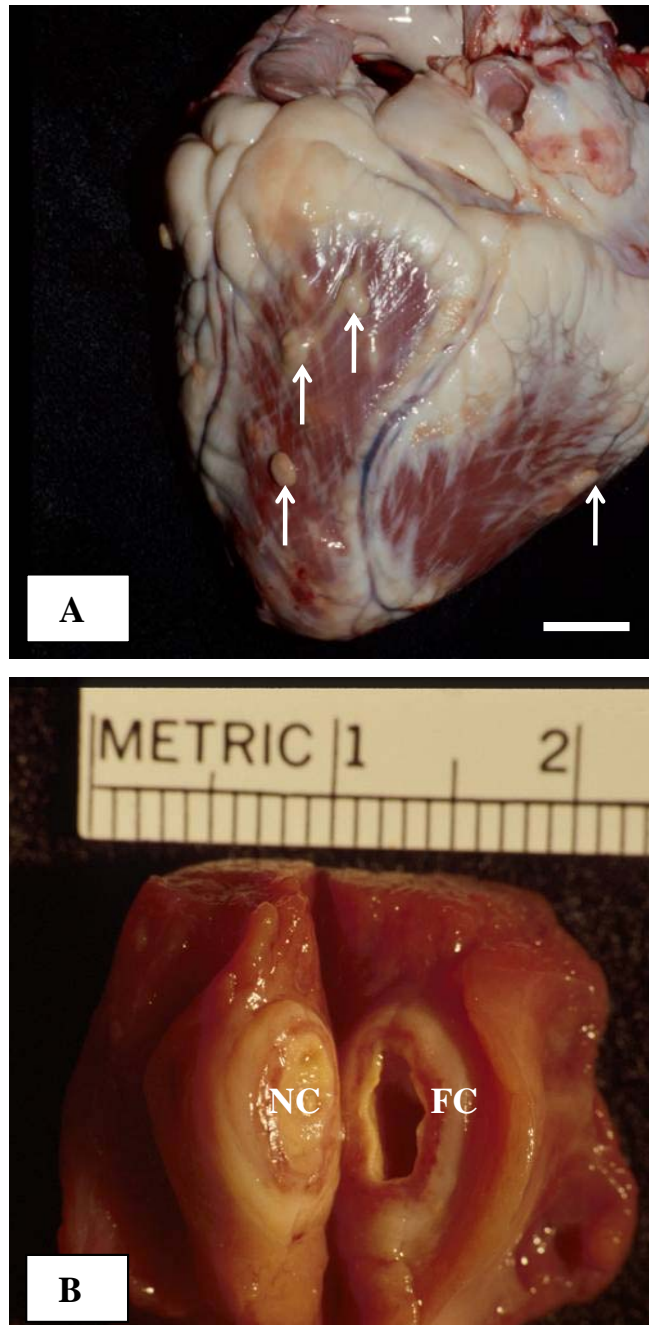


Figure 3.3. Degenerated cysticerci *in situ* (A), and a sectioned degenerated cysticercus demonstrating caseous necrotic core (NC) and fibrous capsule (FC) (B), in heart of an experimentally infected calf killed 117 days post-inoculation with 5000 *Taenia saginata* eggs. Bar = approximately 2 cm.

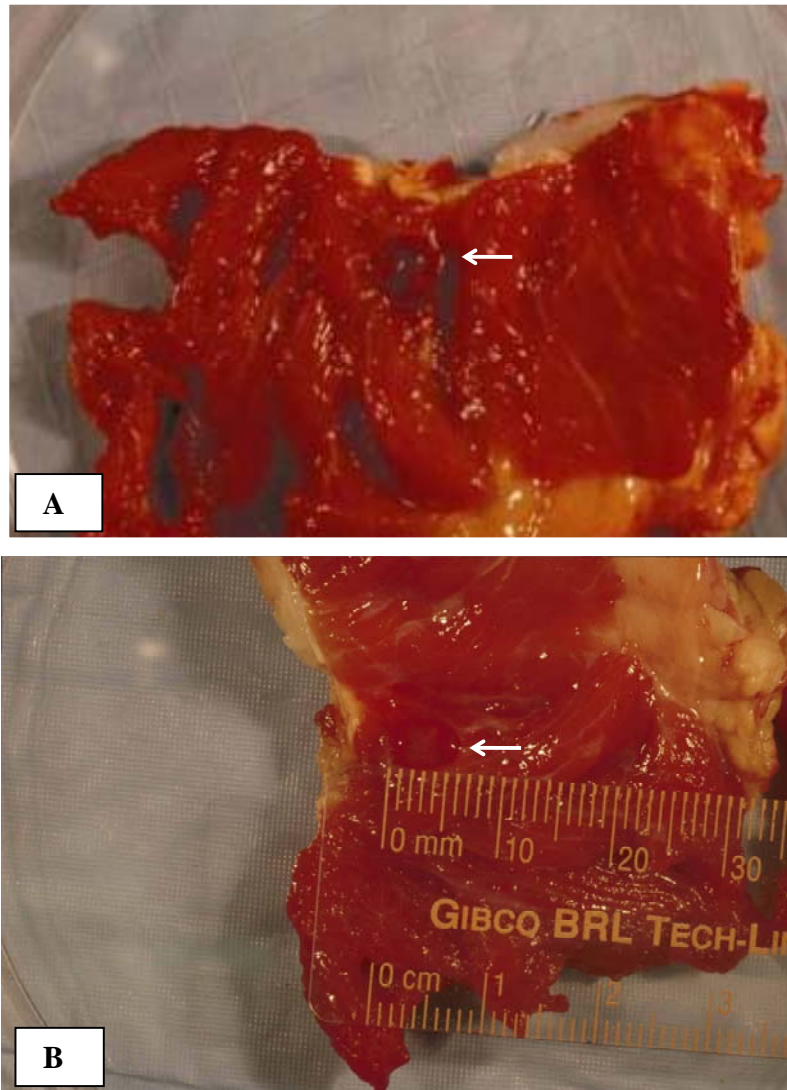


Figure 3.4. Two views (A, B) of a viable cysticercus (arrows) with a superficial gross appearance similar to a hemal lymph node, in masseter muscle of an experimentally infected calf killed 63 days post-inoculation with 5000 *Taenia saginata* eggs.

For the second lot of 32 cattle inoculated with 10 – 10000 eggs, few viable cysts were recovered following comprehensive inspection of the traditional tissue sites. However, intact and grossly and/or stereomicroscopically identifiable larval tapeworms were often recovered from degenerated cysts (Fig. 3.5). Two animals that received 10000 eggs and killed at 434 and 466 DPI were negative for cysticerci on routine inspection of traditional sites, and one animal that received 5000 eggs and killed at 278 DPI had no cysticerci recovered from traditional sites even after comprehensive examination (Table 3.1). Within each dose group there was a trend towards decreasing numbers of total cysts recovered per animal over time (Fig. 3.1).

3.4.2 Enhanced inspection

Incision of each forelimb and hindlimb was assessed in 41 of the 42 cattle. Cysts were detected in one or more of these sites in five animals. Of these animals, four had received 5000 eggs and one had received 10000 eggs.

3.4.3 Comparison of all tissue sites (traditional and non-traditional)

For the ten animals receiving 5000 eggs and for which both traditional and non-traditional sites were examined, there was a significant difference overall in median cyst density among the sites ($p < 0.0001$). The traditional sites, with the exception of the oesophagus, consistently had the highest observed median cyst densities; the oesophagus had amongst the lowest of all the sites examined. No non-traditional site, except for the lesser head muscles, had an observed median cyst density as high as or higher than the traditional sites (other than the oesophagus) (Table 3.2). The difference in median cyst density between lesser head muscles and oesophagus was not significant. Because cyst counts and/or site weights were erroneously not determined for some sites in some animals, median cyst density values for each site were calculated based on data from as few as eight animals. When animals for which there were incomplete data were excluded from the analysis, the conclusions did not change. Therefore, results from analysis of all available data are presented here.

For comparisons within animals, sites were ranked based on cyst density. There was a significant difference in median rank (based on cyst density within calves) among the sites ($p < 0.0001$). The highest ranking non-traditional site was the lesser head muscles (Table 3.2), and this was not significantly different from the tongue which had the next highest median rank. The

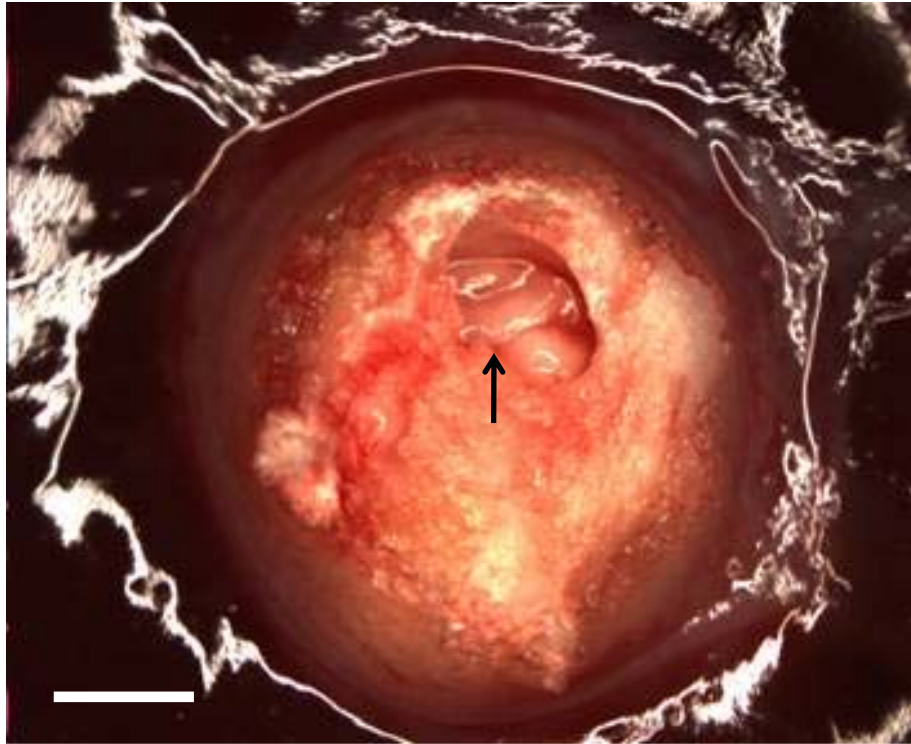


Figure 3.5. Mineralized degenerated cysticercus with grossly identifiable larval tapeworm (arrow) in liver of an experimentally infected calf killed 319 days post-inoculation with 10 *Taenia saginata* eggs. Bar = approximately 1 mm.

only other traditional site that had a median rank below the lesser head muscles was the oesophagus; the difference between these two sites was significant ($p = 0.02$). Data for ten sites of one animal was missing, and this animal was excluded from this analysis. Data for one and two sites for two other animals was also missing; however, these animals were included in the analysis. The missing data points mean that some sites in these two animals may have received a higher rank than they might otherwise have received. This would make the analysis more conservative with differences less likely to be significant, and was deemed acceptable.

3.4.4 Comparison of traditional sites only

Of the 42 animals receiving various doses of eggs, those that received only ten or 100 eggs ($n=20$) had no cysts detected in the majority of traditional sites (Table 3.3). These animals, and the animal which had received 5000 eggs and had no cysts in traditional sites, were excluded from the comparison of these sites based on cyst density. For the remaining animals ($n=21$), there was no significant interaction between the dose of eggs and the distribution of cysts to specific sites. Univariate analysis was therefore used. There was a significant difference overall in the median rank among the sites ($p < 0.0001$). The heart had the highest observed median rank but was not significantly different from the next four highest ranking sites. There were three such groups of sites (1. heart, masseters, pterygoids; 2. pterygoids, diaphragm, tongue; 3. left pterygoid, diaphragm, tongue, oesophagus) within which median ranks were not significantly different (Table 3.4). Although, maximum and mean cyst density values for each traditional site in the group of five animals given 10000 eggs each were lower than those for the group given 5000 eggs, ranking of sites relative to each other in this highest dose group was deemed relevant to the comparison, and included in the analysis.

There was a significant difference in the frequency with which traditional sites contained one or more cysticerci ($p = 0.0001$). Cysticerci were present in the heart (29 animals) more often than in right masseter (20 animals) which was the second most frequently affected site ($p = 0.048$; Table 3.3).

3.4.5 Comparison of routine inspection and comprehensive heart examination

Of the 37 confirmed positive animals in this study, 20 were positive by routine inspection of all traditional sites and 18 by routine inspection of the heart alone (Table 3.1). In animals

Table 3.3. Cyst density for traditional inspection sites from carcasses of cattle inoculated with various doses (10-10000) of *Taenia saginata* eggs.

Traditional Site	Cyst density (per 100 g)									
	Dose:	10 (n=10)		100 (n=10)		1000 (n=5)		5000 (n=12)		10000 (n=5)
Heart		0.00 (0.00-0.04) [1]	0.04 (0.00-0.15) [7]	0.30 (0.06-0.65) [5]	4.54 (0.00-23.74) ^a [11] ^b	0.23 (0.12-6.00) [5]				
Pterygoid - Right		---- ^c	----	----	3.33 (0.00-13.00) [10]	0.00 (0.00-3.44) [2]				
Masseter - Left		0.00 (0.00-0.16) [1]	----	0.32 (0.00-0.82) [3]	2.75 (0.00-12.22) [11]	1.09 (0.00-6.81) [3]				
Pterygoid - Left		----	----	0.00 (0.00-0.30) [1]	2.50 (0.00-7.65) ^a [9]	0.63 (0.00-4.80) [3]				
Masseter - Right		----	----	0.31 (0.16-0.54) [5]	2.39 (0.00-15.43) [10]	1.38 (0.14-5.80) [5]				
Diaphragm- pillars		----	----	0.00 (0.00-0.10) [1]	1.52 (0.00-7.86) [11]	0.00 (0.00-2.47) ^d [1]				
Diaphragm - memb.		----	----	0.07 (0.00-0.13) [4]	1.14 (0.00-5.18) [11]	0.00 (0.00-2.63) ^d [1]				
Tongue		----	----	0.00 (0.00-0.15) [2]	0.93 (0.00-4.86) [9]	0.07 (0.00-0.42) [3]				
Eosophagus		----	----	0.00 (0.00-0.67) [1]	0.25 (0.00-3.81) ^a [6]	----				

^a Values for only 11 animals available for this tissue site

^b Includes 1 animal for which total number of heart cysts not counted

^c No cysts found in this site

^d Values for only 4 animals available for this tissue site

Table 3.4. Site rank (based on cyst density within animal) for traditional inspection sites from carcasses of cattle inoculated with 1000, 5000, or 10000 *Taenia saginata* eggs.

Site	Site rank						
	Median (range)						n
	Dose:	1000 (n=5)	5000 (n=11) ^a	10000 (n=5)	Overall		
Heart	2	(1 - 4)	3 (1 - 5) ^b	2 (2 - 5)	2 (1 - 5)	A ^c	20
Masseter - Left	2	(1 - 6.5)	3 (1 - 7)	4 (1 - 6)	3 (1 - 7)	A	21
Masseter - Right	3	(1 - 3)	3 (1 - 7)	2 (1 - 3)	3 (1 - 7)	A	21
Pterygoid - Right	7.5	(6 - 8)	3 (1 - 7)	6 (1 - 6.5)	5 (1 - 8)	AB	21
Pterygoid - Left	6.5	(5 - 8)	4 (1 - 7) ^d	4 (1 - 6)	5 (1 - 8)	ABC	20
Diaphragm - membranous	6	(3 - 6)	6 (4 - 9)	6 (6 - 8) ^e	6 (3 - 9)	BC	20
Tongue	6	(4 - 8)	7 (5 - 9)	6 (5 - 8)	6.5 (4 - 9)	BC	21
Diaphragm - pillars	6.5	(5 - 8)	7 (1 - 9)	6.5 (6 - 8) ^e	6.75 (1 - 9)	BC	20
Oesophagus	6.5	(1 - 8)	8 (6 - 9) ^d	6.5 (6 - 9)	7.75 (1 - 9)	C	20

^a One animal excluded from analysis since no traditional sites contained cysts

^b One animal excluded from analysis of this site since total number of heart cysts not counted

^c Different letters represent groups where the overall median rank was significantly different (p< 0.05)

^d Values for only 10 animals available for this tissue site

^e Values for only 4 animals available for this tissue site

receiving doses of ten or 100 eggs, no additional infections were detected by routine inspection of all traditional sites versus that of the heart alone. Comprehensive heart dissection alone detected significantly more positive animals than those detected by routine inspection of traditional sites ($p = 0.008$). This difference was most evident in the animals dosed with 100 eggs ($p = 0.04$).

There was no apparent trend to a lower ratio of heart cysts to total cysts recovered per animal (Fig 3.1a), or of heart cysts to total traditional site cysts (within or between the relevant dose groups), over the course of the experiment (Fig 3.1b). In the initial lot of cattle infected with 5000 eggs, the proportion of total cysts recovered from each animal that were found in the heart ranged from 10 - 16 % for eight of nine carcasses. One animal in this lot, killed at 100 DPI, had 27% of total carcass cysts in the heart (Fig. 3.1a). Overall, the heart comprised from 9% to 78% of the total cysts per animal found in all traditional sites ($n=20$; cattle that received ten or 100 eggs had too few cysts for comparison, as did two animals in the second lot that received 5000 eggs, of which one had no cysts in any traditional sites, and one was missing total cyst data for the heart).

3.5 Discussion

This was the first large scale detailed study of the carcass distribution of *T. saginata* cysticerci in experimentally infected Canadian cattle. Although McAninch (1974) reported the distribution of cysticerci in 436 animals infected in an Ontario outbreak, findings were limited to frequency of infection in heads, hearts, and diaphragms. In a subsequent 1986 Ontario outbreak, findings were similarly reported for the heart, masseter, tongue and liver only, in 233 animals histologically confirmed as infected (Bundza et al., 1988). Smith et al. (1991a) determined total numbers of cysticerci in tongue, masseter, diaphragm, heart and liver for four groups of three experimentally infected animals each, but reported only the mean total number of cysticerci recovered per group. The current study demonstrated that most of the traditional inspection sites used to detect this parasite are as good as, or better than, any of the non-traditional sites evaluated. The data also support the absence of any true predilection sites for this parasite as in seven of the infected animals in this study cysticerci were not found in any of the traditional sites. The presence and number of cysts in any given site were quite variable among animals, even when given the same dose of *T. saginata* eggs. Because of this variability, and the relatively

few animals sampled, the observed differences in cyst density were often not significantly different because of the overlap of the distribution of values.

Although the lesser head muscles were found to have a median cyst density comparable to those of some of the traditional sites, these tissues would not be practical to examine under routine inspection conditions. As well, some of the viable cysts recovered from this site were grossly similar to hemal lymph nodes, further confounding detection. This congestion or hemorrhage in the cyst wall may have been a postmortem effect due to hanging the carcass head down prior to exsanguination. The oesophagus is a traditional inspection site that is conveniently accessed during the slaughter process, but in this study it ranked among the poorest sites for cyst recovery. Of the traditional sites examined in this study, heart and masseters had the highest cyst densities, followed by the diaphragm and tongue. The heart was also the most frequently affected site. This is in general agreement with the findings of others despite differences in methodology to detect and quantify infection in various sites. The triceps brachii has been shown to be a useful site for the detection of cysticercosis in African Zebu cattle (Mitchell, 1973; Nyaga, 1979; Pugh and Chambers, 1989; Maeda et al., 1996), but is not considered as such for European breeds. This is possibly due to reduced activity of this muscle in European breeds compared to their African counterparts that are typically grazed on extensive ranges (Maeda et al., 1996). The forelimb site in our study included this muscle and was one of the higher ranking non-traditional sites (Table 3.2); however incision of each triceps brachii muscle, as per the enhanced inspection procedure performed in this study, failed to detect most infected animals.

The relatively low numbers of cysts recovered from traditional sites in the the five animals given 10000 eggs was unexpected. This, the failure to detect infection by routine inspection in two of these animals, and the absence of cysticerci in traditional sites for an animal given 5000 eggs may have been due to normal biological variability among the cattle, variation in egg viability, or undetected technical problems in administration of inocula. Although the two animals given 10000 eggs and negative on routine inspection were killed late in the course of the study (434 and 466 DPI), it is unlikely that significant resorption of pre-existing cysticerci would have occurred by this stage (Penfold and Penfold, 1937). Nor is it likely that this dose was too high to establish the corresponding intensity of infection in these animals, since other studies have generated substantial infections in calves inoculated with similar numbers of eggs (Mango and Mango, 1972; Smith et al., 1991a).

Routine inspection of traditional sites of animals receiving 1000 eggs or less detected 30% of infected animals, which is in agreement with previous estimates of a 15 - 50% detection rate of lightly infected animals (Kyvsgaard et al., 1990; Saini et al., 1997). Since the routine inspection procedure used in this study entailed more incisions into the heart than are normally implemented in Canadian abattoirs, the actual detection rate under field conditions would be lower. The difficulty in detecting translucent viable cysticerci, which blend in with the surrounding host tissue, under abattoir conditions further reduces sensitivity of gross inspection. Routine inspection of all traditional sites detected only two more infected animals than those detected by routine inspection of the heart alone (Table 3.1). Comprehensive inspection (0.5 cm tissue slices) of all traditional sites detected an additional ten animals, but only one more than those detected by comprehensive examination of the heart alone. There was a significant increase in detection by comprehensive examination of the heart of the lightly infected animals inoculated with 100 eggs, compared to routine inspection of all traditional sites. Since these animals had only one to three cysts present in the heart (data not shown), merely increasing the number of inspection incisions in this organ (Juranek et al., 1976), would not reliably detect these animals. More thorough inspection of all traditional sites would be time prohibitive under routine conditions. However, since comprehensive inspection of the heart alone provided comparable sensitivity, this may be a feasible alternative to increase detection rates in abattoirs. Geerts et al. (1980) detected infection by thorough slicing of the heart in 25 of 100 animals that had passed routine inspection in Denmark. Dewhirst et al. (1967) similarly recommended minute inspection of the entire heart in endemic regions.

