**Effect of thermal and ensilation treatments on viability of *Taenia hydatigena* eggs**


BY


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**ABSTRACT**

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In the Pacific Northwest USA feeding of potato co–products has been speculated to result in greater prevalence of beef cysticercosis caused by *Taenia saginata* as compared to rest of the USA. A *Taenia hydatigena* model was used to assess the effect of heat and ensilation treatments on viabilities of eggs. The *T. hydatigena* life cycle was maintained under laboratory conditions by passing the parasite through a canine–ovine cycle. For studying effect of heat, *in vitro* and *in vivo* experiments were carried out at temperatures ranging from room temperature (22°C) to 60°C for five minutes each. To study the effect of ensilation, *in vivo* study was conducted to analyze the effect of 0, 7, 14, 21 and 28 days of ensilation of minced potato on viability of *T. hydatigena* eggs. Effect of *in vitro* heat treatment was analyzed using sigmoidal four–parameter model and resulted in 99.47% reduction in viability at 60.00°C. *In vivo* heat treatments caused linear decrease in viability at the rate of 0.32% per degree Celsius with 100% reduction occurring at 57.38°C. After ensilation, maximum reduction in viability of 0.10±3.72% was attained after 18.59±3.65 days of ensilation. Similar heat and ensilation treatments or a combination of the two may also be effective against *T. saginata* and may help to reduce occurrence of beef cysticercosis.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# Chapter 1

## REVIEW OF LITERATURE

## 1.1 INTRODUCTION

*Taenia saginata*, *T. asiatica* and *T. solium* are three major meat borne zoonotic tapeworms. They cause taeniosis in humans and cysticercosis in meat animals (beef cattle and swine), respectively. Due to zoonotic importance, these parasites detrimentally impact the meat industry. Characteristics like high fecundity, resistant life stages, and co–existence with its hosts make them highly adaptable to their complex two–host life cycle. Also, due to these characteristics, these tapeworms are prevalent globally.

*Taenia saginata* (beef tapeworm) has its major impact on the beef industry. While *T. saginata* is highly prevalent in Latin America, Africa, Asia and some Mediterranean countries, it is also found in other regions with low levels of prevalence (Murrell and Dorny, 2005). Based on unpublished 2009 United States Department of Agriculture (USDA) meat inspection data, the prevalence of bovine cysticercosis in USA and the Northwest USA was 0.003% and 0.052%, respectively. Based on USDA meat inspection data the prevalence of cysticercosis in the Northwest has been the highest in the USA at least since 1984. The relatively high prevalence in the Pacific Northwest (PNW) results in increased economic losses to the feedlot industry in the region. Potato co–products contaminated with *T. saginata* eggs are considered the most likely source of infection leading to higher prevalence of cysticercosis in the PNW cattle (Hancock et al., 1989; Yoder et al., 1994). This relationship provides the primary topic of research that will be described here.

## 1.2 BIOLOGY

*Taenia saginata* is closely related to and has evolved from tapeworms of wild canids. Predecessors of *T. saginata* evolved about 2.0–2.5 million years ago to include humans as the definitive host when ancestors of *Homo sapiens* adopted omnivorous diet to include bovid preys in diet; and later this relation was intensified during domestication of bovids about 10,000 years ago (Hoberg et al., 2000; Hoberg et al., 2001; Hoberg, 2002, 2006). *T. saginata* has evolved to have humans as their only definitive host. Based on phylogenetic cladogram, *T. hydatigena*, a canine tapeworm, is closely related to *T. saginata* (Hoberg et al., 2001) and may be useful as a surrogate organism to study characteristics of *T. saginata*.

Parasitic tapeworms of genus *Taenia* are long, segmented worms with two host life cycle. Body length may vary from less than 10 cm to more than 1,000 cm (Hoberg et al., 2000). The body of an adult tapeworm is divided into distinct scolex (head), neck (unsegmented body) and strobila (segmented body). Taxonomic classification of tapeworms in the genus *Taenia* is shown Table 1.1. Previously two distinct life stages of the parasite, adult and metacestode were considered different species. The morphologically dissimilar life stages and were wrongly assigned to two different genera, *Taenia* and *Cysticercus*. Intermediate life stage of *T. saginata*, *T. solium*, *T. hydatigena*, *T. ovis*, *T. taeniaeformis* were named *C. bovis*, *C. cellulose*, *C. tenuicollis*, *C. ovis* and *C. fasciolaris,* respectively (Soulsby, 1982 p.107-116; Arme and Pappas, 1983). For a recently identified

3

asian tapeworm some confusion exists for an exact scientific name and has been referred to as *T. saginata asiatica* (Flisser et al., 2004; Scandrett, 2007) or *T. asiatica* (Hoberg, 2006; Youn, 2009).

## 1.3 LIFE CYCLE

Epidemiology, disease and control strategies related to *Taenia saginata* are entertwined with the life cycle of the parasite. Life cycle of *Taenia saginata* involves intermediate (cattle) and definitive (Humans) host species; and three distinct stages – eggs in environment, cysticerci in the cardiac and skeletal muscles of the intermediate host and adult tapeworms in the small intestine of definitive host (Figure 1.1).

### 1.3.1 EGGS

Infected humans pass terminal mature segments known as gravid proglottids that contain infective eggs. These proglottids are 6–22 mm in length and 6.5–9.5 mm in width (Hoberg et al., 2000; Flisser et al., 2004; Murrell and Dorny, 2005) and are released in to the intestinal lumen. In *T. saginata* infections about 10 gravid proglottids are released from humans per day and each proglottids contains 50,000–80,000 eggs (Soulsby, 1982; Murrell and Dorny, 2005). A single adult worm can shed 10.7 billion eggs over an average life span of 18 years (Schapiro, 1937). The proglottids of *T. saginata* are motile and can actively crawl out of the anus or be shed with feces (Soulsby, 1982). Once in the environment the eggs can survive for variable time periods depending on the

environmental factors. Eggs of *T. saginata* are infective to ruminant intermediate hosts, especially cattle, leading to bovine cysticercosis.

*Taenia saginata* eggs are oval brown structure and 46–50 µm by 39–41 µm in diameter, made up of a series of outer coverings that bound the hexacanth (six–hooked) tapeworm larva known as oncosphere (Nieland, 1968; Soulsby, 1982 p. 108; Jabbar et al., 2009). The different layers of the egg are shed in three stages. The outer most layer consisting of shell/capsule and outer envelope is fragile and is lost during escape of the egg from the uterus within a gravid proglottid. The second layer is made up of keratin blocks and is known as embryophore. The embryophore is thick and is visible as a radiating brown layer with radial striations. Eggs in the environment retain this layer. Once ingested by the bovine intermediate host, the embryophore is lost by the action of digestive juices in stomach of vertebrate intermediate host. Under laboratory conditions, sodium hypochlorite may be used to remove this layer (Laws, 1967; Wang et al., 1997). The third and the inner most layer is the oncospheral membrane made up of a pair of laminae with closely spaced vesicles (Nieland, 1968). The oncospheral membrane is lost by the process of activation after exposure to bile in the duodenal region of the intermediate host (Soulsby, 1982 p.110; Smyth and McManus, 1989 p.193; Singh and Prabhakar, 2002). Under laboratory conditions, frozen or fresh bile may be used for activation of the oncospheres. Species specificity of *Taenia* spp. for intermediate hosts is attributed to properties of bile although no exact components of bile responsible are known (Smyth and

McManus, 1989 p.193). Activation is indicated by active movement of hooks. The three pairs of keratinized hooks develop from oncoblasts (hook forming cells) and are attached to 18 cell hook muscle system (Jabbar et al., 2009).

The larval stage of *Taenia* spp. is not as host specific as the adult stage is. While cattle are the most important species, other ruminants– llama, reindeer, sheep, goat, roe deer, fallow deer and lagomorphs may also act as intermediate hosts (Soulsby, 1982 p.108; Cabaret et al., 2002). Cattle are infected when they ingest feed or water contaminated with eggs or gravid proglottids from human sources. In cattle, the eggs hatch to release the oncospheres which further develop into cysticerci in striated and cardiac muscles.

### 1.3.2 CYSTICERCI

After activation the *T. saginata* oncospheres penetrate through the intestinal mucosa to reach the general circulation. The oncospheres then reach various tissues. *T. saginata* has preference for striated, cardiac and smooth muscles especially the diaphragm, tongue, esophagus, masseter, triceps, thigh and heart but may be found elsewhere (Soulsby, 1982; Kebede et al., 2008). Based on postmortem examination of 42 beef calves, Scandrett (2009) concluded that among the traditional sites, heart was the most and esophagus was the least reliable site for localization of *T. saginata* cysticerci among the sites listed.

In transition from multicellular oncospheres to fluid filled cysticerci the hooks are lost; there is a rapid increase in surface area with development of

surface microvilli and generation of a strong antigenic response by the host (Engelkirk and Williams, 1982; Jabbar et al., 2009). In muscle tissue, oncospheres develop into encysted cysticerci stage of *T. saginata* (Abuseir et al., 2006). Earlier these cysticerci were thought to be caused by a distinct species, independent of *T. saginata* and was named *Cysticercus bovis* (Kassai, 2001). The lesions caused by these cysticerci usually cause no any clinical disease or may cause mild clinical signs in heavy infections, which includes pyrexia, anorexia, muscular weakness and emaciation (Arme and Pappas, 1983 p. 519; Radostits et al., 2000 p. 1386). The infective stage (mature cysticercus) is reached in ten weeks and can remain viable for up to nine months or more. Cysticerci that die at predilection sites are calcified by the host defense system and are easily detectable post–mortem.

### 1.3.3 ADULT TAPEWORM

Humans usually become infected by consuming cysticerci along with "measley beef" (raw or undercooked infected meat). In the human stomach, the wall of the cysticerci is digested and young tapeworm is released. The parasite reaches the small intestine and attaches to the wall with sucker disks present on the scolex (head). The adult tape worm develops in three months and may grow to 4 to 12m in length (Murrell and Dorny, 2005 p.4). Unlike *T. solium*, a rostellum and hooks are missing from the scolex of *T. saginata*, which provides a diagnostic feature to differentiate these two tapeworms of humans. The adult tapeworms are hermaphroditic and following reproduction, eggs develop in gravid proglottids that are released into the environment.

## 1.4   EPIDEMIOLOGY

All three life stages of *T. saginata* viz. eggs in environment, cysticerci in beef cattle and adult tapeworms in humans play important roles in the epidemiology of the parasite (Murrell and Dorny, 2005). In recent times, intensification of the beef industry and migration of humans have also played important epidemiological role.

### 1.4.1 ROLE OF EGGS

In regions with high prevalence of taeniosis, the possibility of environmental contamination with *T. saginata* eggs is really high due to release of up to 800,000 eggs by each adult worm per day. Subsequently, high resistance to environmental deterioration enables these eggs to survive for a long time and possibly relocate to distant locations via different means. Once passed by humans, eggs can get access to feedstuffs through sewage, water contamination, poor hygienic practices, or mechanical carriers (humans, birds, arthropods, earthworms) (Slonka et al., 1975; Slonka et al., 1978; Fertig and Dorn, 1985; Cabaret et al., 2002; Murrell and Dorny, 2005).