In spite of the relatively high number of cysts found in the heart, our findings support that it is not a suitable matrix for long term parasite survival, and that cysts in cardiac muscle degenerate earlier than in skeletal muscle sites (Soulsby, 1963; Juranek et al., 1976; Sterba et al., 1979; Geerts et al., 1980; Gallie and Sewell, 1983; Lloyd, 1998). The degenerating cysticerci incite a host inflammatory response (Sterba et al., 1979a) resulting in lesions that are easier to detect by gross inspection than viable cysts. Although degenerated lesions are more frequently detected, they pose challenges to definitive diagnosis, since parasite features may no longer be evident on gross or microscopic examination. Contrary to the suggestion of Kyvsgaard et al. (1990) we did not find a decreasing trend in the ratio of heart cysts to total carcass cysts or to total traditional site cysts over time (Figs. 3.1a and 3.1b) providing evidence to support that

resorption of degenerating cysticerci in the heart occurs more slowly than in other sites (Gallie and Sewell, 1983; Harrison et al, 1984). Overall, the findings suggest that the heart is the most reliable “sentinel” site for the detection of infected animals for at least a year after initial exposure to infective eggs. It is reasonable to assume that more thorough inspection of the heart would increase the number of infected animals detected. Although further inspection of the heart may not be necessary for routine screening in low prevalence regions such as Canada, it would have merit in detecting infected animals once a suspect herd is identified. In the event that no grossly or histologically identifiable cysticerci are recovered from an affected carcass, newly developed immunohistochemical and/or molecular methods may be useful in definitive diagnosis of degenerated heart lesions (Ogunremi et al., 2004a; Harrison et al., 2005; Abuseir et al., 2006).

3.6 Conclusion

This study examined the tissue localization of *T. saginata* cysticerci in experimentally infected Canadian beef cattle raised under representative extensive (outdoor) conditions. The traditional site of heart was confirmed as the tissue of choice for routine inspection based on frequency of infection, number of cysts present and increased visibility on gross inspection due to the early inflammatory response to cysts in cardiac muscle. The traditional site of oesophagus was one of the poorest of all carcass sites examined for detection of cysticerci. The data suggest that more thorough inspection of the heart would be an efficient and effective method to improve detection of infected animals at slaughter.

4. VALIDATION OF AN IMMUNOHISTOCHEMICAL ASSAY FOR BOVINE CYSTICERCOSIS

4.1 Abstract

The larval stage (syn *Cysticercus bovis*) of the human tapeworm *Taenia saginata* causes cysticercosis in cattle, which has both aesthetic and food safety implications to consumers of beef. Bovine cysticercosis occurs worldwide, and is a federally reportable disease in Canada with requisite regulations for its control. Nevertheless, diagnostic challenges exist at every step in the control effort, from recognition and confirmation of the parasite at slaughter to identification of the source of infection. Currently, diagnosis of suspect lesions recovered during meat inspection relies on gross, stereomicroscopic, or standard histological examination. Such lesions are often caused by degenerating cysticerci that cannot be definitively identified by these methods. The Centre for Food-borne and Animal Parasitology of the Canadian Food Inspection Agency (CFIA) has developed a monoclonal antibody-based immunohistochemical (IHC) assay to improve the postmortem diagnosis of this parasite. In the present study, both the IHC and currently implemented histological assays were standardized and evaluated using 169 known-positive *T. saginata* cysticerci from experimentally and naturally infected cattle, and known-negative specimens and lesions of various etiologies from non-infected cattle. The IHC assay identified significantly more known positive bovine cysticerci than the histological method (91.7% and 38.5%, respectively), and non-specifically stained only the other cestode species examined. Use of the IHC assay will improve our ability to reliably diagnose lesions caused by degenerated cysticerci, and thus facilitate more effective and efficient control of bovine cysticercosis.

4.2 Introduction

Bovine cysticercosis, caused by the intermediate stage (syn *Cysticercus bovis*) of the human tapeworm *Taenia saginata*, has both aesthetic and food safety implications to consumers of beef and is a reportable zoonotic disease in Canada (CFIA Health of Animals Regulations,

1991). Although the apparent prevalence of *T. saginata* cysticercosis in Canada is relatively low (Pawlowski and Murrel, 2001; Cabaret, 2002, OIE, 2004c), globalization, travel from endemic regions, and recycling of human sewage on livestock pasture (MacPherson, 1978) increase the risk of infection to Canadian cattle. Several Canadian outbreaks have been reported (McAninch, 1974; Bundza et al., 1988; Borman-Eby et al., 1994), and in recent years, there have been cases in Ontario, Quebec, and Alberta (Lees et al., 2002).

Although federal regulations exist for control of bovine cysticercosis, diagnostic challenges occur at every stage of the control cycle, from recognition and confirmation of the parasite at slaughter to identification of the source of infection to the affected cattle (McAninch, 1974; Bundza et al., 1988; Borman-Eby et al., 1994; Scandrett and Gajadhar, 2004). The control measures currently implemented by the Canadian Food Inspection Agency (CFIA) rely on detection of cysticerci in affected carcasses during routine gross postmortem inspection. Confirmed or suspected cases of cysticercosis result in substantial financial costs to affected cattle producers and the CFIA, which is responsible for implementing regulatory measures including trace-back, quarantine, enhanced screening of suspect animals at slaughter, and compensation for condemned carcasses (CFIA Disease Control Manual of Procedures, Section 13, 2000). The inspection protocol involves incision and/or palpation of the tongue, internal (pterygoid) and external masseters, esophagus, heart, and diaphragm, and observation of superficial and cut surfaces of the carcass exposed during routine dressing procedures (CFIA Meat Hygiene Manual of Procedures, Section 4.6.1, 2007). This is similar to inspection protocols implemented for this parasite in the USA (Snyder and Murrel, 1986; Saini et al., 1997) and Europe (Kyvsgaard et al., 1990). These ‘traditional’ inspection sites are easily accessed during routine slaughter and inflict minimal carcass damage. Although it is increasingly accepted that true predilection sites for this parasite do not exist, the traditional sites currently inspected are amongst the best for detecting infection. Recent work in our laboratory at the Centre for Food-borne and Animal Parasitology did not identify any inspection sites more likely than traditional sites to harbour cysts in an infected carcass and confirmed that the heart is the optimal site for detection based on cyst density, frequency of infection at this site, and increased visibility of lesions due to an early inflammatory response against degenerating cysticerci in cardiac muscle (Chapter 3). As well, the heart is traditionally already one of the more thoroughly examined inspection sites (CFIA Meat Hygiene Manual of Procedures, Section 4.6.1, 2007). Cysts that are

non-viable and are undergoing degeneration can vary in appearance depending upon the degree of inflammation, necrosis, and mineralization in the resulting lesion (Geerts et al., 1980). Cattle can harbor both viable (infective) and degenerate cysts concurrently (Juranek et al., 1976); thus recovery of only degenerate cysts does not imply absence of infective cysts in the carcass, or in herd mates. As well, viable cysts can persist in cattle for at least two to three years, and possibly for the life of the host (Penfold, 1937; Dewhirst et al., 1963; Froyd, 1964; Urquhart and Brocklesby, 1965; Van den Heever, 1967). Therefore, it is important to confirm cysticercosis even in cases where suspect lesions are obviously degenerated and non-infective. Degenerated lesions comprise the bulk of diagnostic submissions for this parasite to the CFIA laboratory since such lesions are more likely to be detected by inspectors than viable cysticerci, which are translucent and blend in with the surrounding host tissues. Definitive diagnosis of degenerated lesions is often impossible using current gross or histological methods since the lesions no longer contain identifiable parasite features, and are consistent histologically with chronic lesions of other etiologies. There have been many studies to characterize the histopathology of cysticercosis, most often staining the tissues with haematoxylin-eosin, (Silverman and Hulland, 1961; Retzlaff, 1972; Sterba and Dykova, 1978; Sterba et al., 1979ab; Safranov and Drozan, 1985; Aluja and Vargas, 1988; Zivkovic et al., 1996), some of which describe grading criteria based on characteristic histological features for bovine or porcine cysticerci at various stages of degeneration. However, definitive diagnosis requires that morphological features of the parasite can be identified (Silverman and Hulland, 1961; Geerts et al., 1980; Marty and Chester, 1997). This is especially challenging for bovine cysticercosis because *T. saginata* cysticerci do not have rostellar hooks, and cysticerci in the heart and other tissues may not be fully developed prior to the onset of degeneration (Silverman and Hulland, 1961; Machnicka et al., 1977). Although histoenzymatic, histochemical and immunohistochemical methods have been used in morphological, physiological and immunological studies of cysticercosis, they have not been used for definitive identification of degenerated cysticerci (Gustowska and Pawlowski, 1981; Marty and Chester, 1997; Londono et al., 2002; Perez-Torres et al., 2002).

“Molecular morphology” provided by combining morphologic detail of histological sections with concurrent visualization of target antigens makes immunohistochemistry a powerful diagnostic tool (Haines and West, 2005; Taylor, 2006). In the current study, an immunohistochemical (IHC) assay for degenerated bovine cysticerci previously developed by

Ogunremi et al. (2004a) was optimized, standardized, and compared to the standardized histological hematoxylin-phloxine-safran (HPS) staining method currently implemented by the CFIA for diagnosis of cysticercosis, using known-positive *T. saginata* cysticerci from experimentally and naturally infected cattle and a variety of known-negative lesions from cysticercosis-negative cattle. The results indicate superior performance of the immunohistochemical assay and support its adoption for routine diagnostic use. This improved assay will increase confidence in the final diagnosis, and facilitate more effective and efficient disease control. Selection of such an assay with fitness for intended use is the first step in quality assured immunohistochemical testing (Taylor, 2006). Assays having acceptable accuracy (sensitivity and specificity), and precision (repeatability/reproducibility) for their intended use are considered reliable and are essential not only to ensure confidence in diagnostic results used to ensure domestic food safety and disease control, but increasingly to meet international standards for export of animals and their products (ISO, 1999; Greiner and Gardner, 2000; Dabbs, 2002; Gajadhar and Forbes, 2002; OIE, 2004a, b).

4.3 Materials and methods

4.3.1 Collection and processing of known-positive and known-negative specimens

One hundred and sixty-three cysticerci were collected from five mixed-breed beef calves, of both sexes, from a local research herd with no history of cysticercosis, that had been experimentally infected at approximately 9-11 months of age with 1000 (2 calves) or 10000 (3 calves) *T. saginata* eggs obtained from Thailand, as previously described (Chapter 3). Calves were killed between 412 and 466 days post-inoculation (DPI) and cysticerci were recovered by sectioning of postmortem tissues into 0.5 cm thick slices with a commercial meat slicer (Chapter 3). Cysticerci were carefully excised from the surrounding tissue matrix, and stored in phosphate buffered saline (PBS) at 4° C for up to two days. Specimens were collected from heart, skeletal muscle, and liver; one specimen was collected from the serosal surface of the rumen. The approximately oval cysticerci were sectioned in half perpendicular to the long axis. Those with clear to pinkish translucent cyst walls containing a larval cestode with an opaque scolex-anlagen and fluid-filled bladder were considered viable. Degenerated cysticerci were those with grey, tan, or pinkish translucent to opaque cyst walls of varying thickness, containing green, yellow, white,

to grey/brown, caseous to mineralized contents. Only degenerated cysticerci were collected as test specimens for this study; two viable specimens harvested from the heart of an experimentally infected calf killed 47 DPI in a previous study (Chapter 3) were used in the IHC assay for antibody titration and as a source of positive control sections for both assays. Animal identification, tissue origin and gross descriptions were recorded for each specimen. One half of each cysticercus was placed in a labelled plastic vial and fixed in 10% neutral buffered formalin for 24-48 hrs, after which the formalin was decanted and replaced with 70% ethanol until paraffin embedding of the specimens. The other half of each cysticercus was placed in magnesium-free PCR buffer in a plastic vial and stored frozen at -70° C. Seven cysticerci obtained from seven infected cattle in recent field outbreaks in Alberta and Ontario were also collected and similarly processed.

Tissue specimens representing other infectious or neoplastic etiologies that were grossly similar to *T. saginata* cysticerci (Dukes et al., 1982, Bundza et al., 1986; Smith et al., 1991b) were also collected from a variety of sources. These consisted of specimens of eosinophilic myositis/sarcocystosis (n=14), intracardiac schwannoma (n=1), myocardial epithelial inclusions (n=1), myocardial cyst of unknown etiology (n=1), focal myocardial fibrosis (n=1), focally extensive plasmohistiocytic myocarditis (n=1), and multifocal lymphocytic myocarditis (n=1). Other common bovine parasites that might stain in the immunohistochemistry assay were also collected, consisting of specimens of *Fasciola hepatica* (n=1), *Fascioloides magna* (n=1), *Dicrocoelium dendriticum* (n=1), and *Monezia sp.* (n=1), as were other taeniids *Taenia ovis* (n=4) and *Thysanosoma actinoides* (n=1) from sheep, and *Echinococcus granulosus* (n=1) from an elk. Miscellaneous other specimens included mineralized hepatic granulomata from a muskox (n=1), multifocal hepatic lipomatous metaplasia from a llama (n=1), and a pulmonary cyst from a whitetail deer (n=1). Additionally, samples of bovine lymph node and hemolymph node were obtained from cattle free of bovine cysticercosis to serve as tissue controls. Specimens, if not already fixed and/or blocked, were placed in individual labeled vials, and fixed as described above.

Each known-positive experimentally generated specimen was consecutively numbered and three such specimens were randomly assigned to one of three standardized positions in each tissue cassette (*Histosette II*; Simport Plastics, Beloeil, QC, Canada) for paraffin embedding. The position of each specimen in each cassette was recorded. Specimens were oriented in the cassette

such that their cut surface faced the microtome surface of the block. Each cassette of three specimens was consecutively numbered. Paraffin embedding was performed following standard procedures. Five μm -thick sections were cut and placed on either charged poly-L-lysine (*Superfrost plus*; VWR Scientific, West Chester, PA, USA) or regular glass slides for IHC and histology, respectively. A total of nine sections were cut from each block and each was assigned to a slide for histological or immunohistochemical staining as follows: The first section from the first block was randomly assigned to either the histological or IHC staining method. Assignment of the first section from subsequent consecutive blocks alternated between each method. For a first section assigned to histology, sections 2 and 3 were assigned to IHC, section 4 to histology, sections 5 and 6 to IHC, section 7 to histology, and sections 8 and 9 to IHC. For a first section assigned to IHC, section 2 was also assigned to IHC, section 3 to histology, sections 4 and 5 to IHC, section 6 to histology, sections 7 and 8 to IHC, and section 9 to histology (Fig. 4.1). This process was designed to control for the variability of the diagnostic material in the specimens, and generated three replicates of each specimen for each method, with each IHC replicate consisting of a test section for staining with the relevant primary antibody and an adjacent section for staining with the irrelevant primary antibody (the latter as a control for background staining). Slides were labeled with the tissue block number, section number (1-9), and three specimen numbers in their respective positions in the block. For IHC staining of sequential paired sections, the first section to come off the block received the relevant antibody and the adjacent second section was stained with the irrelevant antibody.

Known-positive and known-negative field-derived specimens were pooled and randomly assigned to consecutively numbered blocks and processed as described above for known-positive experimental specimens, with the following exceptions: the specimens of intracardiac schwannoma, myocardial epithelial inclusions, myocardial cyst of unknown etiology, focally extensive plasmohistiocytic myocarditis, multifocal lymphocytic myocarditis, *F. hepatica*, *F. magna*, *Monezia sp.*, as well as one of the *T. ovis*. specimens were blocked individually. One section of each such specimen was stained with the relevant Mab only, except for the *T. ovis* specimen for which an adjacent control section was also stained with the irrelevant Mab.

Controls consisting of two viable cysticerci, and of lymph node and hemolymph node, were prepared similarly to the known-positive experimental specimens.

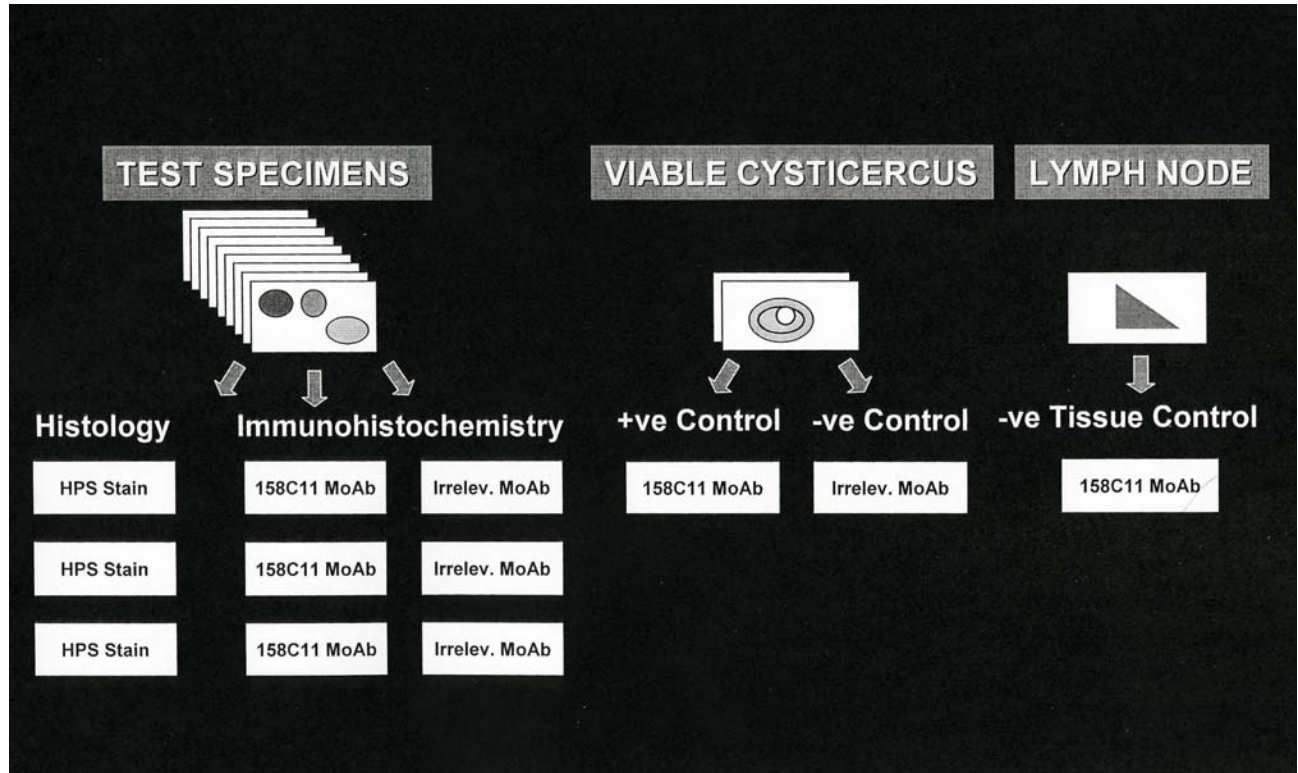


Figure 4.1. Experimental design for allocation of test specimen sections to hematoxylin-phloxine-safran (HPS) histological or immunohistochemical (IHC) staining methods. Each of nine consecutive tissue sections from each paraffin specimen block was assigned to either HPS or IHC and placed on a regular or charged glass slide, respectively. The method to which the first section from each block was assigned alternated between successive blocks. A first section assigned to HPS (as illustrated) was followed by a consecutive section assigned to IHC staining with the relevant (158C11) monoclonal antibody (MoAb) section, followed by a consecutive section assigned to IHC staining with the irrelevant MoAb. A first section assigned to IHC received the relevant MoAb, the next section the irrelevant MoAb, and the next section assigned to HPS. Either pattern was repeated until all 9 sections were allocated, resulting in 3 replicates for each assay. For IHC staining of each replicate from one or more specimens, 2 sections from a viable cysticercus, each stained with the relevant or irrelevant MoAb, served as positive and negative controls, respectively, and a section of hemal lymph node stained with the relevant MoAb served as a negative tissue control.

4.3.2 Antibodies for immunohistochemical analysis

The relevant primary antibody was a purified mouse ascites myeloma-derived monoclonal IgG₁ designated *158C₁₁A₁₀* developed against excretory/secretory (ES) antigens of 12 week old *T. saginata* cysticerci recovered from experimentally infected calves (Draelants et al., 1995), and was kindly provided by Stanny Geerts and Jef Brandt of the Institute of Tropical Medicine, Antwerpen, Belgium. The irrelevant primary antibody was a mouse myeloma-derived monoclonal IgG₁ designated *2BD 4E4* (# HB-8178, American Type Culture Collection (ATCC), Manassas, VA, USA) directed against *Escherichia coli* K99 pilus antigen, kindly provided by Dale Godson, Prairie Diagnostic Services, Saskatoon. Protein concentrations of antibody suspensions (reconstituted in sterile water) were 0.91 and 3.35 mg/ml, respectively (*Bethyl Mouse IgG Elisa Quantitation Kit*; Bethyl Inc., Montgomery, TX, USA). A commercial biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA) was used as the secondary antibody and prepared for use as directed by the manufacturer. All antibody suspensions were diluted in a 2:1 ratio in peroxidase conjugate stabilizer (*Superfreeze*; Pierce, Rockford, IL, USA), and the resulting suspensions dispensed in 150 µl aliquots into 1.5 ml plastic microcentrifuge tubes (Bio-Rad Laboratories, Hercules, CA, USA) and stored at -20° C until used.

4.3.3 Histological method (Hematoxylin-Phloxine-Safran “HPS” Staining)

All HPS staining of sections was performed by Nicole Viau and Yves Robinson of the CFIA Laboratory in St. Hyacinthe, Quebec, following a standardized protocol (Appendix A). Prepared slides were deparaffinized and re-hydrated by sequential immersion in toluene, ethanol (absolute, 95%, and 80%), and distilled water. Slides were then processed manually in the following manner: immersion in picric acid for 5 min followed by a water rinse, staining in Mayer’s hematoxylin for 20 min followed by a water rinse for 7 min, staining in 1.5 % aqueous Phloxine B solution for 1-2 min followed by a tap water rinse for 5 min. After dehydration in three changes of 95 %, followed by one of absolute ethanol, for 1 min each, slides were stained in 2 % alcoholic safran for 4 min, and rinsed in two to three changes of absolute ethanol followed by two changes of toluene, for 2 min each. Coverslips were placed over the sections with mounting medium (*Permount*; Biomedex, Foster City, CA, USA) and slides allowed to dry before viewing under light microscopy at 4-60 X.

4.3.3.1 Interpretation of HPS-stained sections

Stained slides were randomized and assigned a coded identification (blinded) prior to being examined. For sections containing multiple specimens, specimens were examined in the numerical order in which they were positioned in the block. Specimens were classed as mineralized if at least two of the three replicate sections had one or more foci of mineralization. Specimens were considered positive for cysticercosis if one or more sections yielded a positive result. Results were determined using the following criteria:

Positive: Sections were considered positive if calcareous corpuscles and/or putative *T. saginata* cysticercus scolex or bladder (which could be identified as such even in the absence of calcareous corpuscles) were observed. Sections in which suspect parasite tissue was unidentifiable due to degenerative changes, but which formed the typical outline/shape of a cysticercus (cysticercus “ghost”), were also considered positive (Fig. 4.2).

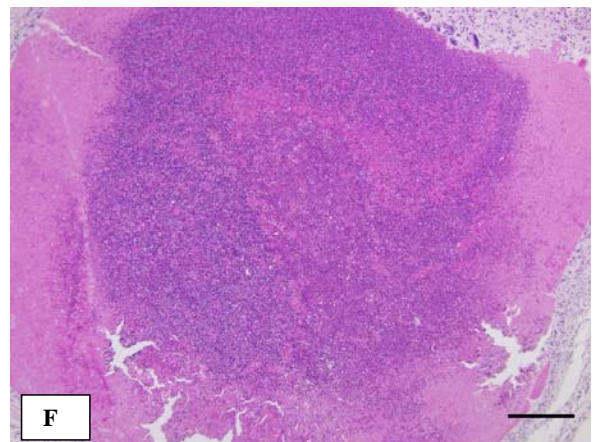
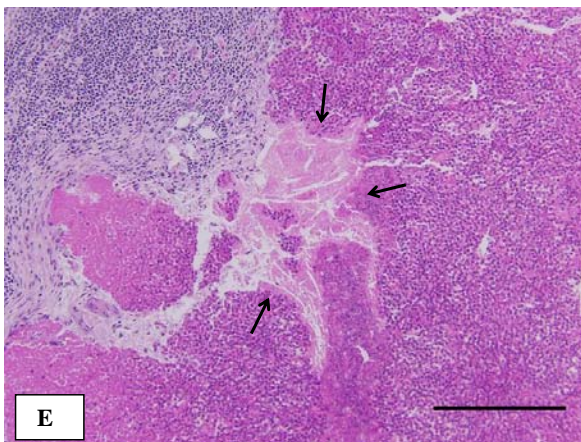
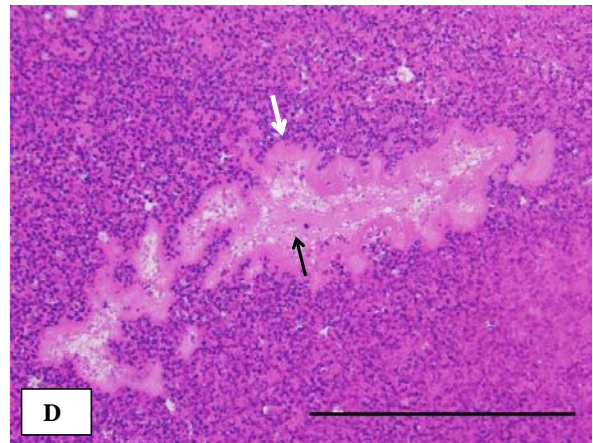
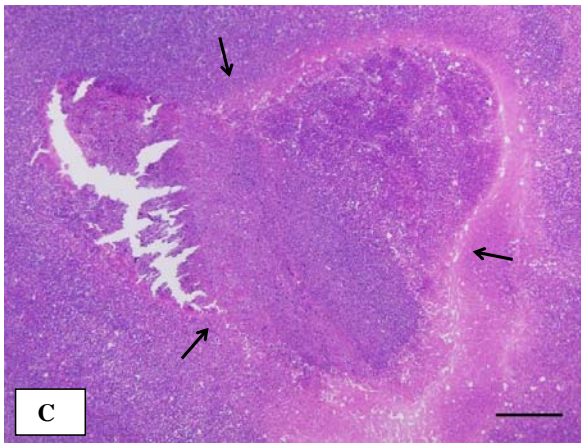
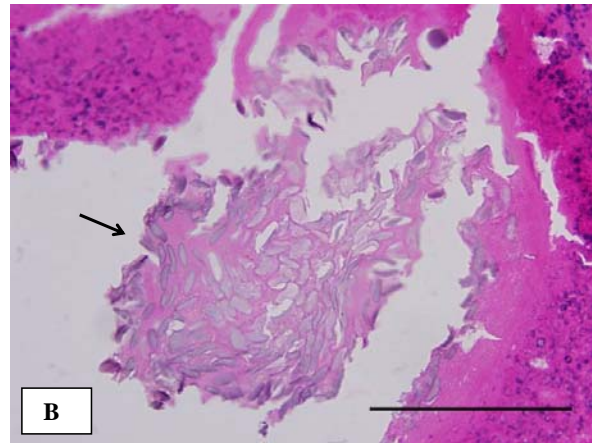
Negative: Sections that did not meet the above criteria, including those for which another etiology was evident, were considered negative (Fig. 4.2).

4.3.4 Immunohistochemical method (IHC)

A standardized method modified from that of Ogunremi et al. (2004) was used for immunohistochemical staining (Appendix B). Each staining batch consisted of the following: paired sections of the positive control and of the test specimens, for which one of each pair received the relevant and the other the irrelevant Mab. A single section of the negative tissue control received the relevant Mab. Sections on charged slides were deparaffinized and re-hydrated by sequential immersion in xylene, ethanol (absolute, 95%, and 70%), and distilled water. Endogenous peroxidases were inactivated by immersion in 4% H₂O₂ in methanol for 12 min at room temperature. Slides were washed three times in 10% 10X automation buffer (Biomedex, Foster City, CA, USA; diluted with distilled water) and incubated in a 0.05% protease XIV solution (Sigma-Aldrich Co., St. Louis, MO, USA) in Dulbecco's phosphate buffered saline (PBS) (Gibco, Invitrogen Canada Inc., Burlington, ON, Canada; prepared with sterile water) at 37° C for 20 min. Slides were washed three times in automation buffer, and non-specific antibody binding sites blocked by sequential immersion in PBS solutions of 4% normal horse serum (Gibco, Invitrogen Canada Inc., Burlington, ON, Canada) and 2% non-fat rehydrated dried milk (Bio-Rad Laboratories, Hercules, CA, USA), each for 10 min. Slides were then

Figure 4.2. Criteria for determination of positive or negative results for study specimens tested by the hematoxylin-phloxine-safran (HPS) histological method.

HPS-stained sections of representative *Taenia saginata* cysticerci from experimentally infected cattle in photomicrographs A-D demonstrate features required for a positive histological result; those in E and F are negative. (A) Section of scolex (thick arrow) and bladder wall (fine arrows) of intact (viable) *Taenia saginata* cysticercus. (B) Aggregate of calcareous corpuscles (arrow) in central necrotic core of degenerated *T. saginata* cysticercus. (C) Outline (delineated by arrows) or “ghost” of a degenerated *T. saginata* cysticercus. (D) Definitive necrotic remnant of bladder wall of a degenerated *T. saginata* cysticercus demonstrating hyalinized tegument (white arrow) and reticular parenchyma (black arrow). (E) Non-definitive necrotic remnant (delineated by arrows) of putative *T. saginata* cysticercus. (F) Chronic granulomatous lesion of putative *T. saginata* cysticercus etiology with no identifiable parasite features. All bars except (B) = 200 μm ; bar in (B) = 100 μm .



quickly rinsed with 4% normal horse serum, blotted on edge to remove excess serum, the sections circumscribed with a PAP pen (Daco Cytomation Inc., Mississauga, ON, Canada), and transferred to a plastic humidity chamber to prevent drying of the sections. Separate humidity chambers were used for sections stained with the relevant (*158C_{11A10}*) or irrelevant (*2BD 4E4*) Mab. Representative sections of viable and degenerated cysticerci had been previously stained using combinations of the relevant primary antibody at dilutions of 1:100, 1:250, and 1:500, and secondary antibody at dilutions of 1:400 and 1:800. The 1:250 dilution of primary antibody and 1:400 dilution of secondary antibody gave the best distinction between specific positive staining and non-specific background staining (Appendix C). Thus, Mab *158C_{11A10}* was used diluted 1:250 in 4% normal horse serum and applied dropwise until the entire section was covered. Mab *2BD 4E4* diluted 1:500 was similarly applied to the irrelevant antibody control sections. Slides were then incubated in the humidity chamber overnight (16-18 h) at 4° C. Slides were removed from the incubation chamber and the antibody solution removed by a gentle stream of 1X automation buffer from a plastic rinse bottle, then washed three times in 1X automation buffer. Slides were blotted on edge to remove excess buffer, and quickly placed back in the humidity chamber. Biotinylated horse anti-mouse secondary antibody diluted 1:400 in 4% normal horse serum was applied dropwise to all slides as described above. Slides were then incubated at 37° C for 30 min. Slides were rinsed, washed, blotted, and avidin-biotin-peroxidase complex solution (Vector Laboratories, Burlingame, CA, USA) prepared and applied to the sections according to the manufacturer's instructions. Slides were incubated in the humidity chamber at 37° C for 45 min, then removed, rinsed, and washed in automation buffer as before, and H₂O₂-activated 3, 3'-diaminobenzidine tetrahydrochloride (DAB; Electron Microscopy Sciences, Washington, PA, USA) added dropwise until sections were covered. The slides were incubated at room temperature for 4 min followed by thorough rinsing in automation buffer to stop the chromogen reaction. Slides were then counterstained with 10% Gill's hematoxylin in distilled water for 1 min, followed by standard dehydration through distilled water, ethanol (70%, 95%, absolute), and xylene. Coverslips were applied to the sections with mounting medium (*Cytoseal*; Richard-Allen Scientific, Kalamazoo, MI, USA). Slides were viewed under light microscopy at 4-60 X.

4.3.4.1 Interpretation of IHC-stained sections

Stained slides were coded to blind the examiner, and specimens assessed as described for

the histological examination. Specimens were considered positive for cysticercosis if one or more sections yielded a positive result. Results were determined using the following criteria:

Positive: Sections were positive if there was focal to extensive, moderate to dark, chromogen staining, in a manner consistent with the expected distribution of the targeted antigen, of a putative *T. saginata* cysticercus (with or without differential staining of tegumental excretory/secretory and parenchymal regions of parasite). There was a clear distinction between stained putative parasite and unstained host tissue with either a well-demarcated or blended border (due to presumed “leaching/diffusion” of antigen) between these two matrices (Fig 4.3).

Negative: Sections were considered negative if there was no chromogen staining present, or if non-specific background staining was present with no apparent preferential staining of a putative *T. saginata* cysticercus. Sections in which another etiology was evident were considered negative, regardless of extent of non-specific background staining, if there was no preferential staining of the etiological agent.

Nonspecific background and cross-reactive staining: For all sections, non-specific background staining was noted if present. Minor nonspecific staining was recorded as focal, multifocal, edge or border (peripheral) staining of the lesion; more extensive to diffuse non-specific staining was recorded as such. Any staining of (presumably similar) parasite antigen in the expected distribution for the etiological agent in known negative specimens was considered cross-reactive.

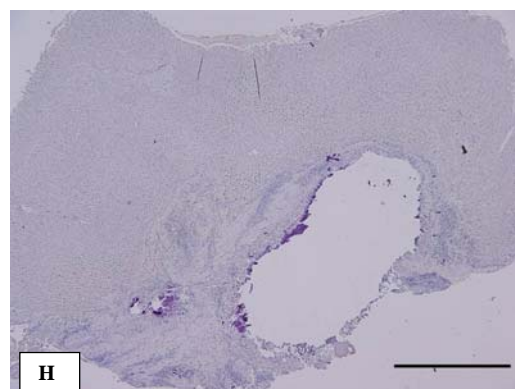
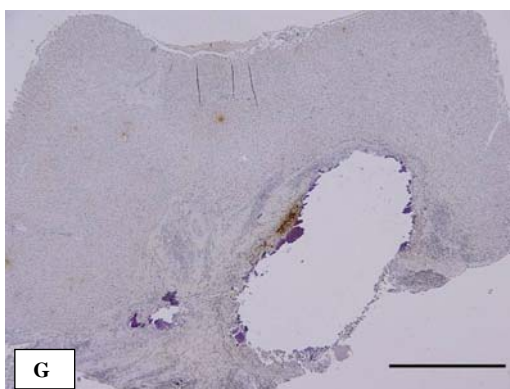
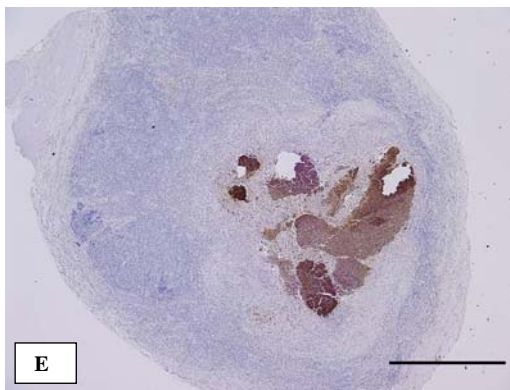
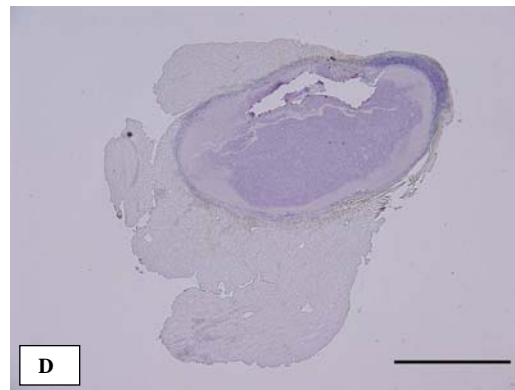
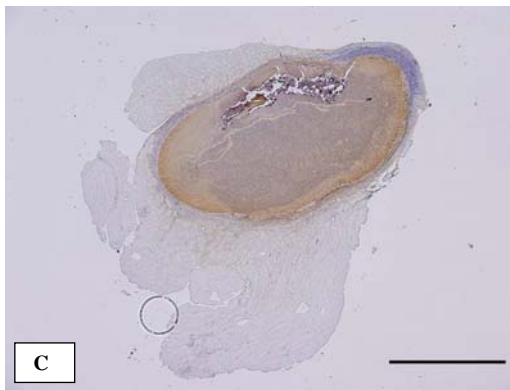
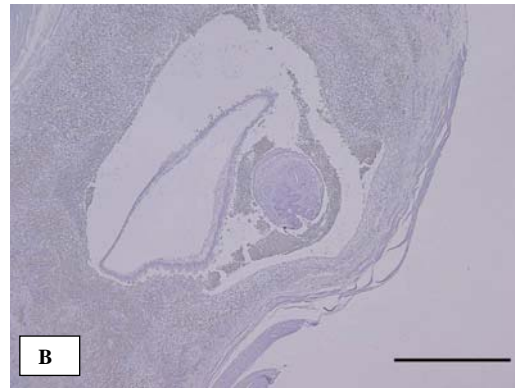
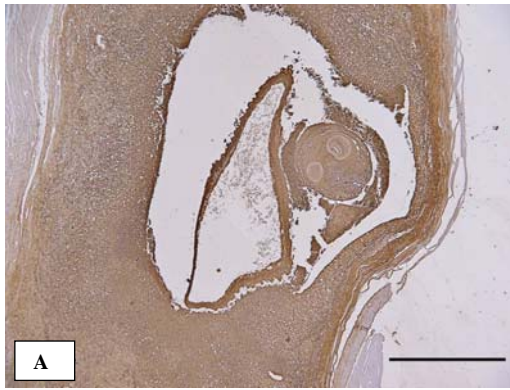
Sections for which there was very light or sparse chromogen staining of a suspect *T. saginata* cysticercus with or without a clear distinction between stained parasite and unstained (counterstained) regions were re-examined following access to specimen and stain identity (relevant vs irrelevant Mab). The sections stained with the relevant Mab were viewed in conjunction with their irrelevant negative control to reach a negative or positive result. Representative sections of such specimens were also stained with Perl’s Prussian blue and Gram’s stains to rule out nonspecific staining of hemosiderin and bacteria, respectively.

Immunostained sections in which calcareous corpuscles were identified were also noted.

4.3.5 Statistical analysis

Statistical analysis was performed by Sarah Parker of the Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine. Since the HPS and IHC methods

Figure 4.3. Criteria for determination of positive or negative results for study specimens tested by the immunohistochemical (IHC) method. IHC relevant antibody-stained sections of representative *Taenia saginata* cysticerci from experimentally infected cattle in photomicrographs A, C, E, and G demonstrate the range of acceptable staining patterns required for a positive result. Photomicrographs B, D, F, and H represent the respective negative control sections stained with the irrelevant antibody and counterstained with hematoxylin. (A) Scolex and bladder wall of intact (viable) *T. saginata* cysticercus showing diffuse IHC staining of parasite and surrounding host capsule and tissue matrix. (C) Degenerated *T. saginata* cysticercus with diffuse staining of necrotic core of lesion. (E) Degenerated *T. saginata* cysticercus with multifocal staining of areas of putative parasite antigen. (G) Degenerated *T. saginata* cysticercus with focal staining of putative residual parasite antigen. Bar = 1mm.



each tested portions of the same specimen, statistical techniques appropriate for comparing paired samples were used. The proportion of specimens identified as positive by each test was compared with McNemar's chi-square test (Shoukri and Pause, 1999). Agreement between tests was measured with Kappa (Shoukri and Pause, 1999). The univariate effect of other factors such as animal, tissue matrix, position of the specimen in the block and source of the specimen on the performance of each test were evaluated using a Fisher's exact chi-square. Factors were considered for multivariate analysis if they had a significant individual effect ($p = 0.2$). Multivariate logistic regression was used to test for whether factors together had an effect on test outcome and to test for the presence of interaction (Dohoo et al., 2003). Statistical tests were conducted with a commercial software package (Statistix, Version 8).

Repeatability between replicates for identifying known-positive specimens was calculated using an analysis of variance based coefficient of agreement (Shoukri and Pause, 1999) which was conducted with a commercial spreadsheet software package (Excel, 2002).

4.4 Results

Grossly, recovered degenerated cysticerci were round, oval or fusiform in shape, and approximately 2 -10 mm in maximum dimension. Most consisted of a grey to tan to pinkish, translucent to opaque capsule surrounding a green to yellow to brown caseous core (Fig. 4.4). In some specimens, some grittiness was apparent when sectioning the lesion. Occasionally specimens appeared homogeneously granulomatous or fibrotic. Histologically, most lesions were characterized by a central mass of necrotic cellular debris, sometimes containing calcareous corpuscles or other parasite remnants, and surrounded by a granulomatous cellular zone consisting mostly of lymphocytes, plasma cells, and eosinophils. Giant cells were often found at the border of the cellular zone and the necrotic core. Fibroblasts, fibrocytes, and collagen formed a distinct capsule peripheral to the cellular zone (Fig. 4.5). All sections from one of the 163 experimentally generated cysticerci consisted of only skeletal muscle and were excluded from further analysis. Seventy-seven (45.6 %) of the specimens demonstrated focal or multifocal mineralization in at least two replicates and were classed as mineralized; the other 92 (54.4 %) specimens were classed as caseous (Table 4.1). The majority of cysticerci recovered from the experimentally infected animals were from skeletal muscle and all but one of the field specimens were from the heart. All but one of the liver cysticerci were recovered from a single



Figure 4.4. Typical gross appearance of a degenerated *Taenia saginata* cysticercus. (A) shows the cysticercus *in situ*. (B) shows the same cysticercus incised to reveal caseous necrotic contents. Specimen was recovered from the skeletal muscle of an experimentally infected animal killed 412 days post-inoculation. Bar = approximately 1 mm.