In the environment, eggs may survive for different lengths of time mainly depending upon temperature and moisture conditions. The best survival rates are at low to moderate temperatures and high moisture conditions. The eggs viability is quite variable depending upon the prevailing environmental conditions. Even under similar conditions the survivability of *Taenia* spp. eggs has been reported to be quite variable (Froyd, 1962). Eggs remain viable for 11 weeks in water at

24°F (-4.44°C) under laboratory conditions (Lucker, 1960), 413 days on pastures in Kenya (Duthy and Someren, 1948), 60-80 days in grass silage at 10°C in Germany (Enigk et al., 1969) and 21 days in stored dry hay (Lucker and Douvres, 1960; Murrell and Dorny, 2005).

Fields fertilized with sewage contaminated with *T. saginata* eggs have been reported to be an important source of infection for feedlot animals (Cabaret et al., 2002). The eggs are able to survive all forms of sewage and sludge treatments (Cabaret et al., 2002; Murrell and Dorny, 2005). Apart from use as a fertilizer, municipal sewage may get access to pastures by leakage or flood (Fertig and Dorn, 1985), or may contaminate the water source of the feedlot (Lees et al., 2002; Scandrett and Gajadhar, 2004). Among sludge treatments, lagooning of sludge at 7°C for 28 days has been reported to be the most successful treatment causing more than a 99% reduction in viability of *T. saginata* eggs (Bruce et al., 1990).

In the Northwest USA, potato co–products are considered the source of infection of *T. saginata* eggs to beef cattle at feedlots (Hancock et al., 1989; Yoder et al., 1994; Nelson, 2003). Potato processing plants in the area generate low cost co–products in high volumes for the feedlots (Bradshaw et al., 2002; Nelson, 2003). Potato co–products are usually stored in pits at the feedlot for a short time and are fed to feedlot cattle without processing. Potato co–products may get contaminated at potato processing plants or at feedlots through sewage water or infected workers. Since the proglottids are motile and can actively migrate out of

infected humans, contamination may not always require direct contact with human feces. Low to moderate temperature conditions in the region and high moisture contents of the potato co–products may prolong the survival of *T. saginata* eggs.

## 1.4.2 ROLE OF CYSTICERCI

Since there are no clinical signs associated with bovine cysticercosis in live animals, the disease is diagnosed postmortem in the form of cysticerci found at time of routine meat inspection. Based on historic data, both endemic and epidemic occurrences of beef cysticercosis are possible. Epidemics usually originate from a point source of infection that infects many animals at the same time. Various sources of infection for cattle may be eggs in silage, cattle pens, feed bins, pastures, and/or water supplies that have been contaminated with infected human feces, sewage, or sewage effluent (Weedon, 1987). Outbreaks of bovine cysticercosis have been reported in the North America (Table 1.2). These outbreaks were reported mostly based on postmortem inspections. Meat inspectors may fully condemn the affected carcass or condemn affected parts depending upon severity of the condition for public health concerns. Condemnation adds to economic losses and leads to heavy unexpected losses to the beef industry.

In 2009, the Northwest contributed 4% of the USA beef slaughter but 85% of the bovine cysticercosis measles cases identified by USDA inspection. The prevalence was 0.052% in the Pacific Northwest compared to 0.003% in the USA.

### 1.4.3 ROLE OF ADULT TAPEWORM

Globally, the prevalence of *T. saginata* infections in humans may be divided into three regions. A highly endemic (>10%) region in central and east Africa, a moderately endemic (0.1–10%) region in Europe, SE Asia and South America and a low endemic (<0.1%) region in USA, Canada and Australia (Murrell and Dorny, 2005). Like bovine cysticercosis, taeniosis in many humans may occur from a point source of infection but there are seldom any reports of epidemics of *T. saginata* taeniosis. Most infected humans are clinically normal but shed gravid proglottids in feces for years (Garcia et al., 2003b). This situation of the disease makes it impossible to prevent cysticercosis outbreaks.

Taeniosis in USA has not been studied extensively and is poorly understood (DeGiorgio et al., 2005). In addition to beef as source of infection, immigrants from endemically infected countries are also a cause of increased cases of bovine cysticercosis and taeniosis in USA (Flisser et al., 2004; DeGiorgio et al., 2005; Macpherson, 2005). Unlike bovine cysticercosis, there are no reports of outbreaks of taeniosis in humans in USA because the infection in most cases is sub–clinical and has a chronic course of infection.

With advancement in knowledge of public health, stricter post mortem regulations have made detection and diagnosis of bovine cysticercosis a standard postmortem practice. If lesions are detected, carcasses are condemned or appropriately treated to prevent transmission of the disease to humans. But this

does not prevent the economical losses to the beef industry. Thus, now the aim of the investigators is to prevent the exposure of feedlot cattle to *T. saginata* eggs.

## 1.5 ECONOMIC IMPACT

Cysticercosis may lead to sever losses to feedlots because of complete condemnation or carcass processing. Table 1.3 shows the estimates of loss per animal due to cysticercosis. These losses have risen 369% in 21 years and thus, on an average, 18% per year.

## 1.6 PRESENT CONTROL STRATEGIES AND THEIR SHORT COMINGS

Potential control strategies may be described as four critical control points in the life cycle of *T. saginata* (Figure 1.1). These critical control points are (A) Preventing cattle from developing infective stages; (B) Preventing human infection; (C) Preventing eggs' dispersal; and (D) Treating feedstuffs to prevent infection of beef animals.

### 1.6.1 PREVENTING CATTLE FROM DEVELOPING INFECTIVE STAGES

This may be achieved by vaccinating or treating animals. Reports of control with vaccination may be a promising control strategy but treatment of infected animals is not possible in large scale production systems since cysticercosis is asymptomatic and the disease cannot be diagnosed in live animals. Substantial work has been done on vaccine development recently. Recombinant vaccine having two antigens TSA–9 and TSA–18 confer 99.8% protection against *T. saginata* eggs in cattle (Lightowlers, 1996; Lightowlers et al., 1996).

While the vaccines against *Taenia* spp. have been reported to be efficacious, it may not be a feasible solution in USA due to various reasons. USA has historically a low prevalence of *T. saginata* that makes vaccination not very cost effective. For instance, the highest prevalence of cysticercosis in the USA in 2006 was 0.067% in the Northwest USA; that is every 1 out of 1493 was infected. Thus to protect a single animal in the northwest, vaccination of 1493 animals would be required. If the vaccine costs $1 per dose, the cost of $1493 would be required to effectively protect a single infected animal which is more than actual present loss per animal ($1186) due to cysticercosis. Economics will discourage production of a commercial vaccine since the demand will be low.

Since the disease involves two hosts, vaccinating cattle alone will never eliminate cysticercosis till human sources that pass eggs are controlled. Further, even if the human population in the area is diagnosed and treated, it may not be possible to stop immigrants from bringing in new *T. saginata* infections to the region.

### 1.6.2 PREVENTING HUMAN INFECTION

The most effective method of killing *T. saginata* cysticerci from meat is cooking to minimum of 71°C (USDA, 2001). Due to personal preferences, cultural or religious practices, proper cooking to recommended temperatures may not be always achieved in human diets. This may expose individuals to taeniosis. To address this, better public health practices have been adopted that has led to the adoption of stricter postmortem inspection techniques against cysticercosis.

To date postmortem meat inspection is the most relied upon method of controlling *T. saginata*. Visual diagnosis is performed postmortem by an authorized meat inspector by dissecting predilection muscle sites. *T. saginata* cysticercosis is usually diagnosed as calcified or rarely as non calcified cysticerci at predilection sites. A heavily infected carcass is totally condemned. In the USA, Food Safety and Inspection Service (FSIS) directive requires post–mortem inspectors to inspect heart, tongue, esophagus and other muscles for live, dead or degenerated *Taenia* spp. cysticerci (FSIS, 2007). Detection of even a single live, dead or degenerated cyst from a carcass originating from a producer, warrants a detailed inspection of all other carcasses originating from that source and to notify the health department of the state and veterinarian in–charge at Animal and Plant Health Inspection Service (APHIS) of USDA. Depending on the extent of infection, the carcass may be fully condemned or held for refrigeration or heat treatment. Mildly infected carcass or boned meat needs to be refrigerated at 15°F (–9.44°C) or less for 10 or 20 days respectively. Throughout heat treatment at 140°F (60°C) of the mildly infected carcass is also acceptable.

Despite rigorous inspection directives, postmortem inspection is not fool proof. Diagnosis of cysticercosis has been reported to be 3–10 times less efficacious depending upon meat inspector's motivation, and experience; and the extent and stage of infection in a particular carcass (Abuseir et al., 2006). Moreover, even if postmortem inspection was fully efficacious, it will not prevent

economic loses to commercial beef producers and feeders due to total condemnation, freezing or heating costs.

### 1.6.3 PREVENTING EGG DISPERSAL

Egg dispersal may be controlled by treating humans, ensuring high standards of personal hygiene or by ensuring that all mechanisms by which egg transmission from humans to beef are blocked to prevent egg dispersal. Human anthelmintic treatment and personal hygiene are particularly important with those in close contact with beef animals. Humans may be effectively treated with common anthelmintics like praziquantel, niclosamide, buclosamide or mebendazole (Garcia et al., 2003a). If diagnosed, the treatment is quite efficacious but the diagnosis of infected humans is difficult. This is because there are almost no clinical signs associated with taeniosis and even if a stool sample is submitted, the sensitivity of stool test is low (Garcia et al., 2003b).

Sewage water application to feedstuffs, water contaminated with infected human feces and direct transfer from infected farm workers are major channels of dispersal of *T. saginata* eggs to beef animals while birds, arthropods and earthworms form a minor channel of transmission (Slonka et al., 1975; Slonka et al., 1978; Fertig and Dorn, 1985; Cabaret et al., 2002; Murrell and Dorny, 2005). Present sewage treatments do not effectively kill *Taenia* spp. eggs (Bruce et al., 1990; Cabaret et al., 2002; Murrell and Dorny, 2005).

**1.6.4 TREATING FEEDSTUFFS TO PREVENT INFECTION OF BEEF ANIMALS**

Since all other control strategies fail to effectively control transmission of eggs to feedstuffs, this control strategy may be implemented by treating feedstuffs before feeding them to animals. Since heat and lack of moisture kills the eggs, feeds may be pelleted to effectively eliminate chances of infection. But some feedstuffs that may harbor *Tania* may not be easy to treat like potato co–products in the Northwest USA. It may not be possible to remove bound water from potato without altering its nutritive value. Excessive heating has been tried by some beef operations in the region but this has gelatinized the potato starch leading to rumen acidosis (Nelson, 2003). A possible control strategy under this situation may be heating the feedstuff to an optimum time temperature combination that will not gelatinize potato co–product. Alternatively, the potato co–products may be ensiled before feeding. The author is not aware of any research studies that have made these measurements.