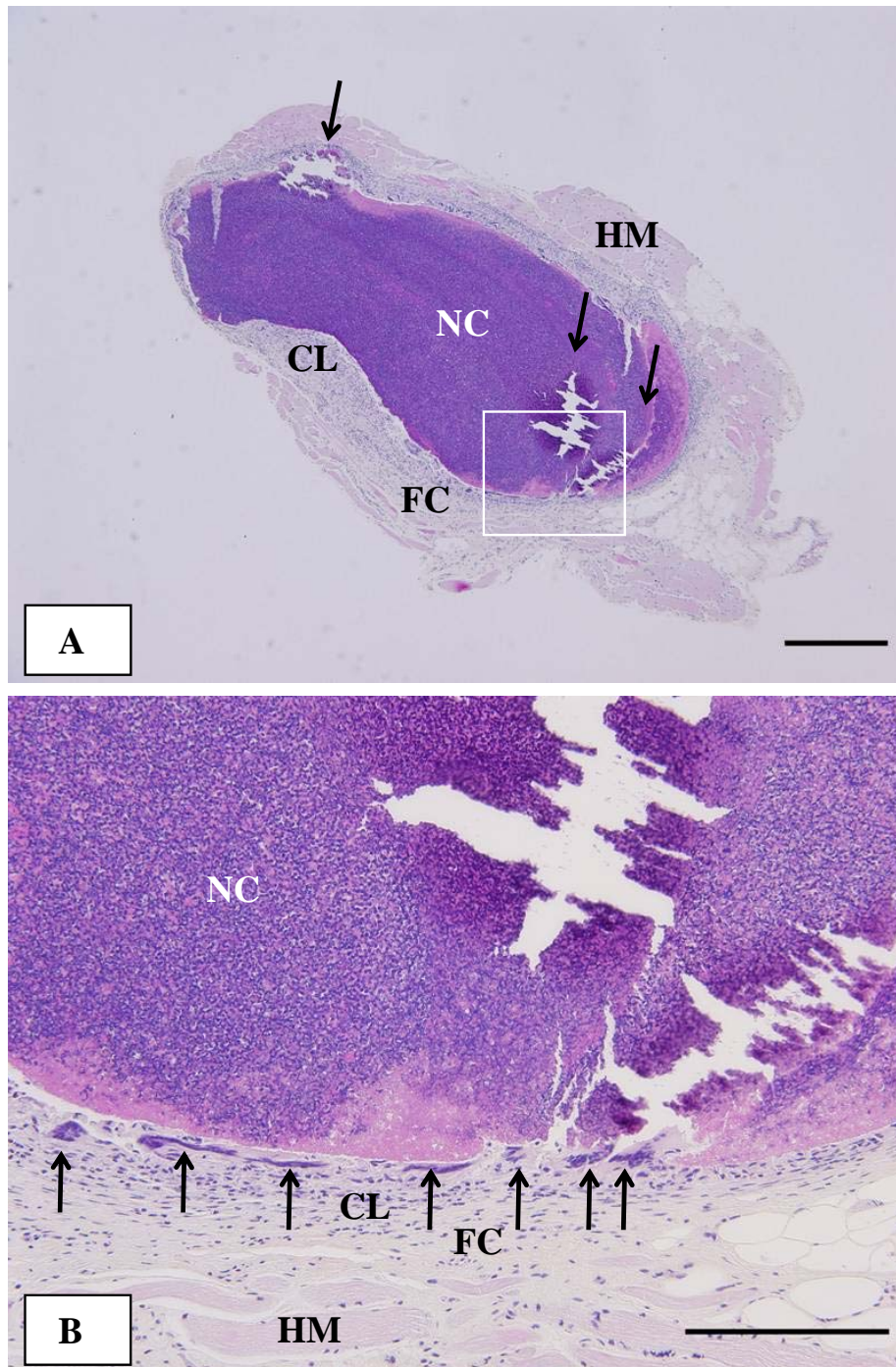


Figure 4.5. Typical histological appearance of a degenerated *Taenia saginata* cysticercus stained with hematoxylin-phloxine-safran method. (A) 4X magnification demonstrates the caseous necrotic core (NC) with focal areas of mineralization (arrows), surrounded by a cellular layer of mixed inflammatory infiltrate, within a fibrous capsule (FC) of host origin. Host matrix (HM), in this case, skeletal muscle, surrounds the cysticercus. Bar = 500 μ m. (B) 20X magnification of inset area in (A) shows a line of giant cells (arrows) at the border between the necrotic core and cellular layer. Bar = 200 μ m. Specimen was recovered from an experimentally infected animal killed 454 days post-inoculation.

Table 4.1: Number, origin and histological determination as mineralized or caseous for known-positive degenerated *Taenia saginata* cysticerci assayed by histological and immunohistochemical methods

Animal ID	Source	Tissue	Caseous ^a	Mineralized ^a	n
Calf # 17	Experimental	Heart		2	2
		Skeletal muscle	1	4	5
		Liver		1	1
Calf # 46	Experimental	Skeletal muscle	66	13	79
		Rumen serosa		1	1
Calf # 129	Experimental	Heart	1	3	4
		Skeletal muscle	13	7	20
Calf # 163	Experimental	Heart		2	2
		Liver	2	27	29
Calf # 401	Experimental	Heart		4	4
		Skeletal muscle	6	9	15
F1	Field	Skeletal muscle	1		1
F3	Field	Heart	1		1
F41	Field	Heart		1	1
F42	Field	Heart		1	1
F5	Field	Heart	1		1
F61	Field	Heart		1	1
F62	Field	Heart		1	1
Total			92	77	169

^a Specimens determined to be mineralized if at least 2 of 3 replicates demonstrated foci of mineralization on histological examination of hematoxylin-phloxine-safran stained sections

experimentally infected animal and all but two from that same animal were mineralized. In many of the sections of cysticerci, and most evident in the irrelevant antibody-stained controls for IHC, were large mononuclear cells containing coarse refractile brown granules. These cells were present in small focal to multifocal aggregates, sometimes forming a complete band, in the cellular zone and amongst the collagen layer at the host-parasite interface (Fig. 4.6). Staining with toluidine blue and Perl's Prussian blue to rule out mast cell granules and hemosiderin, respectively, was negative.

All positive control intact cysticerci sections were stained with the IHC method, in a distribution similar to that described by Ogunremi et al. (2004), and the corresponding irrelevant antibody-stained controls, as well as the lymphatic tissue controls were consistently negative (Fig. 4.7). Thirteen cysticerci from the experimental animals (of which seven were from liver and six were from skeletal muscle) had very limited or pale IHC staining of one or more of the three replicate sections and were assessed as "suspect" positive on initial blinded evaluation. Nine of these specimens were assessed as "suspect" in all three replicates, one specimen in two replicates, and three specimens in one replicate only. After determining specimen identities and the stain used on the sections (relevant vs irrelevant Mab), the sections stained with the relevant antibody were re-examined and interpreted in conjunction with the pertinent irrelevant antibody-stained control. As well, using additional representative sections, nonspecific chromogen staining of hemosiderin (using Perl's Prussian blue stain) or bacteria (using Gram stain) was ruled out. Based on this assessment, all but one of the "suspect" sections were classified as positive.

Specimen position in the block, whether sourced from experimental or natural infections, and animal identification did not have an effect on the proportion of known-positive cysticerci identified as positive by use of the IHC or HPS method, when tissue of origin was accounted for, and were not included in further statistical analysis of test performance. Tissue of origin (heart, skeletal muscle, or liver) did have a significant effect on the proportion of cysts identified as positive with either the IHC or HPS staining method (Fisher's exact chi-square; $p = 0.0008$, $p = 0.0001$, respectively). The difference in proportions identified with each test varied between these tissues and interaction between tissue and test method was detected. However, since for each tissue the use of the IHC method resulted in identification of more specimens as positive than did that of the HPS stain, a summary statistical analysis was used to compare the overall

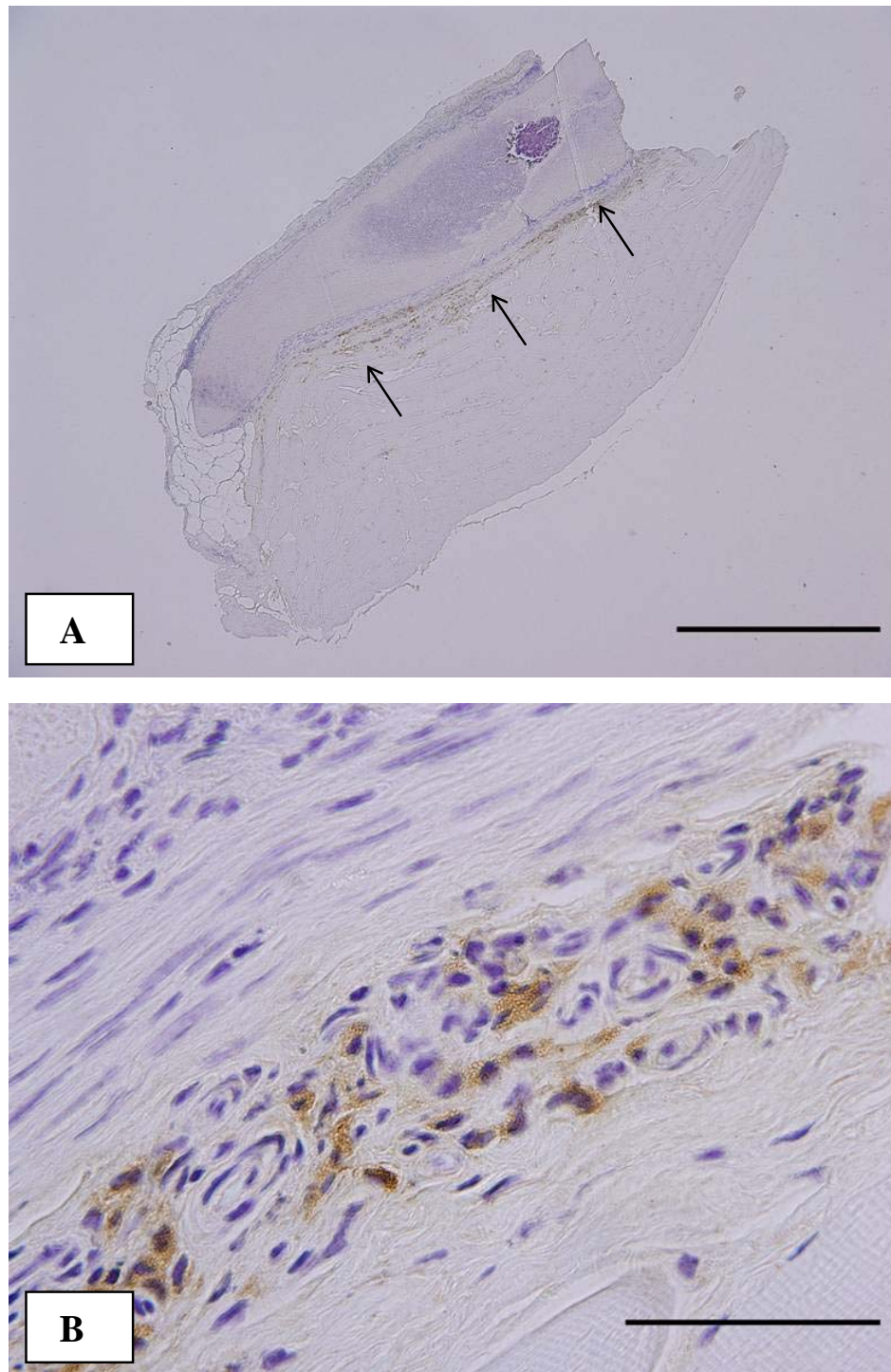
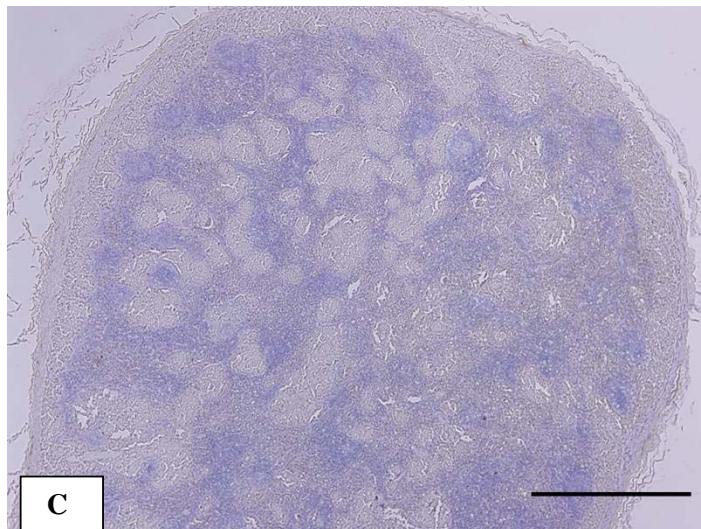


Figure 4.6. Band of cells containing coarse brown granules at the border of fibrous capsule and host skeletal muscle in a tissue section of a degenerated *Taenia saginata* cysticercus. Immunohistochemistry negative control section stained with irrelevant antibody and counterstained with hematoxylin. (A) 4X magnification. Bar = 1 mm. (B) Oil immersion 100X magnification. Bar = 50 μ m. Specimen was recovered from an experimentally infected animal killed 454 days post-inoculation.

Figure 4.7. Representative positive, negative, and tissue control sections used in the immunohistochemical staining method. (A) Positive control tissue section of intact (viable) *Taenia saginata* cysticercus (thick arrow points to scolex; small arrows to bladder wall); tissue is from an experimentally infected animal and was stained with the anti-*T. saginata* cysticercus relevant antibody. (B) Adjacent section to (A) stained with the irrelevant antibody as a negative control. (C) Section of a hemolymph node from an uninfected animal that received the relevant antibody as a tissue control. Bar = 1 mm.



proportion of specimens identified as positive by each method (confounding was controlled for by the experimental design in which each method tested paired portions of the same specimens; Shoukri and Pause, 1999).

In overall assessment of the specimens (i.e. based on results from all three replicates) use of the IHC method resulted in the identification of 155 of the 169 known positive cysticerci for an overall proportion positive of 91.7 %, while use of the histological (HPS) method identified 65 of the 169 known positive cysticerci for an overall proportion positive of 38.5 % (Table 4.2). The IHC method enabled identification of significantly more cysticerci than the HPS stain ($p < 0.0001$; McNemar's chi-square). The proportion positive identified by use of IHC for experimental and field cysticerci was 92.0 % and 85.7 %, respectively, and by HPS, 37.7 % and 57.1 %, respectively. All 18 cysticerci from the heart of experimental or field animals were identified by use of the IHC method, compared to identification of 95.0 % and 73.3 % of cysticerci from the skeletal muscle and liver, respectively. Of the six cysticerci in skeletal muscle not identified using the IHC method, one was a field specimen with sarcocysts and associated inflammation consistent with eosinophilic myositis/sarcocystosis, four were dense mononuclear granulomas interspersed with fibrosis, and one consisted of loose connective tissue. The use of the HPS method identified 66.7% of cysticerci from the heart of experimental or field animals, and 42.5 % and 6.7 % of cysticerci from the skeletal muscle and liver, respectively. Within all three tissues, use of IHC identified significantly more cysticerci than did the HPS stain (McNemar's chi-square; $p = 0.02$, $p < 0.0001$, $p < 0.0001$, for heart, skeletal muscle, and liver, respectively). The single cysticercus recovered from the rumen serosa of an experimentally infected animal was positive by IHC staining and negative by HPS staining. The proportion identified as positive with IHC staining for mineralized and caseous cysticerci was 89.6 % (69/77) and 93.5 % (86/92), respectively, and with HPS staining, 40.3 % (31/77) and 33.7% (34/92), respectively.

Calcareous corpuscles, ellipsoid-shaped and approximately 10-20 μm in maximum dimension, were observed singly or in small to large aggregates in sections, and had a refractile appearance. Individual calcareous corpuscles were either unstained, or variably stained eosinophilic or basophilic with the HPS stain, and unstained or variably basophilic with IHC staining. For the 65 cysticerci identified as positive with the HPS stain (of the three replicate sections examined per specimen), 31(47.7%) had calcareous corpuscles only, eight (12.3 %) had

Table 4.2: Results of histological (HPS) and immunohistochemical (IHC) methods to identify known-positive *Taenia saginata* cysticerci. **(A)** Overall results for all known-positive cysticerci. **(B,C)** Results for experimental or field known-positive cysticerci, respectively. **(D, E, F)** Results for known-positive cysticerci from heart, skeletal muscle, or liver, respectively.

A					B				
IHC	HPS			Total	IHC	HPS			Total
		Positive	Negative				Positive	Negative	
	Positive	64	91 ^a			Positive	60	89	
	Negative	1	13			Negative	1	12	
Total		65	104	169	Total		61	101	162
C					D				
IHC	HPS			Total	IHC	HPS			Total
		Positive	Negative				Positive	Negative	
	Positive	4	2			Positive	12	6	
	Negative	0	1			Negative	0	0	
Total		4	3	7	Total		12	6	18
E					F				
IHC	HPS			Total	IHC	HPS			Total
		Positive	Negative				Positive	Negative	
	Positive	50	64			Positive	2	20	
	Negative	1	5			Negative	0	8	
Total		51	69	120	Total		2	28	30

^a Includes a single cysticercus recovered from the rumen serosa of an experimentally infected animal

other parasite remnants only, and 26 (40 %) had both calcareous corpuscles and other parasite remnants. The parasite remnants, other than calcareous corpuscles, most often observed were sections of bladder wall. Overall, calcareous corpuscles were present in 33.7 % (57/169) of the known-positive specimens tested with the HPS method and in 38.5 % (65/169) of the specimens examined with the IHC stain (Fig. 4.8).

Agreement between the assays was low if all specimens were considered (Kappa = 0.09; $p < 0.00001$), and for the experimentally-generated cysticerci which comprised the bulk of the known-positive specimens (Kappa = 0.08; $p < 0.00001$) (Table 4.2). For the relatively few (seven) field specimens tested, agreement was slightly higher (Kappa = 0.36), but not significantly so due to the small sample size. The use of the IHC stain enabled identification of all the HPS positive specimens except for one cysticercus from the skeletal muscle of an experimentally infected animal. This was a histologically homogenous granulomatous lesion containing a few calcareous corpuscles in the centre which were evident in both HPS and IHC stained sections, but for which there was no chromogen staining of any replicates tested by IHC (Fig. 4.9).

There were no significant differences between the proportion of known-positive specimens identified with the IHC staining for each of the three positions in the block (91.07 %, 89.47 %, and 94.64 % for positions 1, 2, and 3, respectively). Similarly, there were no significant differences between the proportion of known positive specimens identified with the HPS stain (35.7 %, 36.8 %, and 42.9 % for positions 1, 2, and 3, respectively). For the 155 cysticerci identified as positive with the IHC stain, 153 were positive for all three replicates, and two (one each from skeletal muscle and liver) were positive on one replicate only. Concordance among replicates was 95%. The proportion of known-positive specimens identified with the HPS stain per replicate was 31.95 % (54/169), 36.69 % (62/169), and 34.32 % (58/169), for the first, second, and third replicates, respectively. Of the 65 known-positive cysticerci identified with the HPS stain, seven, seven, and 51 specimens were identified as positive in one, two, or all three replicates, respectively. Concordance among replicates was 88%.