**1.6.4.1 Heat treatment**

In a normal life cycle, *Taenia* spp. eggs undergo a sudden change in temperature when they are ingested by vertebrate intermediate host. The eggs are able to survive this change due to presence of heat shock proteins (Benitez et al., 1998; Vargas-Parada et al., 2001; Ferrer et al., 2005). Varagas-Parada et al. (2001) reported that metacestodes of *T. solium* and *T. crassiceps* responded to incubation at different temperatures by increasing production of heat shock proteins and this response was lost after heat treatment at 46°C for 30 minutes

and was thus lethal. Similar or higher temperatures may or may not be lethal to *Taenia* spp. eggs and the author is not aware of any study of the effects of heat on viability of eggs of *Taenia* spp. Williams and Colli (1970) studied effects of heat on activation rates of *T. hydatigena* eggs and reported highly variable results, owing to unknown factors, but did conclude that no activation was observed after 5 minutes at 55°C, 2 minutes at 60°C and 1 minute at 65°C. They also tried an *in vivo* model by intraperitoneal inoculation of heat treated ex–shelled and activated oncospheres into jirds (*Meriones unguiculatus*) but concluded the system was unreliable. One of the effects of temperatures from 7 to 38°C was to promote aging of mature and maturation of immature *Taenia* spp. eggs (Gemmell, 1977).

While heat treating potato co–products to eliminate the *T. saginata* threat, it is important not to cause potato gelatinization. Complete potato gelatinization required 30–60 minutes of heat treatment at 64.2°C (Shiotsubo, 1983, 1984). Further, 60 minutes of *in vitro* heat treatment of potato at 55, 57.5, 60, 62.5 and 65°C results in 0, 46, 73, 88 and 100% gelatinization, respectively (Parada and Aguilera, 2009).

### 1.6.4.2 Ensilation

Personal communication with Washington State Cattle Feeders suggests that feeding fresh potato co–products has led to increased cysticercosis cases in past. Potato co–products have been ensiled for at least 21 days in different experiments at Washington State University and no case of cysticercosis has ever been reported despite higher prevalence of cysticercosis at other feedlot

operations in the region that use similar potato co–products but did not ensile for that long (Nelson, 2009). Lagooning of sludge at 7°C for 28 days causes more than 99% reduction in viability of *T. saginata* eggs (Bruce et al., 1990). Ensilation of potato under similar conditions may yield similar results.

## 1.7   STUDYING *TAENIA* SPP. VIABILITY OF EGGS

Understanding behavior, ultrastructure and mortality characteristics of *Taenia* spp. eggs is critical to devise techniques to control rates of cysticercosis in intermediate hosts. Owing to complex structure of *Taenia* spp. eggs and lack of complete knowledge of structural and behavioral properties, accessing viability of *Taenia* spp. eggs still poses a significant challenge. Different *in vivo* and *in vitro* techniques used to assess viability of *Taenia* spp. eggs include staining, activation analysis, culture, infection of intermediate hosts and lab animals (Williams and Colli, 1970; Wang et al., 1997; Minozzo et al., 2002; Chapalamadugu, 2006; Kyngdon et al., 2006; Chapalamadugu et al., 2008).

### 1.7.1 HATCHING AND ACTIVATION

Hatching and activations of *Taenia* spp. eggs are two important processes that occur under natural conditions that release active oncospheres in the intermediate host. These two processes may be simulated under *in vitro* conditions to isolate oncospheres from two important barriers – embryophore and oncospheral membrane. Hatching refers to removal of the thick brown embryophore of *Taenia* spp. egg caused by gastric juices and activation refers to active movements of the oncosphere leading in escape from the oncospheral

membrane by the action of bile. The methodology for *in vitro* hatching and activation has improved over time. Silverman (1954) initially developed hatching technique and used gastric and intestinal enzymatic preparations but the technique resulted in inconsistent results by many other investigators. Laws (1967) described the use of sodium hypochlorite to effectively hatch *T. hydatigena*, *T. ovis*, *T. pisiformis* and *Echinococcus granulosus* eggs. Exposure of *Taenia* spp. eggs to 2% sodium hypochlorite for 10–30 minutes did not cause any reduction in viability based on activation and culture results (Osborn et al., 1982). Use of sodium hypochlorite for hatching has been widely adopted by investigators as a replacement for gastric enzymes (Lightowlers et al., 1984; Wang et al., 1997; Chapalamadugu et al., 2008). To enhance activation rates, various other substances like carbon dioxide and sodium hypochlorite have been recommended but their use have not been widely accepted and sometimes discouraged. Activation is still a poorly understood process that yields quite variable results with different batches of eggs of same species. A partial reason for this variability has been attributed to difference in maturation levels of *Taenia* spp. eggs, even within a single proglottid. Also, different eggs may require different lengths of incubation before they exhibit activation.

## 1.7.2 STAINING

For vital staining with methylene blue, neutral red, or Janus B green, hatching and activation are required as prerequisites (Smyth and McManus, 1989 p.193) but diazo dye like trypan blue may be used without activation (Wang et al.,

1997; Chapalamadugu, 2006; Chapalamadugu et al., 2008). Owen (1985) compared results of *in vitro* activation with various vital stains and concluded that the subjective differences between stained and unstained eggs caused inconsistent results and that only diazo dyes yielded significant results. Chapalamadugu et al. (2006; 2008) concluded that *T. taeniaeformis* non activated eggs that were heat treated and ex–shelled with 0.5% sodium hypochlorite did not yield consistent results for staining of oncospheres with various non–vital (acridine orange) and vital (trypan blue, propidium iodide) dyes.

### 1.7.3 *IN VITRO* CULTURE

Heath and Smyth (1970) described in *in vitro* culture technique for development of oncospheres of *T. hydatigena*, *T. ovis*, *T. pisiformis*, *T. serialis* and *Echinococcus granulosus* to cystic larvae stages. Despite being a successful technique, it was not quantitative enough to access viability rates and was limited to studying protective antigenic response of relevant intermediate host species and vaccine development (Lightowlers et al., 2003; Kyngdon et al., 2006).

### 1.7.4 *IN VIVO* AND SURROGATE MODELS

*In vivo* studies may yield the most reliable results provided intermediate and definitive hosts are available for the target species or appropriate surrogate species is selected. Due to greater risk of human infection and low availability of *T. saginata*, working with this tapeworm is a challenging task. To resolve this issue, a surrogate model has been adopted for this study that involves a

phylogenetically very similar tapeworm (*T. hydatigena*), which has a ruminant intermediate host (sheep) and a canine definitive host (dog).

*Taenia hydatigena* is phylogenetically very similar to *T. saginata* and falls in the same subclade in a phylogenetic tree of genus *Taenia*. *T. hydatigena* is more phylogenetically similar to *T. saginata* than *T. solium* and *T. taeniaeformis* (Hoberg et al., 2000; Hoberg, 2006). The predilection site of these metacestodes is omentum, mesenteries and liver as compared to muscular tissue in *T. saginata*. Usually *T. hydatigena* does not cause any clinical signs in sheep but upon ingestion of a large dose of eggs or a whole fresh gravid proglottid sudden death may occur due to hepatitis cysticercosis resembling acute hepatic fasciolosis (Radostits et al., 2000 p. 1386).

## 1.8  OBJECTIVES

To justify economical use of the potato co–product, the life cycle of the tapeworm needs to be intercepted to prevent cattle from getting infected with *T. saginata*. For this study our focus was to determine the viability and characteristics of eggs with following objectives:

1.  To study effect of heat treatments ranging from 22 to 60°C for 5 minutes on viability of eggs. The study was carried out *in vitro* and *in vivo*. These heat treatments were not severe enough to alter the nutritive value of the potato co–product.

2.      To study the effect of ensilation of potato co-product for 0 to 28 days on viability of *T. hydatigena* eggs.

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**Table 1.1 Taxonomy of tapeworms of genus *Taenia*.**

| Category | Taxonomic Classification | Characteristics |
|---|---|---|
| Kingdom | Animalia | - |
| Phylum | Platyhelminthes | Flatworms – body is dorso-ventrally flattened |
| Class | Cestoidea | Hermaphrodite, Lack body cavity and alimentary canal |
| Sub-class | Eucestoda | Hexacanth embryo, body of adult divided into scolex, neck and strobila |
| Order | Cyclophyllidea | Two-host life cycle, large tapeworms |
| Family | Taeniidae | Poorly developed eggs shell, thick keratinized embryophore |

(Soulsby, 1982; Smyth and McManus, 1989; Singh and Prabhakar, 2002; Mehlhorn, 2006)

**Table 1.2 Reported bovine cysticercosis outbreaks in North America.**

| Year | Location | Source of Epidemic | Reported by |
|---|---|---|---|
| 1973 | Phoenix, Arizona | Farm worker | (Slonka et al., 1975) |
| 1978 | South California | Farm worker | (Slonka et al., 1978) |
| 1981 | Ohio | Leakage of raw sewage onto pastures after flood | (Fertig and Dorn, 1985) |
| 1984 | Washington | Human fecal contamination of local feed sources | (Hancock et al., 1989) |
| 1987 | West Texas | – Not reported – | (Weedon, 1987) |
| 1992–93 | Idaho | Potato co–products | (Yoder et al., 1994) |
| 2000 | Alberta, Canada | Leakage of sewage onto fields | (Lees et al., 2002; Scandrett and Gajadhar, 2004) |

**Table 1.3 Estimates of economic losses per animal due to cysticercosis.**

| Location | Loss per animal | Reference |
|---|---|---|
| West Texas | $253 | (Weedon, 1987) |
| South–central Idaho | $337 | (Yoder et al., 1994) |
| West US states average | $1186* | (Getz et al., 2008) |

* Based on cost of an average 750 lb carcass. These losses do not include losses due to decrease in reputation; raising, feeding and maintenance cost of the beef animals.
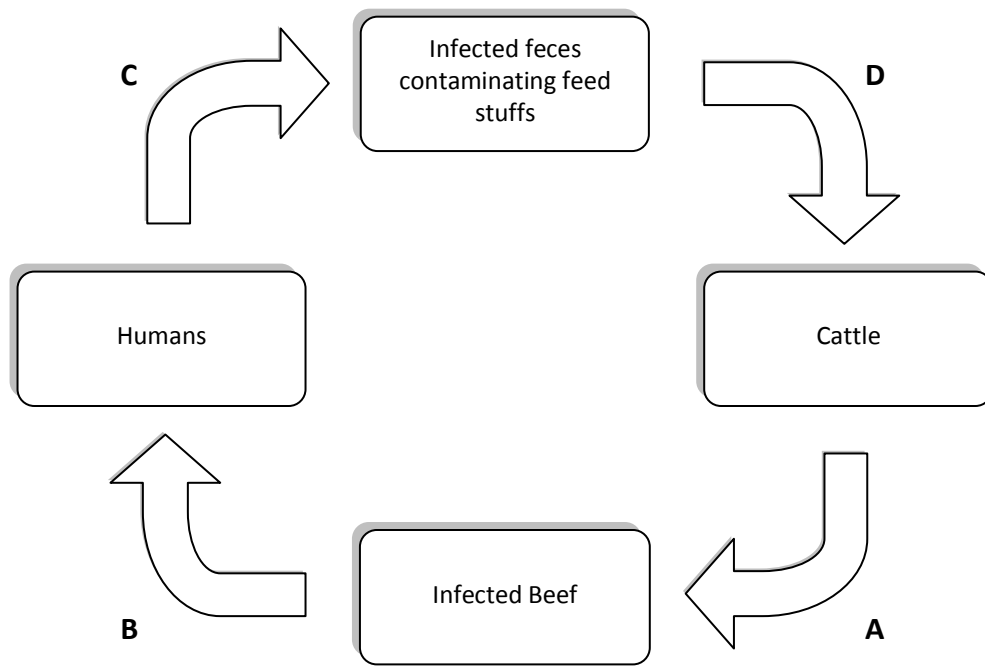
**Figure 1.1 Critical control points in life cycle of *Taenia saginata*.**

**Chapter 2**

**THERMAL KILLING OF *TAENIA HYDATIGENA* EGGS**

**Abstract**

In the Pacific Northwest USA feeding of potato co-products has been speculated to result in greater prevalence of beef cysticercosis caused by *Taenia saginata* as compared to rest of the USA. A *Taenia hydatigena* model was used to assess the effect of heat treatments on viabilities of eggs below temperatures of complete potato gelatinization. The *T. hydatigena* life cycle was maintained under laboratory conditions by passing the parasite through a canine–ovine cycle. The eggs were used for *in vitro* and *in vivo* studies to determine effects of five minutes of heat treatment, ranging from room temperature (22°C) to 60°C, on viabilities of the eggs. The results showed 99.47 and 100% reduction in viabilities after five minutes of heat treatment at 60.00 and 57.38°C under *in vitro* and *in vivo* conditions, respectively. Similar heat treatments may also be effective against *T. saginata* and may help to reduce occurrence of beef cysticercosis.

**Key words:** *Taenia saginata*, *Taenia hydatigena*, cysticercosis, potato co-product, heat treatment, potato gelatinization.

## 2.1 INTRODUCTION

*Taenia saginata* is a tapeworm of humans that causes cysticercosis in intermediate hosts (cattle) and taeniosis in definitive hosts (humans). Being a public health concern, strict post mortem regulations apply at the harvest level that leads to condemnation of infected carcasses and thus economic losses to beef producers. Cattle become infected orally; most commonly through contaminated

water, feed stuffs or infected farm workers. Depending on the extent of exposure the disease may be endemic or epidemic in cattle (Slonka et al., 1975; Slonka et al., 1978; Fertig and Dorn, 1985; Weedon, 1987; Hancock et al., 1989; Yoder et al., 1994; Lees et al., 2002; Scandrett and Gajadhar, 2004). Based on unpublished United States Department of Agriculture (USDA) meat inspection data, in 2006 the prevalence of beef cysticercosis was 0.067% in the Pacific Northwest compared to 0.004% in the USA. There were 720 and 256 reported cysticercosis cases in the Pacific Northwest and rest of the USA, respectively. Based on the same data source, the levels of the disease in the Pacific Northwest have been consistently higher than the rest of the USA at least since 1984. *T. saginata* eggs originating from humans are the infective stage to cattle leading to cysticercosis. Potato co-products are considered a likely source of *T. saginata* eggs leading to elevated levels of disease in the region (Hancock et al., 1989; Yoder et al., 1994). Contamination of potato co-products with *T. saginata* eggs may occur in the field at potato processing plants or at feedlots. Since the proglottids containing eggs are motile and can actively migrate out of infected humans, contamination of potato co-products may not always require direct contact with human feces. To justify economical use of the potato co-product, intervention in the life cycle of the tapeworm is needed to prevent cattle from getting infected. One intervention to prevent transmission to cattle involves heat treatment of the potato co-product to kill viable *T. saginata* eggs before cattle ingest them. In this context, heat treatments were used by some beef operations in the region which were

excessive and caused gelatinization of the potato starch. Gelatinization of this kind caused ruminal acidosis (Nelson, 2003). A possible control strategy under this situation may be heating the potato co-product to an optimum time-temperature combination below the initiation temperature for gelatinization of potato starch that will effectively kill *T. saginata* eggs. The author is not aware of any research studies that have made these measurements.

Measurements used by different investigators in *in vitro* and *in vivo* studies to assess viability of *Taenia* spp. eggs include staining, oncospheres activation analysis, culture, infection of intermediate hosts and lab animals (Williams and Colli, 1970; Wang et al., 1997; Minozzo et al., 2002; Chapalamadugu, 2006; Kyngdon et al., 2006; Chapalamadugu et al., 2008). Williams and Colli (1970) studied effect of heat on infectivity of *T. hydatigena* by inoculating heat treated ex-shelled and activated oncospheres into jirds (*Meriones unguiculatus*) but concluded that the *in vivo* system was unreliable. Chapalamadugu et al. (2008) concluded that accurate assessment of viability of *T. taeniaeformis* eggs using vital dyes was questionable after heat treatment and ex-shelling with 0.5% sodium hypochlorite. *In vivo* studies may yield the most reliable results provided that the intermediate and definitive hosts are available for the target species or an appropriate surrogate species is selected.

For this study, viabilities of eggs were evaluated *in vitro* by measuring activation rates and *in vivo* by counting postmortem cysticerci in infected lambs.

Due to low availability of *T. saginata*, *T. hydatigena* was used as a surrogate species. *T. hydatigena* has a close phylogenetic relationship with *T. saginata* (Hoberg et al., 2001). A major difference between life cycles of *T. hydatigena* and *T. saginata* is the site where cysticerci localize in the intermediate host. *T. hydatigena* larvae have affinity for the liver through which they migrate to reach the abdominal cavity (Soulsby, 1982). To a lesser extent, larvae may reach other tissues such as lungs (Blazek et al., 1985). In contrast, *T. saginata* cysticerci localize in skeletal and cardiac muscles. The objective of this study was to evaluate effect of heat treatments on viability of *T. hydatigena* eggs.

## 2.2 MATERIALS AND METHODS

### 2.2.1 *TAENIA HYDATIGENA* EGGS

*Taenia hydatigena* eggs were obtained from Dr. M.W. Lightowlers (University of Melbourne, Australia) to maintain the life cycle of the parasite under laboratory condition using ovine–canine cycle. Three recently weaned lambs were used for each passage in the intermediate host. The dose of eggs necessary to provide an acceptable assessment of treatments were determined in a preliminary experiment in which 100, 1,000 or 2,000 eggs were administered orally to lambs in 2 ml phosphate buffered saline (PBS, pH=7.2). No morbidity or mortality was observed in any of the groups, with a mean cysticerci recovery rate of 10% of eggs administered initially. Thus, the egg dose was determined to be 2,000 eggs per lamb administered orally in 2ml PBS. All lambs in the study were

maintained on corn–based starter (36% corn for one week), grower (52% corn for three weeks) and finisher (76% corn for three to five weeks) diets which met or exceeded NRC requirements (NRC, 2007). Lambs were housed indoors in temperature controlled room (19–21°C) with 13 hrs of light per day. Lambs were slaughtered eight weeks after infection and calcified (dead) or non–calcified (alive) cysticerci were recovered from omentum, liver surface, peritoneal cavity, diaphragm and lungs. Non–calcified cysticerci were collected in PBS and up to six cysticerci were orally administered to two dogs within 30–60 minutes of collection.

Two dogs were used as the definitive host for the entire study. One dog was a six year old castrated red–tick coonhound, and the other was a seven year old castrated blue–tick coonhound. The dogs were fed a commercial adult dog maintenance diet (2021 Teklad global 21% protein dog diet, Harlan laboratories, USA) and kept in temperature controlled (19–21°C) indoor kennels with 13 hrs of light per day. The dogs began shedding proglottids from seven to nine weeks after infection with cysticerci from lambs, similar to results of Gregory (1976). The proglottids shed by dogs were found motile and were recovered from the floor or occasionally from feces. Up to six proglottids were recovered per dog per day and were collected in 1% Antibiotic–Antimycotic solution (AA solution, Sigma Chem., USA) in PBS and stored at 4°C. Proglottids were not dissected to collect eggs, since active contractions of the proglottids expelled most of the eggs out into the

solution within few hours of collection. The eggs were stored at 4°C and were used within one month of collection.

### 2.2.2 *IN VITRO* EXPERIMENT

In a completely randomized design, temperature treatments were carried out in duplicate at 40, 45, 50, 55 and 60°C for five minutes each. Heat treatments were selected based on a preliminary experiment in which five minutes of heat treatments at 40, 50 and 60°C resulted in activation rates of 5.88, 2.38 and 0.00%. The untreated control was maintained at room temperature of 22°C. Egg concentrations were determined by counting eggs in 10 μl of the egg solution on a ceramic ring slide (Ring Microflocculation Slide, Clay Adams, USA) with a cover slip under a 400× magnification of a light microscope (BH–2, Olympus, USA). Four thousand eggs were diluted to a final volume of 1.2 ml with PBS in 2.0 ml flat bottom micro–centrifuge tubes for each treatment. Two heat treatments were carried out at a time using two heat blocks (VWR, Univar, USA), and temperature was monitored with a real–time digital scanning thermometer (12 Channel Scanning Benchtop Thermometer, Digi–Sense, Cole–Parmer Instrument Company, USA).

Following heat treatment, ex–shelling was carried out to remove the embryophore, using a sodium hypochlorite technique with modifications (Lightowlers et al., 1984; Wang et al., 1997; Chapalamadugu et al., 2008). Sodium hypochlorite (0.3 ml of 6% NaOCl) was added to the heat treated egg solution to a

final concentration of 1.2%. The solution was shaken vigorously using a vortex mixer (SP Deluxe Mixer – S8220, American Scientific Products, USA) to facilitate disintegration of keratin blocks. After five minutes, the solution was diluted to 2 ml with PBS to dilute the sodium hypochlorite to 0.9% and then washed thrice by centrifuging at 1,000×g for 10 minutes (Galaxy 7 Microcentrifuge, VWR international, USA); each time removing 1.6 ml supernatant and diluting to 2 ml with PBS. After the last wash, the supernatant was removed to retain a volume of 100 µl of ex–shelled oncosphere suspension.

Sheep bile, collected at time of slaughter of lambs, was used for activation of oncospheres. Bile was collected from gall bladders using a syringe, pooled and stored at –20°C in 1.5 ml microcentrifuge tubes. For activation, bile was thawed in a water bath at 37°C and was then added (100µl) to the solution of ex–shelled oncospheres (100µl) to final bile concentration of 50%. The solution was incubated in a water bath (Water bath 183, Precision Scientific Inc., USA) at 37°C for two hours. Two hours of incubation was selected based on results of a preliminary study in which maximum observed percent activity of oncospheres was observed at two hours of incubation compared to other time periods of 0, 0.5, 1, 4, and 8 hours of incubation. To observe activity, 20 µl of the solution was transferred on to a ceramic ring slide and a cover slip placed on it. Eggs in each field were individually observed for movement of hooks and contractions (activation) under a light microscope (BH–2, Olympus, USA) at 400×

magnification. Percentage of oncospheres demonstrating activity was then determined.