There was no evidence for cross-reactive immunohistochemical staining of the known-negative specimens with Mab 158C₁₁A₁₀ other than the metacestode larvae of *Taenia ovis* and *Echinococcus granulosus*, and the adult ovine anoplocephalid tapeworm *Thysanosoma actinoides* (Fig. 4.10). Sections of those specimens which were viable at the time of collection

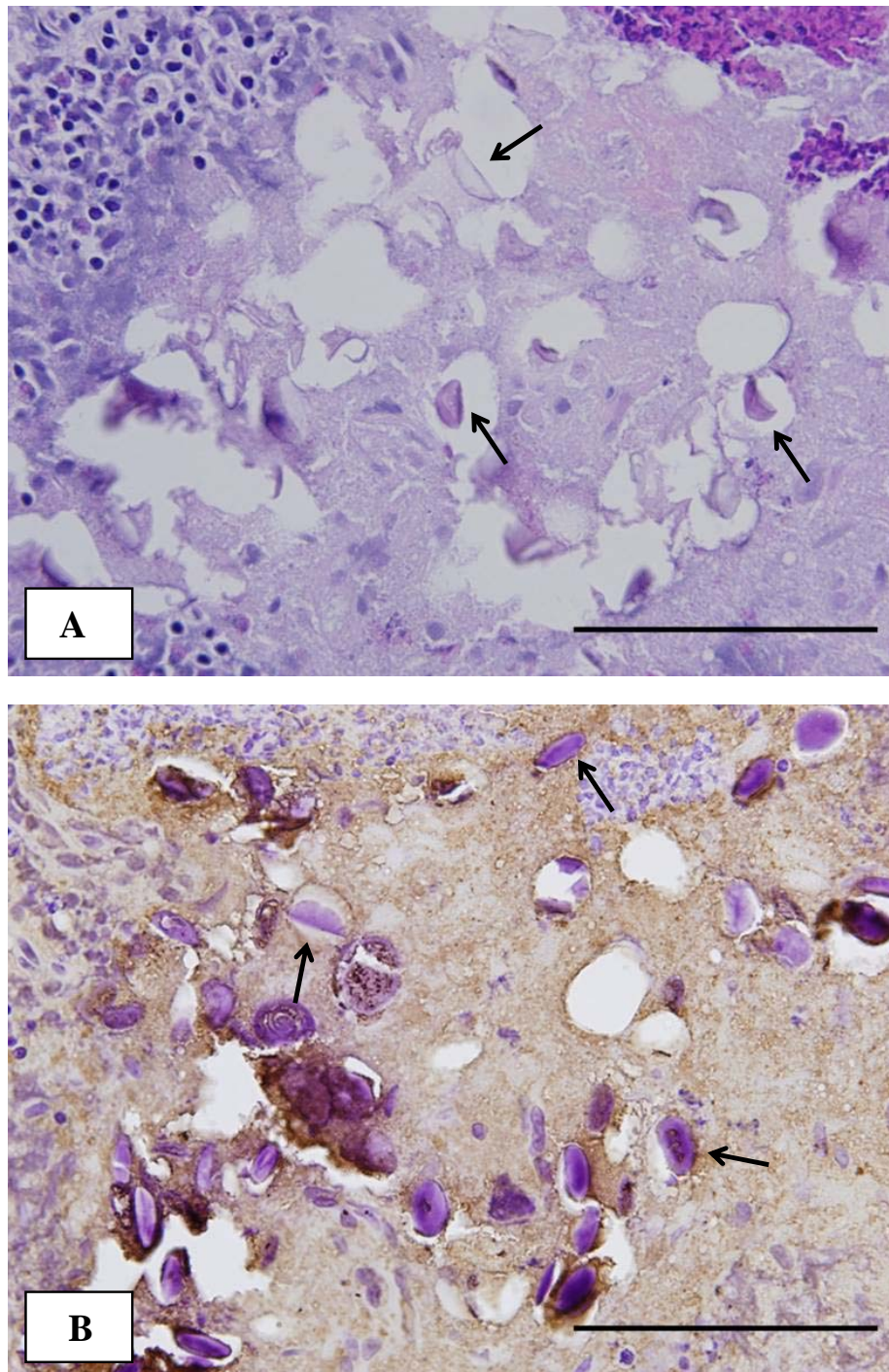


Figure 4.8. Calcareous corpuscles (arrows) in sections of a degenerated *Taenia saginata* cysticercus recovered from an experimentally infected animal killed 412 days post-inoculation. (A) Section stained using hematoxylin-phloxine-safran histological method. (B) Section stained using immunohistochemical method. Bar = 100 µm.

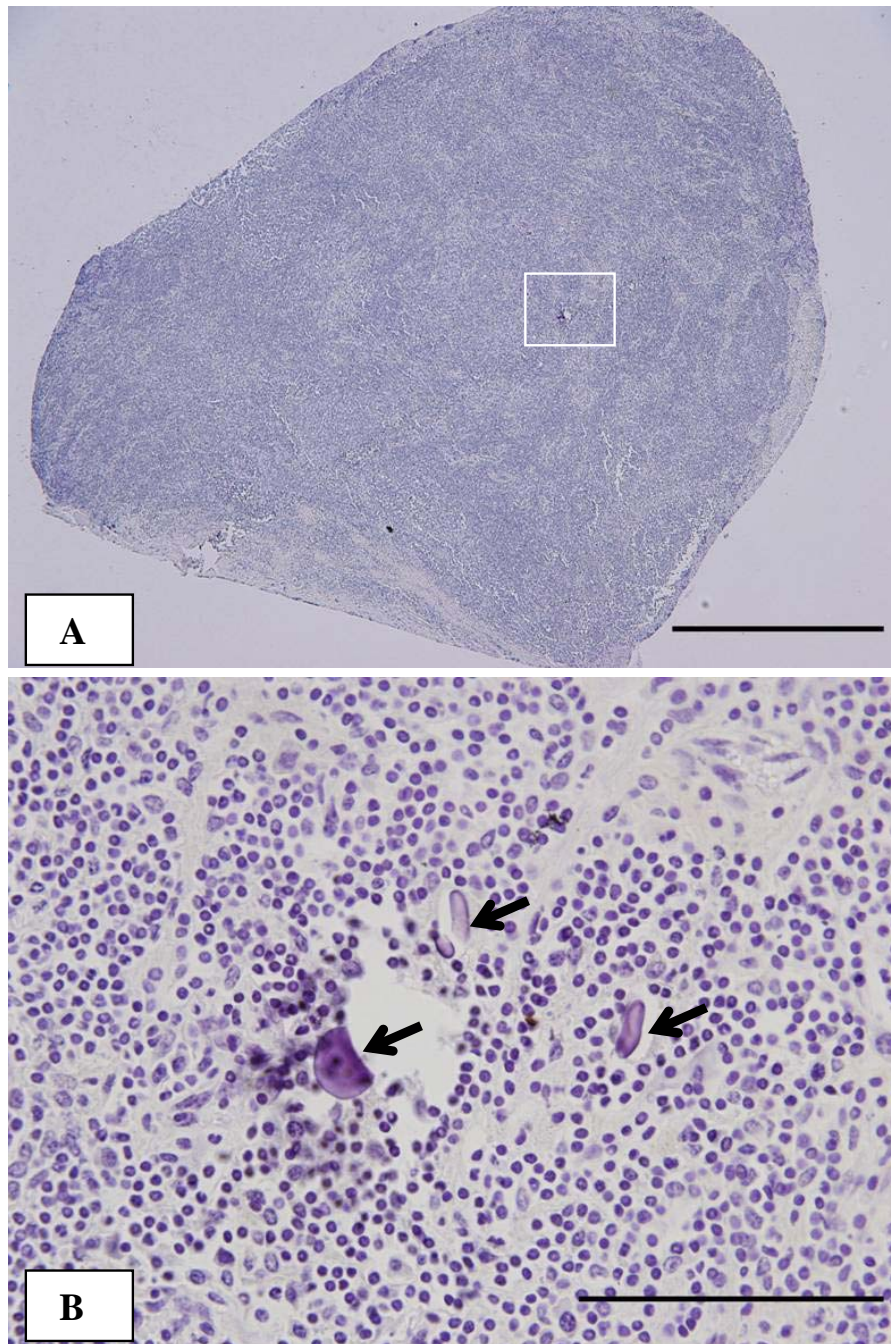


Figure 4.9. Granulomatous lesion from a degenerated *Taenia saginata* cysticercus that was negative using the immunohistochemical method but positive using the hematoxylin-phloxine-safran histological method. (A) IHC relevant antibody-stained section of lesion demonstrating absence of chromogen staining. 4X magnification. Bar = 1 mm. (B) 60X magnification of inset area (A) demonstrating presence of calcareous corpuscles (arrows) in centre of lesion. Bar = 100 μ m. Specimen was recovered from the skeletal muscle of an experimentally infected animal killed 417 days post-inoculation.

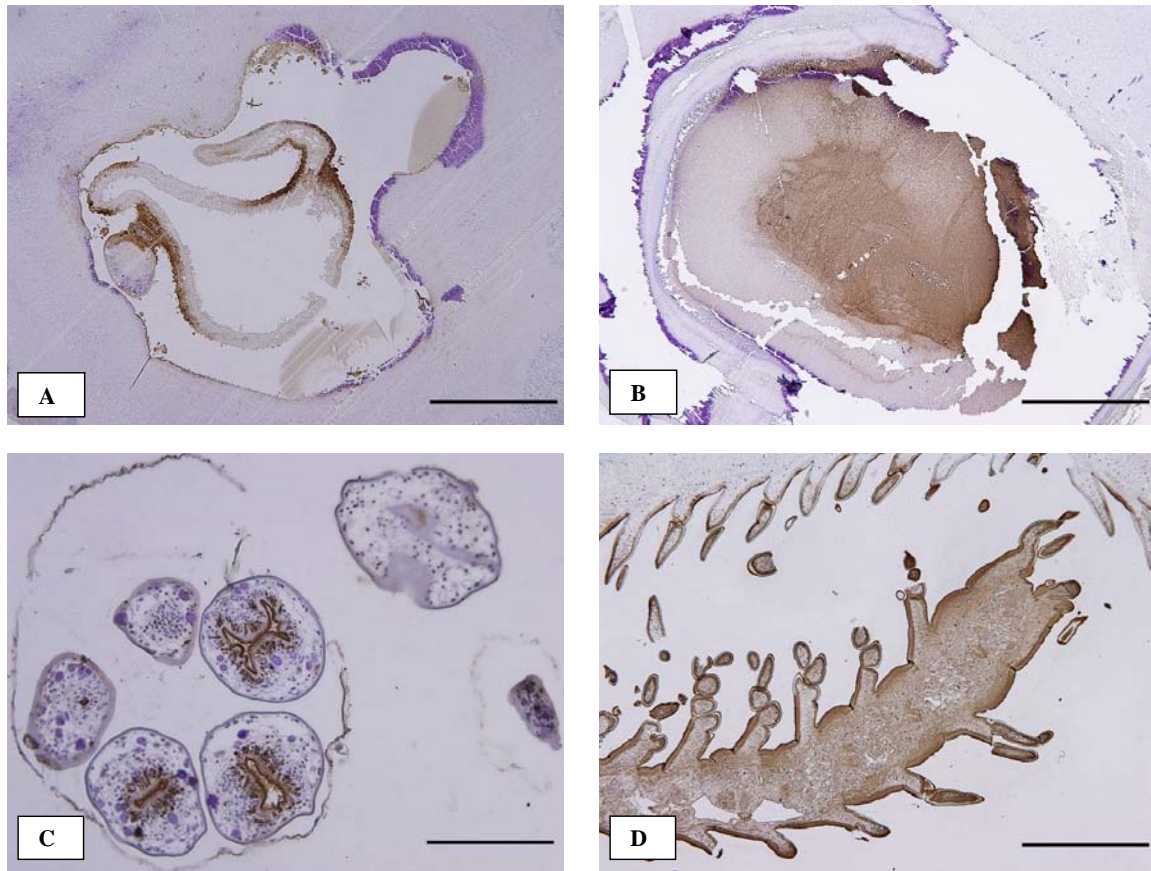


Figure 4.10. Immunohistochemical (IHC) staining of non-*Taenia saginata* cestodes with anti-*T. saginata* monoclonal antibody. (A) IHC-stained section of intact (viable) *Taenia ovis* cysticercus from a sheep. (B) IHC-stained section of degenerated *T. ovis* cysticercus from a sheep. (C) IHC-stained section of *Echinococcus granulosus* protoscolices in hydatid cyst from an elk. (D) IHC-stained section of adult *Thysanosoma actinoides* tapeworm from a sheep. All bars except (C) = 1 mm; bar in (C) = 100 μm.

stained in a distribution similar to that observed for the *T. saginata* cysticercus positive controls, with staining darkest in the tegument and less so in the parenchyma. The *T. ovis* cysticerci which were undergoing degeneration stained primarily in a central necrotic region similar to that for degenerated *T. saginata* cysticerci. All replicate sections of these cestode specimens stained similarly. The single section of the anoplocephalid tapeworm *Monezia* sp from cattle did not stain with this antibody. Basophilic sarcocysts were evident in many of the heart muscle sections, including those of intact *T. saginata* cysticercus positive controls, and were unstained with this antibody.

For most of the sections examined in this study, there was either no background staining, or very minor focal to multifocal background staining with variable staining of the border of the section. None of the known-positive experimentally derived cysticerci had significant background staining in sections stained with the relevant Mab; one replicate section for each of two of these specimens had widespread but pale background staining with the irrelevant Mab. For the known-positive field cysticerci, no significant background staining was observed with the relevant Mab, but five of the seven specimens had widespread pale background staining of all replicates with the irrelevant Mab. For the known-negative specimens, eight had widespread pale background staining of one or more replicate sections with both primary Mabs. Seven of these specimens were of eosinophilic myositis/ sarcocystosis, and one was of focal cardiac fibrosis. In addition, widespread pale background staining was observed in the single section of myocardial cyst of unknown etiology, and of the host tissue matrix only in the single sections of intracardiac schwannoma, myocardial epithelial inclusions, and multifocal lymphocytic myocarditis, stained with the relevant Mab only.

4.5 Discussion

This study demonstrated superior performance of an IHC method compared to conventional histology for identification of degenerated cysticerci. The use of the IHC method enabled identification of almost 2.5 times more known positive specimens than did the HPS staining method. The HPS staining method, which is used as the official histological test in Canada for bovine cysticerci, is similar to the more commonly used hematoxylin and eosin method, but better differentiates by colour various tissue components in the section, particularly collagen which stains yellow against red-stained muscle tissue (Luna, 1968). Use of other special

staining methods, such as Gomori's technique for reticular fibers, have been advocated to demonstrate particular parasite features in lesions in advanced stages of degeneration, or to distinguish partial cestode material, such as body wall, from other pathologies (Slais, 1970, Marty and Chester, 1997). However, Geerts et al. (1980) reported that Gomori's technique performed no better than hematoxylin and eosin on 32 degenerated cysticerci recovered from 25 bovine hearts. Although such special stains may have altered slightly the number of test specimens identified as positive in this study, they are not part of the official diagnostic method currently implemented in Canada and thus would not have provided a relevant comparison with which to evaluate the IHC assay.

When results were analyzed by tissue of origin, the IHC stain resulted in identification of a much higher proportion of positive specimens in each tissue than did HPS staining. All positive specimens from the heart, and most from the skeletal muscle, were identified. This finding is of diagnostic significance, as most of the cysticercosis-suspect lesions submitted for laboratory confirmation originate from these tissues, particularly heart, since these tissue types comprise the majority (except for esophagus) of the carcass sites routinely inspected for cysticercosis, and are amongst the most reliable for detecting infection (Saini et al., 1997). Of these tissue sites, the heart is routinely examined most thoroughly and tends to be most frequently and intensively infected (Chapter 3). The paradoxical earlier death of cysticerci in cardiac muscle compared to skeletal muscle also results in more easily detected gross lesions (Van den Heever, 1967; Sterba et al., 1979a; Lloyd, 1998a). Although this might suggest that the heart cysticerci, most of which had histological foci of mineralization, may have been some of the most chronic degenerated specimens examined in this study, the resorption of such lesions may occur more slowly in heart, thus delaying the dissolution of identifiable parasite features and antigen (Gallie and Sewell, 1983; Harrison et al, 1984). This hypothesis is supported by the finding that the highest proportion of cysticerci identified as positive by HPS staining were also from the heart. The use of the IHC stain was almost as effective in the identification of cysticerci in skeletal muscle as in heart; of the six specimens not identified as positive, one (from a field outbreak) was probably eosinophilic myositis/sarcocystosis, and the other five were chronic granulomatous or fibrotic lesions in which presumably there was no longer sufficient detectable antigen. Antigen retrieval techniques such as heating of sections to restore the integrity of antigens in formalin-fixed tissues prior to IHC staining might have increased the antigenicity in these specimens to the detection

threshold of the assay (Taylor, 2006), but were not attempted in this study. Cysticerci from the liver, most of which were mineralized, had the lowest proportion of positive results by both assays. The liver may be a relatively hostile environment for establishment and persistence of *T. saginata* cysticerci and is often not parasitized in infected animals. In the previous study (Chapter 3), only four of ten animals (of European breed ancestry) experimentally infected with 5000 *T. saginata* eggs had cysticerci in the liver. In a study of naturally infected Zebu cattle in Tanzania, while half of the animals had cysticerci in the liver, 92% of livers had only degenerated cysticerci, compared to 61% of infected hearts (Maeda et al., 1996). Thus, the liver cysticerci in this study may have been in more advanced stages of degeneration than those in other tissues, even heart. As well, the liver is frequently affected with chronic non-specific lesions of various other etiologies (e.g. bacteria) which are grossly indistinguishable from degenerated cysticerci. All but one of the liver specimens were recovered from the same animal, and all but two were mineralized. The majority of liver lesions were identified as cysticerci by IHC staining. Those that were negative may have had too little or no parasite antigen remaining, or may not have been true positive specimens. Although the study was designed to generate cysticerci of known positive status, there is no conclusive test for degenerated specimens. The slightly lower proportion of positive results by IHC staining for mineralized, compared to non-mineralized cysticerci, may have been due in part to the preponderance of liver lesions in that group. Since the liver is not a tissue that is routinely inspected for this parasite, nor from which suspect lesions are commonly submitted for laboratory confirmation, the lower sensitivity of the IHC assay on cysticerci in liver is irrelevant.

The concordance among replicates was high with both HPS and IHC staining. Concordance was highest with the IHC assay, in which all but two specimens were positive for all three replicates. This supports the robustness of this assay even when performing the method manually, as in this study. The findings also suggest that the number of sections of each specimen tested could be reduced with little impact on overall test performance. Conversely, reducing the number of sections viewed by the HPS assay would have reduced the number of positive results by as much as 22 % (14/65) and suggests that increasing the number of sections viewed may have led to identification of more positive specimens. As expected, the most common feature identified in positive specimens with the HPS method were calcareous corpuscles. Only 12% (8/65) of the specimens identified as positive by HPS staining had other

parasite features discernable in the absence of calcareous corpuscles.

Overall agreement between the two assays was low, with a Kappa value of only 0.09, reflecting the high number of false negative results with the HPS stain. One experimentally generated specimen identified as positive with the HPS stain was unstained with the IHC stain. This was a chronic granulomatous lesion containing a few central calcareous corpuscles. Although there was apparently no longer any demonstrable antigen in the sections, calcareous corpuscles were evident in the IHC stained sections, as well as those tested by HPS, and could have been utilized as a criterion to confirm the diagnosis, emphasizing the merit in a method such as IHC which can incorporate traditional diagnostic criteria such as histological features. It is also an exceptional example of a definitive histological feature persisting longer than detectable parasite antigen. Technical errors or reagent irregularities were not responsible for the negative IHC staining in this specimen, as the two other known positive specimens tested concurrently in each of the triplicate sections consistently were stained.

Each known-positive cysticercus from the experimental animals was randomly assigned to one of three positions in the same block, because of the relatively few known-negative specimens to intersperse amongst them, and in order to process and assess the most specimens for minimal cost. This aspect of the experimental design may have generated a positive bias on IHC results for specimens in the second and third positions in the section, based on a positive result in the preceding specimen. Since most of the experimental specimens were IHC-positive regardless of their position in the block, it was not possible to evaluate any position effect; however, the objective criteria for a positive result reduces such bias. Although the known positive field cysticerci were pooled with the known negative specimens and randomly assigned to blocks, most were IHC-positive as well, and there were too few field cysticerci to determine any effect of position in block. When assays were compared for the seven field known-positive lesions only, Kappa was highest (0.36), but not significant because of small sample size. This higher value is likely attributable to the observation that most of these specimens had a histological appearance consistent with a less chronic stage of degeneration compared to the experimentally generated specimens. Thus parasite features still remained which were identifiable by conventional histology. The one field specimen negative in both assays was probably not a cysticercus but was consistent with eosinophilic myositis/sarcocystosis. Removing this specimen from the group of known-positive field specimens would have

increased the proportion identified as positive with the IHC stain to 100% (6/6), and with the HPS stain to 67% (4/6).

Although Silverman and Hulland (1961) reported that calcareous corpuscles were dissolved during routine processing of hematoxylin and eosin stained sections, in this study the calcareous corpuscles often appeared intact, refractile and either unstained, or variably stained eosinophilic or basophilic, or basophilic with HPS and IHC staining, respectively. The preservation of the calcareous corpuscles in the present study is likely attributable to omission of decalcification of specimens, which was not performed due to the negative impact on the IHC assay (Jonsson et al., 1986) and because there was minimal mineralization in most of the cysticerci tested. The slightly higher proportion of IHC-stained sections with detectable calcareous corpuscles compared to those stained with HPS may have been due to enhanced contrast in the IHC method of the unstained or hematoxylin-counterstained structures against the brown chromogen staining of the target antigen.

The dilutions of primary and secondary antibody used in this study were lower than the 1:1000 and 1:800 dilutions, respectively, used in the development of this assay by Ogunremi et al. (2004a,b), even though the protein concentration of the reconstituted stock antibodies was similar. As well, horse-derived secondary antibody was used instead of rabbit antisera used previously. It is customary to use a secondary antibody derived from the same species from which the blocking sera is obtained, in order to reduce background staining (Haines and Chelack, 1991). Our titrations demonstrated that 1:250 dilution of primary antibody and 1:400 dilution of secondary antibody resulted in optimal staining of the target antigen with minimal background. The highest concentration of primary antibody (1:100) assessed during the titrations produced slightly more background staining with no discernible improvement in staining of the target antigen. Because the primary antibody is not commercially available, using a higher concentration than is required to meet the intended use of the assay is not practical. Ogunremi et al. (2004a) reported that 1:1000 dilution of primary antibody yielded positive staining of all 115 degenerate cysticerci tested. In the current study all degenerated specimens were collected >1 year post-inoculation, so it was deemed important to use an antibody concentration that would maximize identification of any residual target antigen. Grossly and histologically most of the cysticerci tested in this study were in an advanced stage of degeneration consistent with Types 3-4 for bovine cysticerci as described by Retzlaff (1972) and histological Grades 5-6 for porcine

cysticerci as described by Aluja and Vargas (1988.)