Gallie and Sewell (1970) cited by Wang (1997) reported that increased levels of carbon dioxide in hatching medium improved activation rates of *T. saginata* oncospheres but Ishiwata et al. (1993) found that carbon dioxide actually reduced activation rates of *T. taeniaeformis* oncospheres. This effect of carbon dioxide may be a species specific effect and was unknown for *T. hydatigena*. Preliminary experiments in the current study demonstrated no effect of carbon dioxide on activation rates of *T. hydatigena* oncospheres and thus carbon dioxide was not used in subsequent experiments.

Heat treatment and exposure to sodium hypochlorite may lead to decreased oncosphere recoveries at higher temperatures, as suggested by Chapalamadugu et al. (2008) with *T. taeniaeformis* at temperatures above 65°C. Therefore the recovery percent, based on an initial egg count of 4,000, was determined and compared among treatments.

### 2.2.3 *IN VIVO* EXPERIMENT

Heat treatments of eggs were carried out at 50 and 60°C for five minutes each. Untreated control eggs were maintained at room temperature (22°C). Following heat treatments, three, recently weaned Suffolk lambs (33.3±1.07kg) per treatment were infected orally, via syringe, with 2,000 heat treated or untreated control eggs in 2 ml PBS. Lambs were weighted on day 0, 14, 21, 35, 49

and 56 post–infections to monitor any effect of treatment on weight gains. Five and four randomly selected lambs were slaughtered on days 62 and 64 post–infection, respectively. Captive bolt stunning was used for humane slaughter of lambs (Welty, 2007; FSIS, 2009). The numbers of cysticerci were counted in omentum, liver surface, diaphragm, mesenteries, lungs and peritoneal cavity. Bulk of the liver cysticerci were found on liver surface with occasional cysticerci under the surface, consequently liver cysticerci counts were restricted to the surface. The viability was calculated as number of cysticerci recovered, as a percentage, of heat treated eggs administered.

### 2.2.4 STATISTICAL ANALYSIS

### 2.2.4.1 *In vitro* analysis

Two replicates per treatment were studied in a completely randomized design (CRD). A sigmoidal four–parameter model (Eqn. 1) (Rutledge, 2004; Chapalamadugu et al., 2008) was fitted using a non linear regression analysis of SigmaPlot 11.0 (SPSS Inc, Chicago, USA). Based on the same model, the four parameters were estimated using Proc NLIN of SAS 9.2 (SAS Institute, Cary, North–Carolina, USA).

$$f = y_0 + \frac{a}{1 + e^{-\left(\frac{x - x_0}{b}\right)}} \qquad ----- \qquad \textbf{[Eqn. 1]}$$

Where, $f$ denotes the dependent variable – percent activity, $x$ denotes independent variable – temperature treatment, and the four parameters are $a$

(total change in percent activity), $b$ (slope of the curve), $x_0$ (inflection temp) and $y_0$ (minimum percent activity).

To determine any interactions between heat treatment temperature and exposure to sodium hypochlorite, the oncosphere recoveries were compared at different temperatures using analysis of variance (Proc GLM in SAS 9.2).

### 2.2.4.2 *In vivo* analysis

Cysticerci recovered as percent of eggs administered (viability) were regressed on temperature of heat treatment of eggs using linear regression (Proc REG in SAS 9.2). A split plot design was used to analyze the number of calcified, non–calcified and total cysticerci recovered from different sites of recovery, with temperature of heat treatment in the whole plot and its interactions in the sub plot with individual lamb within temperature as the whole plot error term using Proc GLM in SAS 9.2. In case a significant temperature × site interaction was found, slice analysis was carried out within temperature for effect of sites of recovery on cysticerci count and within recovery site for effect of temperature on number of cysticerci recovered (Winer, 1971). The weights of lambs on day 0, 14, 21, 35, 49 and 56 post–infection were compared among treatments using analysis of covariance (ANCOVA) with repeated measures and unequal time intervals using Proc MIXED of SAS 9.2.

## 2.3  RESULTS

### 2.3.1 *IN VITRO* RESULTS

Maximum percent activity was observed at 40°C (6.06±0.55%), which decreased with increased temperature to a point at 60°C when no activity was observed (Table 2.1). Based on these observations, a sigmoidal four–parameter model resulted in parameters shown in Table 2.2 ($R^2$ = 0.8718). The data points and non linear regression model, with 95% confidence and prediction bands, are shown in Figure 2.1. Based on the non linear model, it was predicted that percent activity was reduced by 99.47% by heating to at 60°C for five minutes. Total oncospheres recovered were not significantly different among different treatments thus indicating no interaction between the temperature and exposure to sodium hypochlorite.

### 2.3.2 *IN VIVO* RESULTS

Most of the calcified and non calcified cysticerci were recovered from omentum and liver surface of lambs infected with eggs treated at 22°C (control) or 50°C, while no cysticerci were recovered at any site for treatment group dosed with eggs heat treated at 60°C. Comparative visual postmortem inspection of the liver surfaces from three treatment groups was found to be indicative of extent of infectivity across different treatment groups (Figure 2.2).

The mean percentage of cysticerci recovered were 11.55±1.85, 1.22±0.54, 0.00±0.00 % of the total eggs administered for treatments at 22 (control), 50 and

60°C, respectively. Linear regression analysis resulted in the equation Y= −0.318 X + 18.249 ($R^2$ = 0.877) where Y was the cysticerci recovered as percent of eggs administered and X was the temperature of heat treatment for five minutes (Figure 2.3). The slope of the linear equation indicated mortality rate of 0.32% of total eggs administered per degree Celsius rise in temperature from 22 to 60°C for five minutes. Additionally, the statistical model predicted 0% egg viability was attained at 57.38°C.

Split plot analysis of cysticerci (number of calcified, non−calcified and total number) recovered showed significant interactions between the temperature of heat treatment and the site of recovery (Table 2.3). Subsequent slice analysis revealed that at 22°C (control) the mean number of cysticerci recovered from omentum was significantly different from all other sites. However no such difference was observed at 50°C. Also, the mean number of cysticerci recovered from omentum, liver, lungs and diaphragm decreased linearly with temperature of heat treatment but the mean recoveries from mesenteries and peritoneal cavity for all treatments were too low to predict any relation between temperature and recovery. The ratio of the calcified to non−calcified cysticerci was found to be independent of temperature, lamb and site of recovery. The residuals of the count data of cysticerci recovered from different organs were normally distributed with skewness of 1.32 and kurtosis of 14.43. This slight deviation from a normal distribution may be explained by the excessive occurrence of zeros in the count data, after heat treatments at 50 and 60°C (Table 2.3).

The ANCOVA analysis of weights of lambs from different treatment groups (Table 2.4) indicated no difference in slopes of the curves (weight gain per day) or intercepts (weight at day 0) among treatments.

## 2.4 DISCUSSION

In the present study the effects of five minutes of different heat treatments on the viabilities of *T. hydatigena* eggs were analyzed *in vitro* and *in vivo*. The most important observations were made at 60°C at which no activation of oncospheres was observed *in vitro* and no cysticerci were recovered from lambs infected with eggs treated at this temperature. Complete potato starch gelatinization required 30–60 minutes of heat treatment at 64.2°C (Shiotsubo, 1983, 1984). Further, 60 minute treatments of potato starch at 55, 57.5, 60, 62.5 and 65°C resulted in 0, 46, 73, 88 and 100% gelatinization, respectively (Parada and Aguilera, 2009). Thus, treating potato co–product with temperature and time treatments sufficient to neutralize infectivity of oncospheres appears to represent conditions that marginally cause gelatinization of potato starch. It will be important to more accurately determine optimal temperature and time treatments that maximize egg killing and keep gelatinization to minimum. Other experiments indicate that ensilation of potato co–products is detrimental to *T. hydatigena* eggs (Buttar et al., 2009). Hence a combination of heat and ensiling treatments might reduce the intensity of heat required for this purpose.

*In vitro* and *in vivo* statistical models indicated a 99.47 and 100.00% reduction in viabilities after five minutes of heat treatment at 60.00°C and 57.38°C, respectively. The slope of the curve indicated mortality rate of oncospheres in response to increasing temperature was estimated to be 2.25 and 0.32% of total oncospheres per degree Celsius increase in temperature for *in vitro* and *in vivo* models, respectively. Differences in results of *in vitro* and *in vivo* models may be due in part to use of different statistical approaches – non linear sigmoidal four–parameter model for *in vitro* experiment and linear regression for *in vivo* experiment. Nevertheless, it is also possible that the difference reflects sensitivity of *in vitro* assay to detect viable eggs. More data points are recommended for *in vivo* experiment between 22°C and 60°C to better fit a non linear regression model.

When compared to control (22°C), the *in vivo* cysticerci recovery reduced to 10 and 0% for 50°C and 60°C treatment groups, respectively. Similarly, the *in vitro* activation rates reduced to 120, 88, 38, 5 and 0% of activation rates at 22°C, for eggs heat treated at 40, 45, 50, 55 and 60°C, respectively. Similar results were reported by Williams and Colli (1970) that no *in vitro* activation of *T. hydatigena* oncospheres was observed after heat treatment at 55°C for 5 minute, 60°C for 2 minute and 65°C for 1 minute. The results presented here are also similar to the predicted thermal death point (56°C for 5 min) of *T. saginata* cysticerci (Allen, 1947). However, Pike (1988) and Hughes et al. (1985) cited by Bruce et al. (1990) reported a prolonged heat requirement (55°C for 3 hours) to reduced infectivity

of *T. saginata* eggs to 1% in sludge. This difference may be due to lack of penetration of heat through the sludge, protecting the eggs for prolonged period. Similar situation may apply to potato co-products. Percent active oncospheres recovered for heat treatment at 40°C (5.98±0.55%) were similar to that at 22°C (4.98±1.36%). This may indicate that heat treatment at 40°C for five minutes has no effect on viability of *T. hydatigena* eggs. Moreover, for activation all eggs in the *in vitro* study were incubated in bile at 37°C for two hours, which is a thermal treatment that may be more intense than 40°C for five minutes, thus further strengthening the possibility that 40°C may not reduce viability of *T. hydatigena* eggs.