While relatively few known-negative specimens were tested for specificity of the IHC assay, the results suggest that the Mab 158C₁₁A₁₀ cross-reacts only with other cestodes. This is consistent with reports that two IgM Mabs also directed at *T. saginata* cysticercus ES antigen and used in a sandwich ELISA cross-react with sera of sheep infected with *T. ovis* or *E. granulosus* (but not *T. hydatigena*), and pigs and humans infected with *T. solium* cysticerci (Brandt et al., 1992). Serological assays with IgG isotype Mabs (including 158C₁₁A₁₀) to the same antigen demonstrated possible cross-reactions among sera of cattle infected with a variety of hemoparasites and helminths, including *Fasciola hepatica* (Van Kerckhoven et al., 1998). We found no cross-reactive staining with *F. hepatica*, *Fascioloides magna*, or *Dicrocoelium dendriticum*, but there was cross-staining with two other taeniid cestodes, *T. ovis* and *E. granulosus*. Cross-reactivity amongst the cestodes extended beyond the taeniids as evidenced by the positive staining of sections of the anoplocephalid tapeworm *Thysanosoma actinoides*. Why sections of the cattle anoplocephid *Monezia* sp. did not stain is unknown. This particular specimen had been formalin-fixed for several years, which may have adversely affected antigenicity (Werner et al., 2000). Less likely is the possibility that, based on the aforementioned findings of serological cross reactions with *T. ovis* and *E. granulosus* but not *T. hydatigena*, some even closely related cestodes may not express the target antigen. However, it is likely that the IHC assay used in this study will stain most taeniid cestodes, including *T. solium*. The Mab used in this assay reacts with a carbohydrate or carbohydrate/protein epitope present in the metacestode tegument and ES products, that is not stage-specific (Draelants et al., 1995). Similar to the cross-protection to taeniids incurred by vaccination with protective cestode antigens (Lightowlers, 2006), conservation of the antigen recognized by this Mab among cestodes broadens the potential diagnostic application of the assay. Since many cestodes are relatively host and tissue type-specific in their larval stage, identification of a lesion from the heart of a sheep, for example, as a cestode provides a high level of confidence in a diagnosis of *T. ovis*. There is merit in future evaluation of the performance of this assay on degenerated *T. solium* cysticerci.

The most common differential diagnoses for bovine cysticercosis specimens submitted for confirmatory identification in Canada are focal or multifocal granulomatous myocarditis (“eosinophilic myositis”, or EM) consistent with sarcocystosis, focal pyogranulomatous

myocarditis, focal lymphocytic/lymphohistiocytic myocarditis, epithelial inclusions (heterotopic epithelial remnants), intracardiac schwannoma, and various metastatic neoplasms (Yves Robinson, personal communication). Specimens of these lesions in this study, as well as of sarcocysts found incidentally in many of the sections of cysticerci in heart muscle, were negative using IHC.

Although relatively low dilutions of primary and secondary Mabs were used in the IHC assay, there was minimal background staining with either the relevant or irrelevant Mabs for most sections. When more widespread background staining was present in a section, it was generally more pronounced with the irrelevant Mab. Why more widespread background staining occurred most frequently in some of the known negative specimens, particularly specimens of eosinophilic myositis/sarcocystosis, is unknown. Extensive tissue necrosis and collagen, as found in the specimens of EM and cardiac fibrosis, respectively, are recognised causes of non-specific background staining (Dabbs, 2002). Some of the other known-negative specimens that had widespread background were obtained from archived paraffin blocks several years old, and may have been fixed and processed differently from the procedure followed in this study. The relatively long (overnight) incubation of the sections with primary Mab, duration of chromagen staining, and the high sensitivity of the ABC method may also have facilitated the appearance of non-specific staining (Haines and Chelack, 1991; Dabbs, 2002). Regardless, none of the non-specific staining was of an extent which confounded the interpretation of results and almost all sections were easily interpreted even in the absence of the corresponding section stained with the relevant or irrelevant Mab.

Our findings demonstrated the reliable performance of the IHC assay on samples processed manually. Automation of IHC assays is increasingly advocated to facilitate more standardized and efficient processing of large numbers of samples, but is probably not warranted for this assay because the number of submissions for diagnosis of bovine cysticercosis in Canada is low, and automation would require larger quantities of the primary antibody (Haines and Chelack, 1991) which is not commercially available and currently produced in relatively small quantities.

Since many of the sporadic occurrences of this parasite in cattle in Canada are likely due to cases of human taeniosis contracted elsewhere, any one of a variety of strains of this parasite could be responsible for a particular case or outbreak of bovine cysticercosis. Although the

experimentally generated cysticerci which comprised most of the positive specimens in this study were derived from eggs of a single adult *Taenia saginata* tapeworm, it is unlikely that strain differences of this parasite would have affected performance of the IHC assay, given the positive results on the few field specimens examined, and cross-reactive staining of other taeniids. Since the cysticerci in this study were obtained opportunistically from relatively few animals, and disproportionate numbers of specimens from a particular tissue type (i.e. liver) were attributable to single animals, it will be important to continue to increase the numbers and scope of specimens tested to ensure that the performance of the assay in this study is representative of its fitness for use on cysticerci from the bovine population at large.

4.6 Conclusion

The results of this study support that the excretory-secretory antigen targeted by the primary Mab 158C₁₁A₁₀ is preserved in most formalin-fixed degenerated bovine cysticerci originating from a variety of tissues. The IHC assay using this antibody consistently identified more cysticerci than the HPS assay, including all heart cysticerci and all but a few end-stage chronic granulomatous or fibrotic lesions in skeletal muscle. Because of the greater likelihood of more chronic lesions, and of etiologies other than cysticercosis, the IHC assay was less reliable on liver lesions, suggesting that specimens from this tissue should not comprise the sole source of suspect-cysticercus diagnostic submissions. The improved performance of the IHC assay compared to the existing histological method supports its adoption for routine diagnostic use in Canada.

5. GENERAL DISCUSSION AND CONCLUSIONS

In spite of regulated disease control measures currently implemented in Canada for bovine cysticercosis, sporadic outbreaks continue to challenge efforts to ensure food safety for consumers. It is unknown how much “endemic” human taeniosis occurs from consumption of beef in Canada; estimates of the prevalence of taeniosis in Canada are unavailable since a control program similar to that for cysticercosis does not exist. It is likely very low based on the presumably low parasite prevalence and the customary freezing and cooking measures employed by most consumers. Occurrences of both cattle and human infections will likely increase in future, however, as a result of travel and immigration from areas where *T. saginata* is more prevalent, establishment and adoption of culinary habits facilitating parasite transmission, and the trend towards organic livestock production which might increase access of cattle to eggs in the environment. Therefore, more effective control measures for this parasite are needed. The two studies comprising this thesis provide evidence to support recommendations for improved detection of infected animals at slaughter, and more reliable diagnosis of suspect lesions.

Based on the results of experimental infection of calves with *T. saginata* (Chapter 3), a more thorough examination of the heart during the inspection procedure is the most practical method for optimizing the postmortem detection of infected animals. An important feature of infection in the heart is the comparatively early degeneration of cysticerci, resulting in lesions which are more easily seen than viable cysticerci. Although this makes definitive diagnosis more difficult than for viable cysticerci, the immunohistochemical method for confirmation of the identity of degenerated cysticerci (Chapter 4) supports using such a screening procedure for detecting suspect animals.

The results of this study have been incorporated into the CFIA procedures for the postmortem inspection of veal calves for cysticercosis. Because of industry reluctance to incise the masseter muscles based on the market preference for intact “veal cheeks”, an alternative was sought to the existing inspection procedure. Based on the rationale that in infected veal calves (maximum age of approximately 6.5-7 months) any cysticerci in the masseter muscle would

likely be viable and thus difficult to discern visually, and that cysticerci in the heart are more likely to be degenerated and thus more easily detected, cessation of incision of the masseter muscle and increased inspection of the heart was recommended. The revised regulations for veal calves now stipulate that, unless the animal is suspected of having cysticercosis, incisions of the masseters are not performed, and three additional incisions (beyond those in the regular inspection procedure) are made into the heart from the endocardial surface (CFIA Meat Hygiene Manual of Procedures, Section 4.6.4, 2007). No changes have been made to the routine inspection procedure for mature cattle; this may be impractical given the low overall prevalence of infection in the national herd. However, based on this study, CFIA inspectors have been encouraged to more thoroughly examine the heart of suspect animals, and to include the entire heart in laboratory submissions from these animals; the cysticercosis section of the CFIA Disease Control Manual of Procedures should be revised to reflect this. Once received at the laboratory, the heart (and any other submitted suspect tissue) is comprehensively examined for cysticerci using the method described in this study. Both viable and degenerated cysticerci are more likely to be detected in the laboratory (by experienced analysts working under optimal conditions), than at the abattoir. Based on the tendency for cysticerci in heart to degenerate within a few months of infection, the detection of viable parasites in this organ suggests relatively recent exposure, which is relevant to the epidemiology of an outbreak.

In Chapter 4, degenerated cysticerci recovered from the experimental infections in Chapter 3 were used to evaluate the immunohistochemical assay for bovine cysticercosis developed by Ogunremi et al. (2004a), and the histological assay presently implemented by CFIA. The improved performance of the IHC assay compared to the existing histological method, and its reliability for identifying degenerated specimens from heart and skeletal muscle (which comprise most of the diagnostic submissions for this parasite) support its inclusion in the repertoire of official diagnostic tests for bovine cysticercosis in Canada, and potentially elsewhere. Because histological features of the parasite are apparent in the counterstained IHC sections, processing of specimens for conventional histology will only be required to determine etiology for submissions where cysticercosis has been ruled out. Since the longest post-inoculation age of degenerated cysticerci evaluated in this study was 466 days, it is important that the IHC method be assessed on more chronic lesions. This has implications particularly for lesions originating from cull dairy or beef cows which could have been exposed to infective eggs

years prior to slaughter, and in which cysticerci may not yet have been fully resorbed (Penfold and Penfold, 1937). Confidence in a diagnosis of cysticercosis in dairy and cow-calf herds is especially important because of the longer quarantine periods often imposed on these operations, compared to feedlots, from which suspect animals can be removed and slaughtered within a relatively short time.

Since PCR is increasingly being applied to the post-mortem diagnosis of bovine cysticercosis, a logical extension of this study would be to compare PCR with the IHC method. Previous comparisons for a number of other pathogens have demonstrated that PCR is more sensitive than IHC (Brunnert et al., 1994; Bazler et al., 1999; Held et al., 2000; Tegtmeier et al., 2000). The PCR method may more reliably identify chronic end-stage lesions of cysticercosis; however, more work needs to be done to optimize and standardize PCR methods before such a comparison can be made (Abuseir et al., 2006; Geysen et al., 2007). Portions of each of the known positive cysticerci used in this IHC validation study have been stored frozen, for potential future PCR validation. Of particular value would be PCR testing of those specimens in this study which were not identified as positive by the IHC method, the majority of which were from the liver, in an attempt to further elucidate their true (positive or negative) status.

Conclusions

The limitations of current methods for the postmortem detection and definitive diagnosis of bovine cysticercosis are well recognized. By modifying the slaughter inspection procedure for cysticerci to include more thorough examination of the heart, more suspect animals will be detected. By implementing the IHC assay, more suspect lesions will be definitively diagnosed. These innovations will result in more efficient and effective disease control for this parasite and better assurance of food safety to consumers. However, ongoing efforts to develop effective vaccines, reliable methods for antemortem diagnosis of infected cattle and identification of environmental contamination with *T. saginata* eggs, as well as the implementation of surveillance programs for human taeniosis, are also required to most effectively mitigate the impact of this parasite.

6. REFERENCES

- Abuseir, S., Epe, C., Schnieder, T., Klein G., Kuhne, M. 2006. Visual diagnosis of *Taenia saginata* cysticercosis during meat inspection: is it unequivocal? Parasitology Research 99, 405-409.
- Allan, J.C., Velasquez-Tohom, M., Fletes, C., Torres-Alvarez, R., Lopez-Virula, G., Yurrita P., Soto-De-Alfaro, H., Rivera, A., Garcia-Noval, J. (1997). Mass chemotherapy for intestinal *Taenia solium* infection: effect on prevalence in humans and pigs. Transactions of the Royal Society of Tropical Medicine and Hygiene 91, 595-598.
- Aluja, A.S. de, Vargas, G. 1988. The histopathology of porcine cysticercosis. Veterinary Parasitology 28, 65-77.
- Aubry, P., Bequet, D., Quequiner, P. 1995. Cysticercosis: a frequent and redoubtable parasitic disease. Medecine Tropicale (Mars) 55, 79-87.
- Babalola, D.A. 1976. Prevalence of *Cysticercus bovis* in the north-eastern state of Nigeria. Bulletin of Animal Health and Production in Africa Vol. 24, 159-162.
- Baszler, T.V., Gay, L.J., Long, M.T., Mathison, B.A. 1999. Detection by PCR of *Neospora caninum* in fetal tissues from spontaneous bovine abortions. Journal of Clinical Microbiology 37, 4059-4064.
- Bogh, H.O., Gronvold, J., Maeda, G.E., Nansen, P. 1996. Experimental single and trickle infections of cattle with *Taenia saginata*: studies of immunodiagnosis. Research in Veterinary Science 60, 64-68.

Borman-Eby, H.A.C., Ayim, D.S., Small, C. 1994. *Cysticercus bovis* in cattle in two beef feedlots in southern Ontario. Canadian Veterinary Journal 35, 711-713.

Bowles, J., McManus, D.P. 1994. Genetic characterization of the Asian *Taenia*, a newly described taeniid cestode of humans. American Journal of Tropical Medicine and Hygiene 50, 33-44.

Bowles, J., McManus, D.P. 1993. Rapid discrimination of *Echinococcus* species and strains using a PCR-based RFLP method. Molecular and Biochemical Parasitology 57, 231-240.

Brandt, J.R.A., Geerts, S., De Deken, R., Kumar, V., Ceulemans, F., Brijs, L., Falla, N. 1992. Monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. International Journal for Parasitology 22, 471-477.

Brunnert, S.R., Dai, Y., Kohn, D.F. 1994. Comparison of polymerase chain reaction and immunohistochemistry for the detection of *Mycoplasma pulmonis* in paraffin-embedded tissue. Laboratory Animal Science 44, 257-260.

Bundza, A., Dukes, T.W., Stead, R.H. 1986. Peripheral nerve sheath neoplasms in Canadian slaughter cattle. Canadian Veterinary Journal 27, 268-271.

Bundza, A., Finley, G.G., K.L. Easton. 1988. An outbreak of cysticercosis in feedlot cattle. Canadian Veterinary Journal 29, 993-996.

Cabaret, J., Geerts, S., Madeline, M., Ballandonne, C., Barbier, D. 2002. The use of urban sewage sludge on pastures: the cysticercosis threat. Veterinary Research, 33, 575-597.

Canadian Food Inspection Agency (CFIA). Disease Control Manual of Procedures, Section 13, Cysticercosis. March 2000. Available from the Canadian Government Publishing Centre, Supply and Services Canada, Ottawa.

- Canadian Food Inspection Agency (CFIA). Meat Hygiene Manual of Procedures.
<http://www.inspection.gc.ca/english/anim/meavia/mmopmmhv/mane.shtml>. Accessed May, 2007. Also available from the Canadian Government Publishing Centre, Supply and Services Canada, Ottawa.
- Chung, W.C., Lin, C.Y., Fan, P.C. 1996. Ectopic locations of *Taenia saginata asiatica* cysticerci in the abdominal cavity of domestic pig and monkey. *Journal of Parasitology* 82, 1032-1034.
- Cornford, E.M., Cornford, M.E., Wright, E.M., Bruckner, D.A., Sampogna, S., Hirayama, B.A. 2001. Human cerebral cysticercosis: immunolocalization of a sodium-dependent glucose cotransporter (SGLT) in larval and adult tapeworms. *Journal of Parasitology* 87, 510-521.
- Crookshanks, J.L., Taylor, S.M., Haines, D.M., Shelton, G.D. 2007. Treatment of canine pediatric *Neospora caninum* myositis following immunohistochemical identification of tachyzoites in muscle biopsies. *Canadian Veterinary Journal* 48, 506-508.
- Cruikshank, J.J., Haines, D.M., Palmer, N.C., St. Aubin, D.J. 1990. Cysts of a *Toxoplasma*-like organism in an Atlantic bottlenose dolphin. *Canadian Veterinary Journal* 31, 213-215.
- Dabbs, D.J. 2002. Techniques of Immunohistochemistry: Principles, Pitfalls, and Standardization. In: Zorab, R., Strauss, M., Kaye, J. (Ed.), *Diagnostic Immunohistochemistry*, Churchill Livingstone, Philadelphia, pp. 3-43.
- Dewhirst, L.W., Cramer, J.D., Pistor, W.J. 1963. Bovine cysticercosis. 1. Longevity of cysticerci of *Taenia saginata*. *Journal of Parasitology* 49, 297-300.
- Dewhirst, L.W., Cramer, J.D., Sheldon, J.J. 1967. An analysis of current inspection procedures for detecting bovine cysticercosis. *Journal of the American Veterinary Medical Association* 150, 412-417.
- Dohoo, I., Martin, W., Stryhn, H. 2003. *Veterinary Epidemiological Research*. AVC Inc,

Charlottetown, PEI. Canada. 706 pp.

Dorny, P., Vercammen, F., Brandt, J., Vansteekiste, W., Berkvens, D., Geerts, S. 2000. Sero-epidemiological study of *Taenia saginata* cysticercosis in Belgian cattle. *Veterinary Parasitology* 88, 43-49.

Draelants, E., Brandt, J.R.A., Kumar, V., Geerts, S. 1995. Characterization of epitopes on excretory-secretory antigens of *Taenia saginata* metacestodes recognized by monoclonal antibodies with immunodiagnostic potential. *Parasite Immunology* 17, 119-126.

Dubey, J.P. 1997. Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. *Veterinary Parasitology* 71, 307-310.

Dubey, J.P., Hamir, A.N. 2000. Immunohistochemical confirmation of *Sarcocystis neurona* infections in raccoons, mink, cat, skunk, and pony. *Journal of Parasitology* 86, 1150-1152.

Dukes, T.W., Bundza, A., Corner, A.H. 1982. Bovine neoplasms encountered in Canadian slaughterhouses: a summary. *Canadian Veterinary Journal* 23: 28-30.

Enoe, C., Georgiadis, M.P., Johnson, W.O. 2000. Estimation of the sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. *Preventative Veterinary Medicine* 45, 61-81.

Fan, P.C. 1988. Taiwan *Taenia* and taeniasis. *Parasitology Today* 4, 86-88.

Fan, P.C., Chung, W.C., Lin, C.Y., Wu, C.C. 1990. The pig as an intermediate host for Taiwan *Taenia* infection. *Journal of Helminthology* 64, 223-231.

Fan, P.C., Lin, C.Y., Chen, C.C., Chung, W.C. 1995. Morphological description of *Taenia saginata asiatica* (Cyclophyllidae: Taeniidae) from man in Asia. *Journal of Helminthology* 69, 299-303.

- Fan, P.C., Lin, C.Y., Chung, W.C. 1992. Experimental infection of Philippine *Taenia* in domestic animals. *International Journal for Parasitology* 22, 235-238.
- Fan, P.C., Lin, C.Y., Kosman, M.L., Kosin, E. 1989. Experimental infection of Indonesia *Taenia* (Samosir strain) in domestic animals. *International Journal for Parasitology* 19, 809-812.
- Ferrer, E., Benitez, L., Foster-Cuevas, M., Bryce, D., Wamae, L.W., Onyango-Abuje, J.A., Garate, T., Harrison, L.J.S., Parkhouse, R.M.E. *Taenia saginata* derived synthetic peptides with potential for the diagnosis of bovine cysticercosis. *Veterinary Parasitology* 111, 83-94.
- Flisser, A., Sarti, E., Lightowlers, M. & Schantz, P. (2003). Neurocysticercosis: regional status, epidemiology, impact and control measures in the Americas. *Acta-Tropica*, 87, 43-51.
- Flisser, A., Viniegra, A-E., Aguilar-Vega, L., Garza-Rodriguez, A., Maravilla, P., Avila, G. 2004. Portrait of human tapeworms. *Journal of Parasitology* 90, 914-916.
- Froyd, G. 1964. The longevity of *Cysticercus bovis* in bovine tissues. *British Veterinary Journal* 120, 205-211.
- Gajadhar, A.A., Forbes, L.B. 2002. An internationally recognized quality assurance system for diagnostic parasitology in animal health and food safety, with example data on trichinellosis. *Veterinary Parasitology* 103, 133-140.
- Gallie, G.J., Sewell, M.M.H. 1983. Duration of immunity and absorption of cysticerci in calves after treatment of *Taenia saginata* cysticercosis with praziquantel. *Research in Veterinary Science* 34, 127-130.
- Gasser, R.B., Chilton, N.B. 1995. Characterisation of taeniid cestode species by PCR-RFLP of ITS2 ribosomal DNA. *Acta Tropica* 59, 31-40.
- Geerts, S., Kumar, V., Ceulemans, F., Mortelmans, J. 1981. Serodiagnosis of *Taenia saginata*

cysticercosis in experimentally and naturally infected cattle by enzyme linked immunosorbent assay. Research in Veterinary Science 30, 288-293.

Geerts, S., Kumar, V., Van den Abbeele, O. 1980. *Taenia saginata* cysticercosis in slaughter cattle in Belgium. Vlaams Diergeneeskundig Tijdschrift 49, 365-374.

Geysen, D., Kanobana, K., Victor, B., Rodriguez-Hidalgo, R., De Borchgrave, J., Brandt, J., Dorny, P. 2007. Validation of meat inspection results for *Taenia saginata* cysticercosis by PCR-restriction fragment length polymorphism. Journal of Food Protection 70, 236-240.

Ginsberg, A., Grieve, I.M. 1959. Two unusual cases of liver cysticercosis. Veterinary Record. 71, 1959.

Gonzalez, A.E., Cama, V., Gilman, R.H., Tsang, V.C.W., Pilcher, J.B., Chavera, A., Castro, M., Montenegro, T., Verastegui, M., Miranda, E., Bazalar, H. 1990. Prevalence and comparison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in Peru. Am. J. Trop. Med. Hyg. 43, 194-199.

Gonzalez, A.E., Falcon, N., Gavidia, C., Garcia, H.H., Tsang, V.C.W., Bernal, T., Romero, M., Gilman, R.H. (1998). Time-response curve of oxfendazole in the treatment of swine cysticercosis. American Journal of Tropical Medicine and Hygiene, 59, 832-836.

Gonzalez, A.E., Gauci, C.G., Barber, D., Gilman, R.H., Tsang, V.C.W., Garcia, H.H., Verastegui, M., Lightowers, M.W. 2005. Vaccination of pigs to control human neurocysticercosis. Am. J. Trop. Med. Hyg., 72, 837-839.

Gonzalez, A.E., Gavidia, C., Falcon, N., Bernal, T., Verastegui, M., Garcia, H.H., Gilman, R.H., Tsang, V.C. 2001. Protection of pigs with cysticercosis from further infections after treatment with oxfendazole. Am J Trop Med Hygiene 65, 15-18.

Gonzalez, L.M., Montero, E., Harrison, L.J.S., Parkhouse, R.M.E., Garate, T. 2000. Differential

diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR. Journal of Clinical Microbiology 38, 737-744.

Gonzalez, L.M., Montero, E., Morakote, N., Puente, S., Diaz De Tuesta, J.L., Serra, T., Lopez-Velez, R., McManus, D.P., Harrison, L.J., Parkhouse, R.M., Garate, T. 2004. Differential diagnosis of *Taenia saginata* and *Taenia saginata asiatica* taeniasis through PCR. Diagnostic Microbiology and Infectious Disease 49, 183-188.

Gonzalez, L.M., Montero, E., Puente, S., Lopez-Velez, R., Hernandez, M., Sciutto, E., Harrison, L.J.S., Parkhouse, R.M.E., Garate, T. 2002. PCR tools for the differential diagnosis of *Taenia saginata* and *Taenia solium* taeniasis/cysticercosis from different geographical regions. Diagnostic Microbiology and Infectious Disease 42, 243-249.

Gottstein, B. 1994. Immunodiagnosis of infections with cestodes. In: Hui, Y.H., Gorham, J.R., Murrell, K.D., Cliver, D.O. (Ed.). Foodborne Disease Handbook, Volume 2, Marcel Dekker, NY, pp. 393-427.

Government of Canada. Health of Animals Regulations. Canada Gazette, Part II (25-9-91, S.O.R./91-525). 1991. Ministerial Regulations, Regulations Prescribing Certain Diseases as Reportable Diseases Schedule (Section 2). Published under authority of the Speaker of the House of Commons by the Queen's Printer for Canada. Available from the Canadian Government Publishing Centre, Supply and Services Canada, Ottawa.

Greiner, M., Gardner, I.A. 2000. Epidemiologic issues in the validation of veterinary diagnostic tests. Preventative Veterinary Medicine 45, 3-22.

Grove, D.I. 1990. *Taenia saginata* and Taeniasis saginata In: A History of Human Helminthology, C-A-B International, Wallingford Oxon, UK. 848 pp.

Gustowska, L., Pawlowski, Z. 1981. Histopathological and histoenzymatic studies on experimental *Taenia saginata* cysticercosis. Veterinary Parasitology 8, 211-218.

Haines, D.M., Chelack, B.J. 1991. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin-fixed paraffin-imbedded tissues for diagnostic pathology. *Journal of Veterinary Diagnostic Investigation* 3, 101-112.

Haines, D.M., Clark, E.G., Dubovi, E.J. 1992. Monoclonal antibody-based immunohistochemical detection of bovine viral diarrhea virus in formalin-fixed, paraffin-imbedded tissues. *Veterinary Pathology* 29, 27-32.

Haines, D.M., Clark, E.G. 1991. Enzyme immunohistochemical staining of formalin-fixed tissues for diagnosis in veterinary pathology. *Canadian Veterinary Journal* 32, 295-302.

Haines, D.M., Waters, E.H., Clark, E.G. 1993. Immunohistochemical detection of swine influenza A virus in formalin-fixed and paraffin-embedded tissues. *Canadian Journal of Veterinary Research* 57, 33-36.

Haines, D.M., West, K.H. 2005. Immunohistochemistry: Forging the links between immunology and pathology. *Veterinary Immunology and Immunopathology* 108, 151-156.

Hammerberg, B., MacInnis, G.A., Hyler, T. 1978. *Taenia saginata* cysticerci in grazing steers in Virginia. *Journal of the American Veterinary Medical Association* 173, 1462-1464.

Harrison L.J., Gallie G.J., Sewell M.M. 1984. Absorption of cysticerci in cattle after treatment of *Taenia saginata* cysticercosis with praziquantel. *Research in Veterinary Science* 37, 378-380.

Harrison L.J.S., Garate T., Bryce D.M., Gonzalez L.M., Foster-Cuevas M., Wamae L.W., Onyango-Abuje J.A., Parkhouse R.M.E. 2005. Ag-ELISA and PCR for monitoring the vaccination of cattle against *Taenia saginata* cysticercosis using an oncospherical adhesion protein (HP6) with surface and secreted localization. *Tropical Animal Health and Production*, 37, 103-120.

Harrison, L.J.S., Joshua, G.W.P., Wright, S.H., Parkhouse, R.M.E. 1989. Specific detection of circulating surface/ secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. *Parasite Immunology* 11, 351-370.

Harrison, L.J.S., Sewell, M.M.H. 1981. Antibody levels in cattle naturally infected with *Taenia saginata* metacestodes in Britain. *Research in Veterinary Science* 31, 62-64.

Hayunga, E.G., Wong, M.M., Sumner, M.P., Isenstein, R.S. 1991. Evaluation of a 'dipstick' immunoassay to detect cysticercosis in experimentally infected cattle. *Veterinary Parasitology* 38, 13-22.

Heath, D.D., Smyth, J.D. 1970. *In vitro* cultivation of *Echinococcus granulosus*, *Taenia hydatigena*, *T. ovis*, *T. pisiformis* and *T. serialis* from oncosphere to cystic larva. *Parasitology* 61, 329-343.

Heckerroth, A.R., Tenter, A.M. 1999. Development and validation of species-specific nested PCRs for diagnosis of acute sarcocystosis in sheep. *International Journal for Parasitology* 29, 1331-1349.

Held, T.K., Kruger, D., Switala, A.R., Beyer, J., Kingreen, D., Busemann, C., Janitschke, K., Siegert, W. 2000. Diagnosis of toxoplasmosis in bone marrow transplant recipients: comparison of PCR-based results and immunohistochemistry. *Bone Marrow Transplantation* 25, 1257-1262.

Hilwig, R.W., Cramer, J.D., Forsyth, K.S. 1978. Freezing times and temperatures required to kill cysticerci of *Taenia saginata* in beef. *Veterinary Parasitology* 4, 215-219.

Hird, D.W., Pullen, M.M. 1979. Tapeworms, meat and man. A brief review and update of cysticercosis caused by *Taenia saginata* and *Taenia solium*. *Journal of Food Protection* 42, 58-64.

Hoos A., Urist M.J., Stojadinovic A., Mastorides S., Dudas M.E., Leung D.H.Y., Kuo D.,

Brennan M.F., Lewis J.J., Cordon-Cardo C. 2001. Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. *American Journal of Pathology* 158, 1245-1251.

Hsu, S.M., Raine, L., Fanger, H. 1981. The use of avidin-biotin peroxidase complex in immunoperoxidase techniques. *American Journal of Clinical Pathology* 75, 816-861.

Huerta, M., De Aluga A.S., Fragoso G., Toledo A., Villalobos N., Hernandez M., Gevorkian G., Acero G., Diaz A., Alvarez I., Avila R., Beltran C., Garcia G., Martinez J.J., Larralde C., Sciutto E. (2002). Synthetic peptide vaccine against *Taenia solium* pig cysticercosis: successful vaccination in a controlled field trial in rural Mexico. *Vaccine* 20, 262-266.

Hui, S.L., Walter, S.D. 1980. Estimation of error rates of diagnostic tests. *Biometrics* 36, 167-171.

Ilsoe B., Kyvsgaard N.C., Nansen, P., Henriksen, S.A. 1990. A study on the survival of *Taenia saginata* eggs on soil in Denmark. *Acta Veterinaria Scandinavica* 31, 153-158.

International Organization for Standardization (ISO). 1999. ISO/IEC 17025, In: General Requirements for the Competence of Calibration and Testing Laboratories. International Organization for Standardization, Geneva, Switzerland.

Ito A., Nakao M., Wandra T. 2003. Human taeniasis and cysticercosis in Asia. *The Lancet* 362, 1918-1920.

Jonsson, R., Tarkowski, A., Klareskog, L. 1985. A demineralization procedure for immunohistopathological use: EDTA treatment preserves lymphoid cell surface antigens. *Journal of Immunological Methods* 88, 109-114.

Juranek, D.D., Lewis, S.F., Keller, U. 1976. *Taenia saginata* cysticerci in muscles of beef cattle. *American Journal of Veterinary Research* 37, 785-789.

Karim, M.A. 1979. A survey of bovine cysticercosis in northern Iraq. Tropical Animal Health and Production 11, 239-240.

Kearney, A. 1970. *Cysticercus bovis*- Some factors which may influence cyst distribution. Journal of Parasitology 56, 183.

Kirichek V.S. (1985). Peculiarities of the biology of *Taenia saginata* and of the disease that it causes. Veterinariya 2, 50-52.

Kyvsgaard, N.C., Ilsoe, B., Henriksen, S.A., Nansen, P. 1988. An attempt to evaluate the spreading of *Taenia saginata* eggs in the environment. Acta Veterinaria Scandinavica 29: 511-513.

Kyvsgaard, N.C., Ilsoe, B., Henriksen, S.A., Nansen, P. 1990. Distribution of *Taenia saginata* cysts in carcasses of experimentally infected calves and its significance for routine meat inspection. Research in Veterinary Science 49, 29-33.

Laclette, J.P., Merchant, M.T., Willms, K. 1987. Histological and ultrastructural localization of antigen B in the metacestode of *Taenia solium*. Journal of Parasitology 73, 121-129.

Lawson J.R., Gemmell M.A. 1985. The potential role of blowflies in the transmission of taeniid eggs. Parasitology 91, 129-143.

Lees, W., Nightingale, J., Brown, D., Scandrett, B., Gajadhar, A. 2002. Outbreak of *Cysticercus bovis* (*Taenia saginata*) in feedlot cattle in Alberta; Cross Canada Disease Report. Canadian Veterinary Journal 43, 227-228.

Liang, K.Y., Zeger, S. 1986. Longitudinal Data Analysis using Generalized Linear Models. Biometrika 73, 13-22.

Lightowlers M.W. 2006. Vaccines against cysticercosis and hydatidosis: foundations in taeniid cestode immunology. *Parasitology International* 55: S39-S43.

Lightowlers M.W., Rolfe, R., Gauci, C.G. 1996. *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant oncosphere antigens. *Experimental Parasitology* 84, 330-338.

Lloyd, S. 1998a. Cysticercosis and taeniosis; *Taenia saginata*, *Taenia solium*, and Asian *Taenia*. In: Palmer, S.R., Lord Soulsby E.J.L., Simpson, D.I.H. (Ed.), *Zoonoses. Biology, Clinical Practice, and Public Health Control*, Oxford University Press, Oxford, UK, pp. 635-649.

Lloyd, S. 1998b. Other cestode infections. Hymenolepsis, diphyllbothriosis, coenurosis, and other adult and larval cestodes. In: Palmer, S.R., Lord Soulsby E.J.L., Simpson, D.I.H. (Ed.), *Zoonoses. Biology, Clinical Practice, and Public Health Control*, Oxford University Press, Oxford, UK, pp. 651-653.

Londono, D.P., Alvarez, J.I., Trujillo, J., Jaramillo, M.M., Restrepo, B.I. 2002. The inflammatory cell infiltrates in porcine cysticercosis: immunohistochemical analysis during various stages of infection. *Veterinary Parasitology* 109, 249-259.

Luna, L.G. 1968. *Manual of the histologic staining methods of the Armed Forces Institute of Pathology*, third edition, McGraw-Hill Book Company, Toronto 258 pp.

Machnicka, B, Slais, J, Zdarska, Z, Schramlova, J, Hulinska, D, Sterba, J. 1977. Experimental studies on bovine cysticercosis. *Acta Parasitologica Polonica* 25, 55-62.

MacPherson, R. 1978. Bovine cysticercosis storm following the application of human slurry. *Veterinary Record* 102, 156-157.

Maeda, G.E., Kyvsgaard, N.C., Nansen, P., Bogh, H.O. 1996. Distribution of *Taenia saginata* cysts by muscle group in naturally infected cattle in Tanzania. *Preventative Veterinary*

Medicine 28, 81-89.

Mango, A.M., Mango C.K.A. 1972. Recovery rate and distribution of *Cysticercus bovis* in calves artificially infected with *Taenia saginata* eggs. Research in Veterinary Science 13, 284-286.

Marty, A.M., Chester, A.J. 1997. Distinguishing lipid pseudomembranes from larval cestodes by morphologic and histochemical means. Archives of Pathological Laboratory Medicine 121, 900-907.

McAninch, N.H. 1974. An outbreak of cysticercosis in feedlot cattle. Canadian Veterinary Journal 15, 120-122.

McCool, C.J. 1979. Distribution of *Cysticercus bovis* in lightly infected young cattle. Australian Veterinary Journal 55, 214-216.

McIntosh, A., Miller, D. 1960. Bovine cysticercosis, with special reference to the early developmental stages of *Taenia saginata*. American Journal of Veterinary Research 21, 169-180.

McManus, D.P. 1990. Characterisation of taeniid cestodes by DNA analysis. Revue Scientifique et Technique (International Office of Epizootics) 9, 489-510.

McManus, D. 1963. Pre-natal infection of calves with *Cysticercus bovis*. Veterinary Record 75, 697.

McManus, D. 1960. Pre-natal infection of calves with *Cysticercus bovis*. Veterinary Record 72, 847-848

Mitchell, J.R. 1973. Some aspects of epidemiology and the prevalence of *Cysticercus bovis* in Africa. Bulletin of Epizootic Diseases of Africa 21, 133-139.

- Monteiro, L.L., Pinto, P.S.A., Dias, F.S. 2006. Evaluation of the ELISA test for the antibody detection in cattle naturally and experimentally infected with *Cysticercus bovis*. *Veterinary Parasitology* 141, 260-263.
- Mygind, T., Birkelund, S., Falk, E., Christiansen, G. 2001. Evaluation of real-time quantitative PCR for identification and quantification of *Chlamydia pneumoniae* by comparison with immunohistochemistry. *Journal of Microbiological Methods* 46, 241-251.
- Norman, G.R., Streiner, D.L. 2000. *Biostatistics: The Bare Essentials*. B.C. Decker, Hamilton, Ontario, Canada. 324 pp.
- Nunes, C.M., Dias, A.K.K., Dias, F.E.F., Aoki, S.M., De Paula, H.B., Lima, L.G.F., Garcia J.F. 2005. *Taenia saginata*: differential diagnosis of human taeniasis by polymerase chain reaction-restriction fragment length polymorphism assay. *Experimental Parasitology* 110, 412-415.
- Nyaga, P.N., Gathuma, J.M. 1979. Some observations on *Taenia saginata* cysticercosis in Kenya slaughter cattle. *Bulletin of Animal Health and Production in Africa* Vol. 27: 51-59.
- Office Internationale des Epizooties (OIE). 2004a. Chapter 1.1.2, Quality management in veterinary testing laboratories. In: *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Fifth Edition, Paris, France.
- Office Internationale des Epizooties (OIE). 2004b. Chapter 1.1.3, Principles of validation of diagnostic assays for infectious diseases. In: *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Fifth Edition, Paris, France.
- Office Internationale des Epizooties (OIE). 2004c. Chapter 2.10.1, Cysticercosis. In: *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Fifth Edition, Paris, France.
- Ogunremi, O., MacDonald, G., Geerts, S. & Brandt, J. 2004a. Diagnosis of *Taenia saginata* cysticercosis by immunohistochemical test on formalin-fixed and paraffin-embedded bovine

lesions. Journal of Veterinary Diagnostic Investigation 16, 438-441.

Ogunremi, O., MacDonald, G., Scandrett, B., Geerts, S., Brandt, J. 2004b. Bovine cysticercosis: preliminary observations on the immunohistochemical detection of *Taenia saginata* antigens in lymph nodes of an experimentally infected calf. Canadian Veterinary Journal 45, 852-855.

Oryan, A., Moghaddar, N., Gaur, S.N.S. 1995. *Taenia saginata* cysticercosis in cattle with special reference to its prevalence, pathogenesis and economic implications in Fars province of Iran. Veterinary Parasitology 57, 319-127.

Pampiglione, S., Rivasi, F., Canestri-Trotti, G. 1999. Pitfalls and difficulties in histological diagnosis of human dirofilariasis due to *Dirofilaria (Nochtiella) repens*. Diagnostic Microbiology and Infectious Disease 34, 57-64.

Pawlowski, Z.S., Murrel, K.D. 2001. Taeniasis and Cysticercosis. In: Hui, Y.H., Sattar, S.A., Murrell, K.D., Nip, W-K., Stanfield, P.S. (Ed.), Foodborne Disease Handbook, Volume 2, 2nd Edition, Marcel Dekker, NY, USA, pp. 217-227.

Pawlowski, Z., Schultz, M. 1972. Taeniasis and Cysticercosis (*Taenia saginata*). In: Advances in Parasitology, Vol 10, Academic Press, NewYork, USA, pp. 269-343.

Penfold, H. 1937. The life history of *Cysticercus bovis* in the tissues of the ox. Medical Journal of Australia 24th year, 579-583.

Penfold, W.J., Penfold, H.B. 1937. *Cysticercus bovis* and its prevention. Journal of Helminthology 15, 37-40.

Perez-Torres, A., Ustarroz, M., Constantino, F., Villalobos, N., Aluga, A.S.de. 2002. *Taenia solium* cysticercosis: lymphocytes in the inflammatory reaction in naturally infected pigs. Parasitology Research 88, 150-152.

Pfeifer, J.D., Hill, D.A., O'Sullivan, M.J., Dehner, L.P. 2000. Diagnostic gold standard for soft tissue tumors: morphology or molecular genetics? *Histopathology* 37, 485-500.

Pozio, E., Foggin, C.M., Gelanew, T., Marucci, G., Hailu, A., Rossi, P., Morales, M.A.G. 2006. *Trichinella zimbabwensis* in wild reptiles of Zimbabwe and Mozambique and farmed reptiles of Ethiopia. *Veterinary Parasitology* 143, 305-310.

Pramanik, A.K., Bhattacharyya, H.M., Sengupta, D.N. 1984. Occurrence of *Cysticercus bovis* in slaughtered cattle and buffaloes in Calcutta and its public health significance. *Indian Journal of Animal Health* 23(2): 141-145.

Proctor, E.M. 1972. Identification of tapeworms. *South African Medical Journal* 46, 234-238.

Pugh, K.E., Chambers, P.G. 1989. Observations on *Cysticercus bovis* in slaughter cattle in the Matabeleland province of Zimbabwe. *Veterinary Record* 125, 480-484.

Retzlaff, N. 1972. The histological structure of *Cysticercus bovis* at various stages of encapsulation and calcification. *Fleischwirtschaft* 52, 883-886, 889.

Rickard, M.D., Arundel, J.H., Adolph, A.J. 1981. A preliminary trial to evaluate the use of immunisation for the control of naturally acquired *Taenia saginata* infection in cattle. *Research in Veterinary Science* 30, 104-108.

Rickard, M.D., Davies, C., Bont, D.T., Smyth, J.D. 1977. Immunohistological localisation of two hydatid antigens (antigen 5 and antigen B) in the cyst wall, brood capsules and protoscoleces of *Echinococcus granulosus* (ovine and equine) and *E. multilocularis* using immunoperoxidase methods. *Journal of Helminthology* 51, 359-364.

Robinson, Yves. Pathologist, CFIA Ste. Hyacinthe Laboratory, Quebec. Personal communication.

Rodrigues, J.J.S., Ferreira, H.B., Farias, S.E., Zaha, A. 1997. A protein with a novel calcium-binding domain associated with calcareous corpuscles in *Echinococcus granulosus*. Biochemical and Biophysical Research Communications 237, 451-456.

Roman, G., Sotelo, J. Brutto, O.D., Flisser, A., Dumas, M., Wadia, N., Botero, D., Cruz, M., Garcia, H., De Bittencourt, P.R.M., Trelles, L., Arriagada, C., Lorenzana, P., Nash, T.E., Spina-Franca, A. (2000). A proposal to declare neurocysticercosis an international reportable disease. Bulletin of the World Health Organization 78, 399-406.