In vitro exshelling and activation are important steps required to study viability of *Taenia* spp. The most widely adopted *in vitro* approaches have been exshelling with sodium hypochlorite (Lightowlers et al., 1984; Wang et al., 1997; Chapalamadugu et al., 2008) and activation with species-specific bile (Ishiwata et al., 1993; Wang et al., 1997; Kyngdon et al., 2006). For this study we used 1% sodium hypochlorite for exshelling and 50% sheep bile for activation *in vitro*. Viabilities of *T. hydatigena* eggs were not assessed using *in vitro* vital staining in the current study since accurate assessment of viability with staining after heat treatment is questionable (Chapalamadugu, 2006; Chapalamadugu et al., 2008). Instead activation rates were used to assess viability. Wang et al. (1997) reported activation rates of 4, 1% and viabilities (based on trypan blue staining) of 80, 87% for enzymatic and sodium hypochlorite ex-shelling techniques, respectively.

All viable oncospheres may not activate at same time and this may be the reason for the difference in percent activation rates and staining observed by Wang et al. (1997). Different approaches have been suggested by many investigators to improve activation rates but methods that yield consistent results remain unresolved. In the results presented here, *in vitro* activation rates were found consistent with maximum activation rate of 6% after two hours of incubation in bile. Activation of all viable oncospheres may not have been observed but comparison of results among treatments and comparison to *in vivo* results strengthens the *in vitro* technique used for this study.

Dead cysticerci in an intermediate host often calcify in response to the host's immune system. The ratio of calcified to non–calcified mainly depends on host's age, immune response and number of days since infection (Minozzo et al., 2002; Mehlhorn, 2006). Since the lambs used in the study were of same age and were slaughtered only two days apart, the ratio of calcified to non–calcified cysts did not vary significantly. Also, there was no effect of temperature of treatment of eggs and site on calcification status of cysticerci.

No morbidity or mortality was observed in the lambs used in *in vivo* experiment. The rates of weight gain did not vary significantly among treatments even though cysticerci recovered did differ significantly. These were expected observations since *T. hydatigena* does not cause any clinical signs in sheep (Soulsby, 1982).

The results reported here present a promising foundation for application of mild heat treatments that effectively kill *T. saginata* eggs without causing significant starch gelatinization of potato co-products. Further studies should be conducted to better understand the effect of various time-temperature combinations of heat treatments without potato gelatinization. This may help to understand the relationships of time and temperature combinations on egg viabilities of *Taenia* spp. eggs. The thermal treatments may be investigated further by using *T. saginata* instead of surrogate species. Such studies may help in predicting more time-temperature combinations for practical application at level of potato processing plants to render potato co-products free of infectious *T. saginata* eggs.

Lagooning of sludge at 7°C for 28 days caused more than a 99% reduction in viability of *T. saginata* eggs (Bruce et al., 1990). A similar approach involving ensilation of potato co-products may prove beneficial to control *T. saginata* and needs to be explored further. Additionally, analysis of current epidemiological data of beef cysticercosis in the Pacific Northwest, similar to Yoder et al. (1994) and Hancock et al.(1989), will help better understand the current disease dynamics in the region.

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**Table 2.1** *In vitro* **effect of five minutes of thermal treatment of** *T. hydatigena* **eggs on percent recovery of oncospheres and percent activation ex–shelled with 1% sodium hypochlorite and activated with 50 % bile treatments.**

| Temperature (°C) | Recovery ± SE (%) [a] | Activation ± SE (%) [b] |
|---|---|---|
| 22 | 3.76 ± 0.46 | 4.98 ± 0.96 |
| 40 | 3.07 ± 1.02 | 5.98 ± 0.31 |
| 45 | 3.34 ± 0.74 | 4.87 ± 1.63 |
| 50 | 2.37 ± 0.45 | 2.11 ± 0.34 |
| 55 | 3.84 ± 0.66 | 0.33 ± 0.28 |
| 60 | 1.51 ± 0.33 | 0.00 ± 0.00 |

a    The percent recovery was calculated based on total egg count of subsample compared to initial count of eggs used.

b    The percent activation is based on number of oncospheres found active out of total number of eggs counted in subsample. The effect of temperature was non–significant on the percent recovery (p=0.2139) and significant on percent activation (p<0.0001).

**Table 2.2 Sigmoidal four–parameter model parameter estimates for *in vitro* percent activation of ex–shelled *T. hydatigena* eggs in response to heat treatment for five minutes at various temperatures.**

| Parameter | Estimate | Standard Error | 95% Confidence Limits | |
| --- | --- | --- | --- | --- |
| *a* | 5.700 | 0.799 | 3.942 | 7.459 |
| *b* | −2.247 | 1.101 | −4.670 | 0.177 |
| $x_0$ | 48.666 | 1.147 | 46.142 | 51.190 |
| $y_0$ | −0.028 | 0.534 | −1.203 | 1.146 |

*a* = Total change in percent activity (%)

*b* = Slope of the curve (% /°C)

$x_0$ = Inflection temperature (°C)

$y_0$ = Minimum percent activity (%)

**Table 2.3 Average number of calcified and non-calcified cysticerci recovered from lambs infected with *T. hydatigena* eggs heat treated for five minutes at 22 (control), 50, 60°C.**

| | Temperature of heat treatment for 5 minutes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 22°C (Control) | | | 50°C | | | 60°C | | |
| Organ | Calcified | Non-Calcified | Total | Calcified | Non-Calcified | Total | Calcified | Non-Calcified | Total |
| | SE =12.86 | SE =8.08 | SE =17.04 | SE =2.91 | SE =0.96 | SE =3.56 | SE =0.00 | SE =0.00 | SE =0.00 |
| Omentum* | 124.0 [a] | 54.3 [a] | 178.3 [a] | 10.0 | 2.7 | 12.7 | 0.0 | 0.0 | 0.0 |
| Liver* | 25.3 [b] | 10.7 [b] | 36.0 [b] | 9.3 | 2.0 | 11.3 | 0.0 | 0.0 | 0.0 |
| Diaphragm* | 5.7 [b] | 2.7 [b] | 8.3 [b] | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lungs* | 5.0 [b] | 1.0 [b] | 6.0 [b] | 0.0 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 |
| Mesenteries | 0.7 [b] | 0.3 [b] | 1.0 [b] | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| PC | 0.3 [b] | 1.0 [b] | 1.3 [b] | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Within a column different superscripts indicate difference among sites of recovery (p<0.05)

* Increasing temperature had linear effect on total recover (p<0.05)

SE = Standard error

PC = Peritoneal cavity

**Table 2.4 Average weight (±SE) of lambs infected with heat treated *T. hydatigena* eggs at different temperatures. The effect of heat treatment on weight gains was not significant.**

| Treatment Group | Day 0 (kg) | Day 14 (kg) | Day 21 (kg) | Day 35 (kg) | Day 49 (kg) | Day 56 (kg) |
|---|---|---|---|---|---|---|
| 22°C (Control) | 34.02 ± 1.13 | 35.49 ± 1.25 | 37.99 ± 1.93 | 43.54 ± 3.63 | 48.53 ± 4.31 | 51.71 ± 4.99 |
| 50°C | 31.9 ± 2.69 | 33.87 ± 2.56 | 36.29 ± 2.75 | 39.76 ± 3.27 | 43.85 ± 3.94 | 46.87 ± 4.58 |
| 60°C | 34.17 ± 1.71 | 37.19 ± 1.49 | 39.61 ± 1.88 | 45.96 ± 2.55 | 51.03 ± 3.32 | 54.81 ± 4.06 |
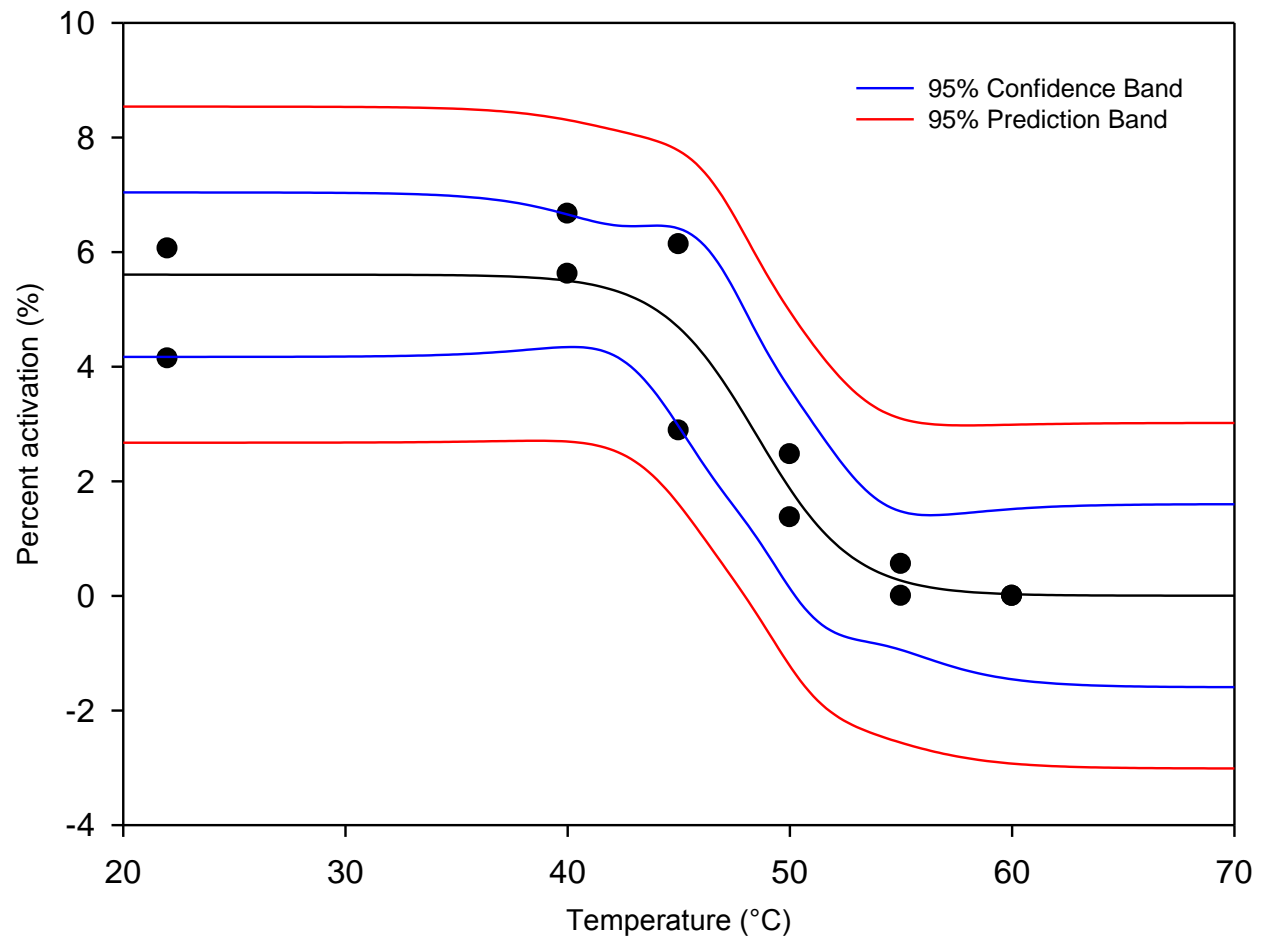
**Figure 2.1 Sigmoidal four–parameter model for observed *In vitro* percent activation of *T. hydatigena* oncospheres after heat treatment for five minutes at various temperatures ($R^2 = 0.8718$).**

a
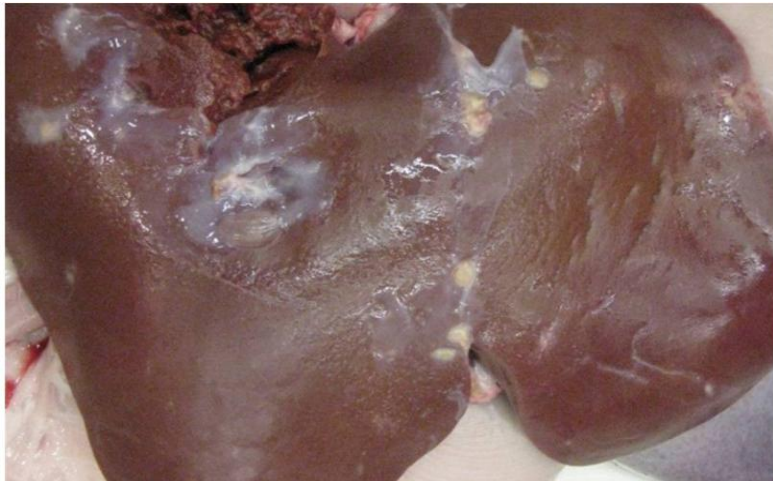


b



c

**Figure 2.2 Comparison of liver surfaces of lambs infected with *T. hydatigena* eggs heat treated at (a) 22 (control), (b) 50 and (c) 60°C.**

**Figure 2.3. Linear regression response of percent recovery of *T. hydatigena* cysticerci recovered as a percent of heat treated eggs administered at various temperatures. (Y= –0. 318 X + 18.249, $R^2$ = 0.8767)**

# Chapter 3

## EFFECT OF ENSILATION OF POTATO ON VIABILITY OF *TAENIA HYDATIGENA* EGGS

**Abstract**

A *Taenia hydatigena* model was used to assess the effect 0, 7, 14, 21, and 28 days of ensilation of minced potato on viability of tapeworm eggs. For infection of lambs, 2,000 *T. hydatigena* eggs were ensiled in 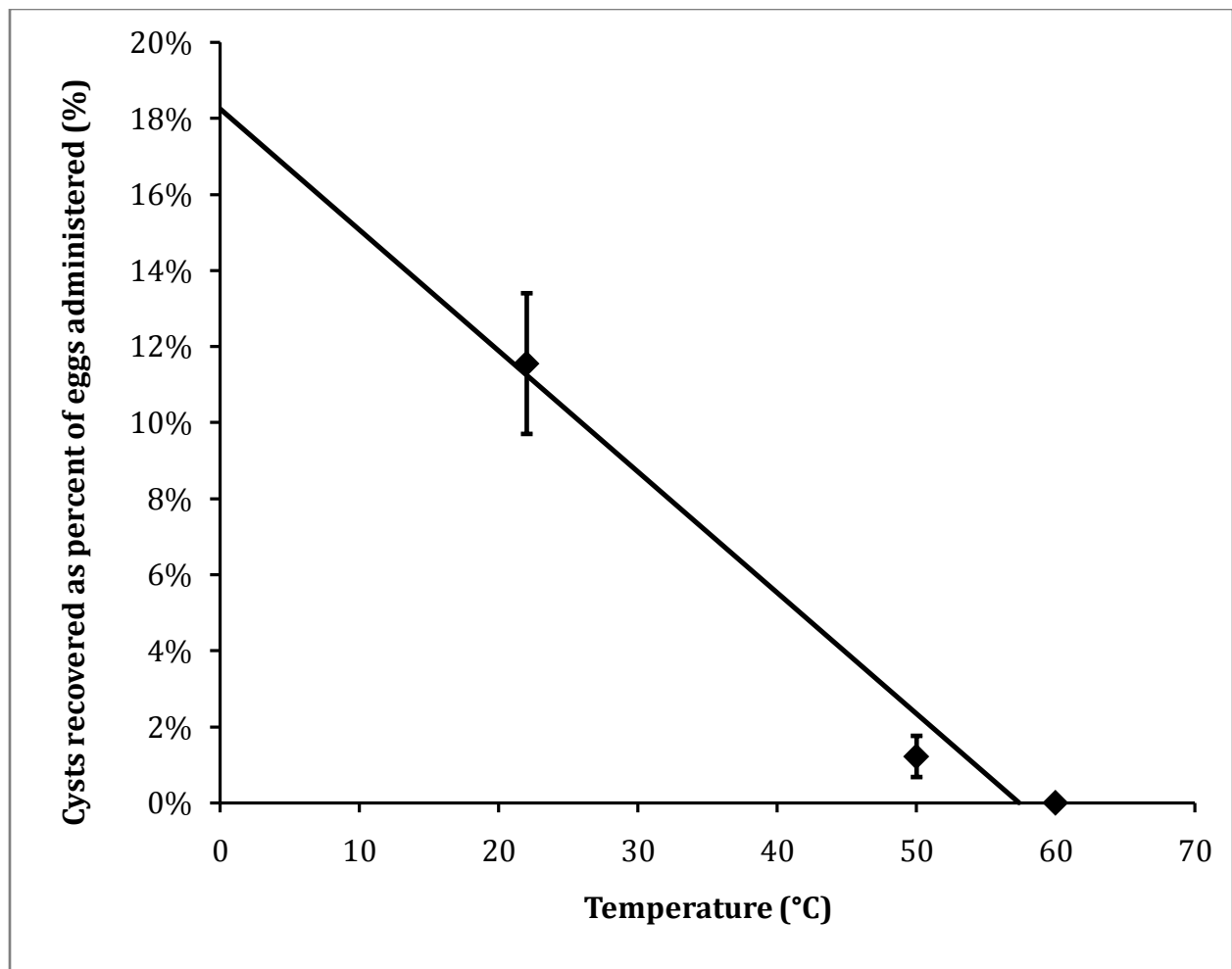minced potato at room temperature and fed to recently weaned lambs (29.9±0.76kg). At slaughter after 49 days, no cysticerci were recovered from lambs infected with eggs ensiled for 28 days while a mean of 5.0±5.0 cysticerci (0.25% of the initial egg dose) were recovered from lambs infected with eggs ensiled for 21 days. For lambs fed eggs ensiled for 0 days (control), 359.3±55.6 cysticerci were recovered (18.0% of the initial egg dose). Regression analysis revealed that maximum reduction in viability was attained after 18.59 days of ensilation.

**Key words:** *Taenia saginata*, *Taenia hydatigena*, cysticercosis, potato co–product, ensilation.

## 3.1 INTRODUCTION

*Taenia saginata* is a worldwide prevalent cestode, with humans as definitive host and cattle as intermediate host. Cattle are infected by ingesting eggs shed by humans. Various factors that may contribute to transmission of eggs from humans to cattle include poor sewage disposal (Cabaret et al., 2002); poor hygienic practices of farm workers (Slonka et al., 1975; Slonka et al., 1978; Murrell and Dorny, 2005); water contamination (Fertig and Dorn, 1985); and transmission of eggs by birds, arthropods and earthworms (Murrell and Dorny, 2005). Based on USDA data, the Pacific Northwest USA has a relatively high

prevalence rate of beef cysticercosis in finishing cattle compared to the rest of USA. This high prevalence was attributed to widespread feeding of potato co-products in the region (Hancock et al., 1989; Yoder et al., 1994). Potato co-products have excellent feeding value (Nelson et al., 2000), no detrimental effect on meat quality (Busboom et al., 2000) and is readily available available in the Pacific Northwest. Thus potato co-products present an economic feeding option for the feedlots and also helps to dispose of the co-product generated by the potato industry in an efficacious and profitable way (Nelson, 2009). Considering the economic justification of use of the potato co-products in the region it is important to control the problem of beef cysticercosis by preventing contamination or treating potato co-products. Since it is not easy to determine the exact origin of contamination of potato co-products with *T. saginata* eggs, treatments to kill *T. saginata* eggs in potato co-products may be necessary steps in eliminating beef cysticercosis.

The survivability of *Taenia* spp. eggs in the environment is very dependent on environmental conditions and has been reviewed by Murrell and Dorny (2005). While the survivability of *Taenia* spp. eggs may be more than 400 days on pastures (Duthy and Someren, 1948), feed treatments like drying and ensilation reduce their survivability. The survivability is reduced to 21 days in stored dry hay (Lucker and Douvres, 1960; Murrell and Dorny, 2005) and 60–80 days in grass silage at 10°C in Germany (Enigk et al., 1969). By lagooning at 7°C for 28

days more than a 99% reduction in infectivity of *T. saginata* was reported (Bruce et al., 1990).

Potato co-products have high moisture contents that are not easy to dewater without losing nutritive value. Additionally, moderate to low environmental temperature in the Pacific Northwest favors survivability of *T. saginata* eggs in wet feedstuffs like potato co-products. Potato co-products have been ensiled for at least 21 days in different experiments at Washington State University, and in no case has cysticercosis has ever been reported from cattle fed these products. In contrast higher prevalence of cysticercosis at other feedlot operations in the region that use similar potato co-products but do not ensile for that long (Nelson, 2009). Thus ensilation treatment of potato co-products may provide a pre feeding treatment that is effective in reducing transmission of *T. saginata* to cattle in potato co-products.

The objective of this study was to determine the effect of ensilation of potato on viability of *Taenia* spp. eggs using a surrogate species *T. hydatigena*, a canine-ovine tapeworm that has a close phylogenetic relationship to *T. saginata* (Hoberg et al., 2001; Hoberg, 2006). *T. hydatigena* was used here because of the unavailability of *T. saginata* eggs which are obtained from humans with an adult tapeworm. A major difference in the life cycle of the two species is the site of recovery of the cysticerci in the intermediate host – muscle tissue for *T. saginata* and the abdominal cavity for *T. hydatigena* (Soulsby, 1982) making *T. hydatigena* cysticerci easier to find and quantify. Therefore, *T. hydatigena* eggs were ensiled

for different lengths of time with potato co–products and fed to lambs to assess the change in viability of *T. hydatigena* eggs over length of ensilation.

## 3.2 MATERIALS AND METHODS

### 3.2.1 EGGS

*Taenia hydatigena* eggs were obtained from Dr. M. W. Lightowlers (University of Melbourne, Australia). Life cycle of the parasite was maintained under laboratory condition by passing parasite stages between lambs and dogs and eggs were recovered from dogs as described by Buttar et al. (2009). A fresh batch of eggs was used for each treatment. All the batches came from a single dog in same infection period. While it may be argued that different batches of eggs might have variation in viability, the method used here ensured that there was no variation due to prolonged storage and likely resembled the natural process of contamination of potato co–products.