Safranov, I.V., Drogun, A.G. 1985. Morphological characterization of degenerated and calcified *Cysticercus bovis* in cattle. Veterinaraya Nauka Proizvodstvu 23, 76-77.

Saini, P.K., Webert, D.W., McCaskey, P.C. 1997. Food safety and regulatory aspects of cattle and swine cysticercosis. Journal of Food Protection 60, 447-453.

Scandrett, W.B. & Gajadhar, A.A. 2004. Recovery of putative taeniid eggs from silt in water associated with an outbreak of bovine cysticercosis. Canadian Veterinary Journal, 45, 758-760.

Schandevyl, P., Vercruysse, J. 1982. Cysticercosis in cattle in Senegal. Veterinary Parasitology 11, 267-270.

Schantz, P.M., Moore, A.C., Munoz, J.L., Hartman, B.J., Schaefer, J.A., Aron, A.M., Persaud, D., Sarti, E., Wilson, M., Flisser, A. 1992. Neurocysticercosis in an Orthodox Jewish community in New York City. The New England Journal of Medicine 327, 692-695.

Schillhorn van Veen, T.W. 1979. The occurrence of *Cysticercus bovis* in cattle livers. Veterinary Record 104, 370.

Sciutto, E., Martinez, J.J., Villalobos, N.M., Hernandez, M., Jose, M.V., Beltran, C., Rodarte, F., Flores, I., Bobadilla, J.R., Fragoso, G., Parkhouse, M.E., Harrison, L.J.S., De Aluja, A.S. (1998). Limitations of current diagnostic procedures for the diagnosis of *Taenia solium* cysticercosis in

rural pigs. *Veterinary Parasitology*, 79, 299-313.

Sewell, M.M.H., Harrison, L.J.S. 1978a. Bovine cysticercosis. *Veterinary Record* 102, 223

Sewell, M.M.H., Harrison, L.J.S. 1978b. Predilection sites for bovine cysticercosis. *Veterinary Record* 103, 18-19.

Shoukri, M.M., Pause, C.A. 1999. *Statistical Methods for Health Sciences*. 2nd Ed. CRC Press, New York. 384 pp.

Silverman, P.H., Hulland, T.J. 1961. Histological observations on bovine cysticercosis. *Research in Veterinary Science* 2, 248-252.

Singh, B. 1997. Molecular methods for diagnosis and epidemiological studies of parasite infections. *International Journal for Parasitology* 27, 1135-1145.

Slais, J. 1970. The morphology and pathogenicity of the bladder worms: *Cysticercus cellulosae* and *Cysticercus bovis*. Czechoslovak Academy of Sciences, Junk Publishers, The Hague, 144 pp.

Sloan, L., Schneider, S., Rosenblatt, J. (1995). Evaluation of enzyme-linked immunoassay for serological diagnosis of cysticercosis. *Journal of Clinical Microbiology* 33, 3124-3128.

Smith, H.J., Snowdon, K.E., Finlay, R.C. 1991a. Serological diagnosis of cysticercosis by an enzyme-linked immunosorbent assay in experimentally infected cattle. *Canadian Journal of Veterinary Research* 55, 274-276.

Smith, H.J., Snowdon, K.E., Finley, G.G. 1991b. Eosinophilic myositis in Canadian cattle. *Canadian Journal of Veterinary Research* 55, 94-95.

Snyder, G.R., Murrel, K.D. 1986. Bovine cysticercosis. In: *Practices in Veterinary Public Health and Preventative Medicine in U.S.A.* 1st Volume, Ames, Iowa, Iowa State University

Press, pp. 161-170.

Sorvillo, F.J., DeGiorgio, C., Waterman, S.H. 2007. Deaths from cysticercosis, United States. *Emerging Infectious Diseases* (www.cdc.gov/eid) 13, 230-235.

Soulsby, E.J.L. 1963. Immunological unresponsiveness to helminth infections in animals. *International Veterinary Congress 17th*, Hannover, 761-767.

Soulsby, E.J.L. 1982. Cestodes. In: Soulsby, E.J.L. (Ed.), *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th Edition, Bailliere Tindall, London, pp. 87-136.

Sripa, B., Kaewkes, S. 2000. Localisation of parasite antigens and inflammatory responses in experimental opisthorchiasis. *International Journal for Parasitology* 30, 735-740.

Steele, Tom. CFIA Veterinarian, Regional Office, Alberta. Personal communication.

Sterba, J., Dykova, I. 1978. Tissue reaction of the skeletal muscles of cattle both to a spontaneous and experimental infection with *Cysticercus bovis*. *Folia Parasitologica* 25, 347-354.

Sterba, J., Dykova, I., Machnicka, B. 1979a. Tissue reaction in the heart of cattle with a spontaneous and artificial *Cysticercus bovis* infection. *Folia Parasitologica* 26, 27-33.

Sterba, J., Dykova, I., Machnicka, B. 1979b. Tissue reaction to *Cysticercus bovis* in the lung of artificially infected cattle. *Folia Parasitologica* 26, 125-128.

Stevenson, P. 1983. Observations on the hatching and activation of fresh *Taenia saginata* eggs. *Annals of Tropical Medicine and Parasitology* 77, 399-404.

Sutton, Mary. Senior pathologist, USDA, Athens, Ga. Personal communication.

- Takemoto, Y., Negita, T., Ohnishi, K., Suzuki, M., Ito, A. 1995. A simple method for collecting eggs of taeniid cestodes from fresh, frozen or ethanol-fixed segments. *International Journal for Parasitology* 25, 537-538.
- Taylor, C.R. 2006. Standardization in immunohistochemistry: the role of antigen retrieval in molecular morphology. *Biotechnic and Histochemistry* 81, 3-12.
- Tegtmeier, C., Angen, O., Ahrens, P. 2000. Comparison of bacterial cultivation, PCR, in situ hybridization and immunohistochemistry as tools for diagnosis of *Haemophilus somnus* pneumonia in cattle. *Veterinary Microbiology* 76, 385-394.
- Thornton, I.I. 1979. Give yourself a tapeworm? *Veterinary Record* 104, 287.
- Urquhart, G.M., Brocklesby, D.W. 1965. Longevity of *Cysticercus bovis*. *Journal of Parasitology* 51, 349.
- Van den Heever, L.W. 1967. On the longevity of *Cysticercus bovis* in various organs in a bovine. *Journal of Parasitology* 53, 1168.
- Van der Logt, P.B., Gottstein, B. 2000. Unidentified parasite cysts in cattle. *Veterinary Record* 146, 610-612.
- Van Kerckhoven, I., Vansteenkiste, W., Claes, M., Geerts, S., Brandt, J. 1998. Improved detection of circulating antigen in cattle infected with *Taenia saginata* metacestodes. *Veterinary Parasitology* 76, 269-274.
- Walther, M. 1980. *Taenia saginata* cysticercosis: A comparison of routine meat inspection and carcass dissection results in calves. *Veterinary Record* 106, 401-402.
- Wang, I.C., Ma, Y.X., Kuo, H., Fan, P.C. 1997. A comparative study on egg hatching methods and oncosphere viability determination for *Taenia solium* eggs. *International Journal for*

Parasitology 27, 1311-1314.

Wanzala, W., Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H., Harrison, L.J.S. 2002. Serodiagnosis of bovine cysticercosis by detecting live *Taenia saginata* cysts using a monoclonal antibody-based antigen-ELISA. Journal of the South African Veterinary Association 73, 201-206.

Werner, M., Chott, A., Fabiano, A., Battifora, H. 2000. Effect of formalin tissue fixation and processing on immunohistochemistry. The American Journal of Surgical Pathology 24, 1016-1019.

White, A.C. (2000). Neurocysticercosis: updates on epidemiology, pathogenesis, diagnosis, and management. Annual Review of Medicine 51, 187-206.

Yoder, D.R., Ebel, E.D., Hancock, D.D., Combs, B.A. 1994. Epidemiologic findings from an outbreak of cysticercosis in feedlot cattle. Journal of the American Veterinary Medical Association 205, 45-50.

Zarlenga, D.S., McManus, D.P., Fan, P.C., Cross, J.H. 1991. Characterization and detection of a newly described Asian taeniid using cloned ribosomal DNA fragments and sequence amplification by the polymerase chain-reaction. Experimental Parasitology 72, 174-183.

Zivkovic, J., Velimirovic, D., Dzaja, P., Grabarevic, Z. 1996. Prevalence of *Cysticercus bovis* s. *inermis* Measles with particular reference to histopathological changes in meat. Archiv fur Lebensmittelhygiene 47, 66-68.

APPENDIX A

HISTOLOGICAL METHOD (HEMATOXYLIN-PHLOXINE-SAFRAN) FOR THE DIAGNOSIS OF *TAENIA SAGINATA* CYSTICERCOSIS

Equipment/Instrumentation

precleaned glass microscope slides 25 x 75 x 1 mm
coverslips
Coplin staining jars
water bottles with rinse nozzle
Permout mounting medium

Reagents

toluene
100 % ethanol
saturated aqueous picric acid
1.5 % aqueous phloxine B solution
2.0 % alcoholic safran
distilled water
tap water

Preparation for the Test

1. Preparation of the sample

Fresh-chilled samples (maximum dimension 1cm x 1cm) are to be formalin-fixed for 24 hours, and paraffin-embedded into tissue blocks. Embedded tissue is cut at 5 µm, floated on a waterbath and picked up on microscope slides. Excess water is drained from the slide and placed on a slide warmer or in an incubator at $45 \pm 5^{\circ}\text{C}$ to dry.

2. Preparation and storage of reagents

Reagents are prepared and diluted as specified in the procedure below. Ensure all reagents are used within any applicable expiry dates, and stored as per the manufacturer's instructions.

3. Preparation of technical personnel

Individuals should be familiar with good laboratory practices and the maintenance of a safe and efficient laboratory.

All individuals performing this assay must have successfully completed a formal training session on the procedure, provided by authorized staff at the CFIA St. Hyacinthe Laboratory.

4. Performance of the Test

Tissue Pretreatment:

Prepared slides are deparaffinized by standard histological methods and rehydrated in fresh distilled water in the following sequence:

Immerse one or more slides in Coplin jar filled (approx. 50 ml) with 2 changes of toluene for 3 min each.

Transfer to Coplin jar filled with 100 % ethanol and immerse for 2 min.

Transfer to Coplin jar filled with 95 % ethanol and immerse for 2 min.

Transfer to Coplin jar filled with 80 % ethanol and immerse for 2 min.

Transfer to Coplin jar filled with distilled water and immerse for 2 min.

Hematoxylin, Phloxin, and Saffran Staining:

Immerse in picric acid (2.0 g picric acid in 100 ml distilled water) in Coplin jar for 5 min.

Rinse in tap water until all the picric acid is removed.

Stain in Mayer's hematoxylin (1.0 g hematoxylin crystals, 1000.0 ml distilled water, 0.2 g sodium iodate, 50.0 g ammonium or potassium alum, 1.0 g citric acid, 50.0 g chloral hydrate) for 20 min.

Wash in running tap water for 7 min.

Stain in 1.5 % aqueous phloxine B solution (1.5 g phloxine B, 100.0 ml distilled water) for 1-2 min.

Wash in tap water for 5 min.

Dehydrate in 3 changes of 95 % alcohol, and once in absolute alcohol (1 min each).

Stain in 2 % alcoholic safran (2.0 g safran du Gatinais, 100.0 ml absolute alcohol) for 4 min.

Rinse with 2-3 changes of absolute alcohol.

Toluene, 2 changes of 2 min each.

Mount in Permount. A drop or line of Permount is applied to one edge of the slide and a glass coverslip positioned along this edge and gently placed over the tissue section. Slide is placed between 2 layers of absorbent paper tissue and even pressure applied to blot out excess medium. Slide is then laid flat to dry.

APPENDIX B

IMMUNOHISTOCHEMICAL METHOD FOR THE DIAGNOSIS OF *TAENIA SAGINATA* CYSTICERCOSIS

Equipment/Instrumentation

precleaned superfrost plus charged slides 25 x 75 x 1 mm
coverslips
timer
vortex
Coplin staining jars
water bottles with rinse nozzle
Eppendorf (or comparable) pipets
disposable plastic pipettes
PAP pen
humidity chamber
37° C incubator

Reagents

xylene
100% ethanol
distilled water
sterile water
30% hydrogen peroxide
absolute methanol
10X automation buffer
Dulbecco's buffer
protease XIV
normal horse serum
skim milk powder
MoAb 158C₁₁A₁₀ (IgG1 mouse anti-T. saginata cysticercus ES antigen)
MoAb 2BD4E4 (ATCC HB-8178; IgG1 mouse anti- E. coli pilus antigen)
biotinylated secondary MoAb (horse anti-mouse)
avidin-biotin-peroxidase solution kit (Vectastain Elite)
3, 3'-diaminobenzidine-4 Hcl (DAB)
Gill's Hematoxylin
Cytoseal mounting medium
Superfreeze peroxidase conjugate stabiliser

Preparation for the Test

1. Preparation of the sample

Fresh-chilled samples (maximum dimension 1cm x 1cm) are to be formalin-fixed for 24-48

hours, and paraffin-embedded into tissue blocks. If there is a delay in the processing of the sample, store in 70 % ethanol after formalin fixation and prior to embedding. Embedded tissue is cut at 5 µm, floated on a waterbath and picked up on microscope slides which are positively charged. Excess water is drained from the slide and placed on a slide warmer or in an incubator at $45 \pm 5^{\circ}\text{C}$ to dry.

2. Preparation and storage of reagents

Reagents are prepared and diluted as specified in the procedure below. Ensure all reagents are used within any applicable expiry dates, and stored as per the manufacturer's instructions.

3. Preparation of technical personnel

Individuals should be familiar with good laboratory practices and the maintenance of a safe and efficient laboratory.

All individuals performing this assay must have successfully completed a formal training session on the procedure, provided by authorized staff at the Centre for Food-borne and Animal Parasitology.

4. Performance of the Test

Tissue Section Pretreatment:

Prepared slides are deparaffinized by standard histological methods and rehydrated in fresh distilled water in the following sequence:

Immerse one or more slides in coplin jar filled (approx. 50 ml) with xylene for 5 min.

Transfer to Coplin jar filled with 100 % ethanol and immerse for 2 min.

Transfer to Coplin jar filled with 95 % ethanol and immerse for 2 min.

Transfer to Coplin jar filled with 70 % ethanol and immerse for 2 min.

Transfer to Coplin jar filled with distilled and immerse for 2 min.

Inactivate endogenous peroxidases by immersing slide(s) in a 4 % solution of hydrogen peroxide in methanol (4 ml 30 % H_2O_2 in 96 ml absolute methanol- use 2 ml 30 % H_2O_2 in 48 ml absolute methanol) for 12 min at room temperature. Do not let slide(s) dry between any of the following steps.

Wash 3 times in 1x Automation Buffer (10 ml of 10x Automation Buffer concentrate in 90 ml distilled water- use 100 ml 10x Automation Buffer concentrate in 900 ml distilled water).

Immerse slides in a solution of 50 mg Protease XIV in 100 ml Dulbecco's Buffer (use 25 mg/50 ml) and incubate at 37 C for 20 min.

Wash slides 3 times in 1x Automation Buffer to rinse all traces of protease from slides.

Block non-specific antibody-binding sites by immersing slides in 4 % normal horse serum diluted in Dulbecco's Buffer (4 ml horse sera in 96 ml Dulbecco's Buffer- use 2 ml horse sera in

48 ml Dulbecco's) for 10 min at room temperature.

Blocking is enhanced by immersing slides in a 2 % solution of skim milk diluted in Dulbecco's Buffer (2 g skim milk / 100 ml Dulbecco's Buffer- use 1 g / 50 ml) for 10 min at room temperature.

Slides are quickly rinsed with 4 % normal horse serum and excess liquid is blotted away with a paper towel ensuring that the section is not disturbed.

Using a PAP pen, the tissue section(s) on the slide are circumscribed to conserve antibody and reagents in the staining procedure.

Immunostaining:

Dilute primary relevant antibody 158C₁₁A₁₀ (1:250; 12 µl of 2:1 dilution of antibody in Superfreeze/ 988 µl) and primary irrelevant negative control antibody 2BD4E4 (1:500; 6 µl of 2:1 dilution of antibody in Superfreeze/ 994 µl) in 4 % normal horse serum. Mix on vortex mixer to ensure uniform distribution of antibody immediately prior to application. Place test specimen section slides, positive and negative control *T. saginata* cysticercus section slides, and tissue control lymph node section slide in a humidity chamber containing distilled water to a depth of 3-5 mm. Separate chambers are to be used for slides receiving either the relevant or irrelevant primary antibody. Add either relevant or irrelevant diluted primary antibody dropwise until entire tissue section is covered but not overflowing PAP pen borders. One of each pair of test sections, one positive control *T. saginata* section, and the tissue control section receive the relevant antibody; the remaining test sections and negative control *T. saginata* section receive the irrelevant antibody.

Place humidity chambers in a 4° C cooler overnight, or alternatively in a 37° C incubator for 2 hours.

Wash slides with 1X Automation Buffer using a water bottle with the stream directed at the slide just above the tissue section. Do not spray directly at the section as this can dislodge the tissue from the slide. Place spray washed slides in Coplin Jar and immerse with gentle agitation in 1X Automation Buffer. Repeat wash 3 times.

Prepare biotinylated secondary antibody in 4 % horse serum and dilute to 1:400 (9 µl of 2:1 dilution of antibody in Superfreeze/ 1191 µl) or as prescribed in product insert. The biotinylated secondary antibody is directed against the primary antibody. Place slide in humidity chamber and add secondary antibody dropwise until tissue is completely covered. Incubate for 30 min at 37° C.

Prepare avidin biotin complex peroxidase solution as prescribed by the product insert. Add 1 drop each of Reagents A and B to 2.5 ml Dulbecco's buffer in plastic dropper bottle and mix with vortex. When using Vectastain Elite ensure that the product is prepared 30 min prior to use as stated in product information.

Wash slides with 1X Automation Buffer using a water bottle with the stream directed at the slide just above the tissue section. Do not spray directly at the section as this can loosen tissue from the slide. Place spray-washed slides in Coplin Jar and immerse with gentle agitation in 1X Automation Buffer. Repeat wash 3 times.

Apply the avidin biotin complex peroxidase solution dropwise until all tissue is completely covered. Incubate slides in humidity chambers for 45 min or as instructed by product insert. Wash slides with 1X Automation Buffer using a water bottle with the stream directed at the slide just above the tissue section. Do not spray directly at the section as this can loosen tissue from the slide. Place spray-washed slides in Coplin Jar and immerse with gentle agitation in 1X Automation Buffer. Repeat wash 3 times.

Thaw 100 µl vial of 3, 3-diaminobenzidine-4 Hcl (DAB) and add to 10 mls of Dulbecco's Buffer. Immediately prior to use add 3.5 µl of 30 % H₂O₂ to the DAB and mix on vortex for 10 sec.

Add DAB dropwise until all tissue is completely covered and incubate at room temperature for approximately 4 min (may range from 2-5 min depending on antibody batch, and requires titration while monitoring positive and negative tissue controls with each new batch of antibody). After the pre-determined allotted time period (when desired intensity is obtained), stop the reaction by washing slides numerous times with 1X Automation Buffer. Ensure the same period of incubation is applied to both the relevant antibody- and irrelevant antibody-stained sections.

Counterstain with Gill's hematoxylin diluted 1:10 with distilled water (5 ml hematoxylin in 45 ml distilled water) for 1 min.

Dehydrate through 70 %, 95 %, 100 % ethanol prepared with distilled water (2 min each) and finish in xylene bath.

When slides are adequately cleared in xylene (10 min), mount with compatible (Cytoseal) mounting media and coverslip. Allow mounted slides to air-cure for at least 30 min prior to viewing.

APPENDIX C

TITRATION OF PRIMARY AND SECONDARY MONOCLONAL ANTIBODIES IN IMMUNOHISTOCHEMICAL METHOD FOR *TAENIA SAGINATA* CYSTICERCOSIS

A. Section of degenerated cysticercus from an experimentally infected animal and stained with relevant primary IgG1 monoclonal antibody 158 C₁₁A₁₀ and horse anti-mouse IgG1 secondary antibody at dilutions of 1:250 and 1:800, respectively.

B. Section of same specimen stained with relevant primary IgG1 monoclonal antibody 158 C₁₁A₁₀ and horse anti-mouse IgG1 secondary antibody at dilutions of 1:250 and 1:400, respectively.

C. Section of same specimen stained with relevant primary IgG1 monoclonal antibody 158 C₁₁A₁₀ and horse anti-mouse IgG1 secondary antibody at dilutions of 1:100 and 1:800, respectively.

D. Section of same specimen stained with relevant primary IgG1 monoclonal antibody 158 C₁₁A₁₀ and horse anti-mouse IgG1 secondary antibody at dilutions of 1:100 and 1:400, respectively.

E. Negative control section adjacent to that in A. above and stained with irrelevant primary IgG1 monoclonal antibody 2BD4E4 and horse anti-mouse IgG1 secondary antibody at dilutions of 1:500 and 1:800, respectively.

F. Negative control section adjacent to that in B. above and stained with irrelevant primary IgG1 monoclonal antibody 2BD4E4 and horse anti-mouse IgG1 secondary antibody at dilutions of 1:500 and 1:400, respectively.

Bar = 500 μ m.