### 3.2.2 ENSILATION

A completely randomized design was used to compare four ensilation treatments and a non–ensiled control with three replicates. Treatments involved ensilation of *T. hydatigena* eggs in minced potato for 0, 7, 14, 21 and 28 days. Fresh eggs, collected within a week from an infected dog, were used for each treatment. On day 0, 7, 14 and 21, 2,000 eggs were transferred to freshly minced red potatoes in each of three labeled, snap–top 30ml plastic vials with a sealable lid (Olympia Plastics, Los Angles, USA) for ensilation treatments of 28, 21, 14 and 7 days, respectively. The containers were flushed with $CO_2$ and sealed to promote

70

ensilation. To ensure proper ensilation pH measurements were taken on day 0 and day 5. The containers were immersed into a 5L plastic bucket with a lid containing the stock of minced potato and then sealed at room temperature. Three replicate were produced for each length of ensilation. On day 28, contents were emptied from vials, separately mixed with 0.5 kg corn and individually fed to one of the 15 different randomly selected, recently weaned lambs (29.9±0.76kg) off feed for 24 hours but provided with water ad libitum.

### 3.2.3 LAMBS

Lambs were fed a corn–based starter (36% corn for one week), a grower (52% corn for three weeks) and a finisher (76% corn for four weeks) diets which met or exceeded NRC requirements (NRC, 2007). Throughout the study, lambs were housed indoors in a temperature controlled room (19–21°C) with 13 hours of light per day. Lambs were weighed on day 0, 13, 27, 36 and 49. Eight lambs were randomly selected for harvest at WSU Meats laboratory on day 49 and seven on day 50 post infection. Captive bolt stunning was used for humane slaughter of lambs (Welty, 2007; FSIS, 2009). At slaughter, calcified (dead) and non–calcified (alive) cysticerci were recovered from omentum, liver surface, diaphragm, lungs, mesenteries, peritoneal cavity, visceral surfaces of rectum, small intestine and large intestine of each carcass. Percent viability of eggs was calculated for each lamb by comparing total number of cysticerci recovered to number of eggs (2,000) initially administered.

### 3.2.4 STATISTICAL ANALYSIS

Cysticerci recovered, as percent of eggs dosed (viability), were regressed against days of ensilation of eggs in potato co–products using a linear plateau model with Proc NLIN of SAS 9.2 (SAS institute, Cary, North–Carolina, USA). The equation used for the regression was $Y = \beta X + i$ where Y was dependent variable (percent cysticerci recovered), X was the independent variable (days of ensilation), $\beta$ was slope of the non-plateau curve (percent change in number of cysticerci recovered per day) and i was Y intercept (percent cysticerci recovered at zero days of ensilation). The inflection point in the model, where the plateau started was also iterated by Proc NLIN of SAS 9.2.

Assuming a normal distribution, a split plot design was used to analyze the number of calcified, non-calcified and total cysticerci recovered from different sites, with days of ensilation in the whole plot and site of recovery and its interactions in the subplot using Proc GLM Proc GLM of SAS 9.2. Lamb within temperature was the whole plot error term. In case a significant days of ensilation × site of recovery interaction was found, data were analyzed to compare recoveries from different sites, within each treatment, using slice analysis along with Tukey's multiple comparison test for means in Proc GLM of SAS 9.2 (Winer, 1971). The average daily weight gains of lambs were compared among treatments using Proc GLM of SAS 9.2.

## 3.3 RESULTS

The pH of the minced potato dropped from 6.38 on day 0 to 4.81 on day 5, ensuring proper ensilation (Nelson, 2009). Larval stages were recovered as calcified (dead) or non-calcified (alive) cysticerci from omentum (45%), liver surface (21%) and other sites from all treatments except when ensiled for 28 days (Table 3.1). The cysticerci recovered as a percent of initial dose of 2,000 eggs ensiled, decreased with time of ensilation for 0, 7, 14, 21 and 28; which averaged 17.97±2.78%, 13.15±4.19%, 3.97±2.21%, 0.25±0.25% and 0.00±0.00%, respectively. Non-linear regression analysis indicated that cysticerci recovery decreased at a rate of 1.00±0.24 % per day (slope of curve) with 18.69±2.19% eggs initially viable at day 0 (intercept). The plateau of the model was reached at 18.59±3.65 days of ensilation at which the percent recovery rate was 0.10±3.72 % (Figure 3.1). Nevertheless, a 28 day treatment produced no cysticerci.

Split plot analysis of number of calcified, non-calcified and total cysticerci recovered resulted in a significant interaction between days of ensilation and site of recovery. Subsequently, slice analysis showed that the means for total recovery of cysticerci from omentum and liver of control lambs were significantly different from all other sites. For lambs with eggs ensiled for 7 days, total mean recovery of cysticerci from the omentum was significantly different from all other sites, except liver (Table 3.1). For lambs fed eggs from all other treatments (14, 21 and 28 days) the mean recoveries were not significantly different among different sites. The residuals of the count data of cysticerci recovered from different organs

had skewness of 1.19 and kurtosis of 6.74. This slight deviation from the normal distribution may be explained by the excessive occurrence of zeros in the count data, especially from the longest ensilation treatments (Table 3.1).

Lambs in all treatment groups and control gained significant weight post infection till day of slaughter; and there was no effect of ensilation treatment time on average daily weight gains of lambs (Table 3.2).

## 3.4   DISCUSSION

The present study was conducted to determine the effect of 0, 7, 14, 21, and 28 days of ensilation of potato co–products on *in vivo* viabilities of *T. hydatigena* eggs. The most striking result was when no cysticerci were recovered, after 28 days of ensilation of *Taenia* spp. eggs in minced potato indicating 100% reduction in viability of *T. hydatigena* eggs by 28 days of ensilation. These results are similar to the effect of lagooning on sludge at 7°C for 28 days, which caused more than a 99% reduction in viability of *T. saginata* eggs (Bruce et al., 1990). Also in the present study, the percent recovery after 21 days of ensilation was 0.25±0.25% indicating a substantial decrease in viability by day 21. Further, the linear plateau model analysis showed that maximum reduction in viability was reached by 18.59±3.65 days with the reduction in viability at the rate of 1.00±0.24 % per day.

The recovery of cysticerci was mostly from omentum and liver similar to that reported in a previous study (Buttar et al., 2009)**.** However the percent

recovery of cysticerci of control treatments with an initial dose of 2,000 eggs was different for the two studies. In the current study, *T. hydatigena* eggs were administered by feeding contaminated minced potato mixed with corn. This method resulted in recovery of a maximum of 19% of the dose as cysticerci in control lambs compared to recovery of 12% in a previous study in which 2,000 *T. hydatigena* eggs were administered orally to lambs in 2 ml phosphate buffered saline. Although minimal, this difference may be due to variable infective potential of different batches of eggs, different methods of administration or differences in susceptibility of lambs. Despite the high rate of infection in day 0 and 7 lambs, average daily weight gain was not impacted by treatment (p>0.05).

The survivability of *Taenia* spp. eggs is highly dependent on environmental conditions (Murrell and Dorny, 2005) but even under similar conditions the survivability of different batches of eggs may be quite variable (Froyd, 1962). High and low temperatures have a detrimental effect on survivability of *Taenia* spp. eggs in the environment. Under laboratory conditions, mild temperatures in range 7 to 38°C promote maturation of juvenile eggs and degeneration of mature eggs whereas at –9°C, the maturation of juveniles is limited but survival time does not increase (Gemmell, 1977). Various ensilation treatments in the present study were carried out at room temperature (22°C). Ensilation in the field with different temperatures may change survivability of *Taenia* spp. eggs and needs to be explored further.

Effects of higher temperatures were studied by Buttar et al. (2009), in which heating *T. hydatigena* eggs for five minutes at 60.6°C and 57.4°C rendered them completely unviable based on *in vitro* and *in vivo* assays, respectively. The detrimental effect of ensilation at feedlots, when coupled with heat treatment, may indicate both milder heat and shorter ensilation times to effectively kill *Taenia* spp. eggs. Because ensilation is a natural process and less resource intensive, this hypothesis should be explored further.

The results obtained with *T. hydatigena* eggs identify two complementary treatments that have potential for practical application because, although the experiments were not conducted on *T. saginata*, the results are likely to control beef cysticercosis in feedlot cattle in which potato co–products are fed. It will be difficult to conduct similar experiments on *T. saginata* without reliable source of eggs. Nevertheless, treatment parameters defined here should make analysis much more efficient with any related experiments on *T. saginata* when they become available. In addition, the effect these treatment methods have on *T. saginata* transmission to cattle in the field can be monitored by using epidemiological methods described by Yoder et al. (1994) and Hancock et al. (1989). Analysis of current epidemiological data in the Pacific Northwest will help better understand the current disease dynamics in the region.

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**Table 3.1 Average number of calcified and non-calcified cysticerci recovered from lambs infected with *T. hydatigena* eggs ensiled in minced potato for different time periods.**

| Site of recovery | Days of ensilation | | | | | | | | | | | |
| | 0 (control) | | | 7 | | | 14 | | | 21 | | |
| | Cal | N.Cal | Total | Cal | N.Cal | Total | N.Cal | N.Cal | Total | Cal | N.Cal | Total |
| | SE= 9.00 | SE = 7.58 | SE= 10.67 | SE= 10.61 | SE= 9.69 | SE= 15.49 | SE= 2.67 | SE= 6.78 | SE= 7.66 | SE= 0.16 | SE= 0.76 | SE= 0.84 |
| Omentum | 38.33 [a] | 119.67 [a] | 158.00 [a] | 44.33 | 76.00 [a] | 120.33 [a] | 9.00 | 25.33 | 34.33 | 7.00 | 2.00 | 9.00 |
| Liver | 79.00 [b] | 37.67 [b] | 116.67 [a] | 25.67 | 21.00 [b] | 46.67 [ab] | 10.67 | 10.33 | 21.00 | 5.33 | 1.33 | 6.67 |
| Lungs | 12.67 [a] | 0.67 [b] | 13.33 [b] | 11.67 | 0.00 [b] | 11.67 [b] | 6.00 | 0.67 | 6.67 | 0.00 | 0.33 | 0.33 |
| DI | 19.00 [a] | 7.33 [b] | 26.33 [b] | 30.67 | 7.00 [b] | 37.67 [b] | 1.67 | 4.67 | 6.33 | 0.00 | 0.00 | 0.00 |
| Rectum | 11.67 [a] | 12.33 [b] | 24.00 [b] | 12.67 | 11.67 [b] | 24.33 [b] | 1.33 | 1.00 | 2.33 | 0.00 | 1.00 | 1.00 |
| ME | 3.33 [a] | 14.33 [b] | 17.67 [b] | 12.67 | 5.00 [b] | 17.67 [b] | 0.33 | 0.00 | 0.33 | 0.00 | 0.00 | 0.00 |
| PC | 1.67 [a] | 1.67 [b] | 3.33 [b] | 0.67 | 3.00 [b] | 3.67 [b] | 0.67 | 2.00 | 2.67 | 0.00 | 0.33 | 0.33 |
| SI | 0.00 [a] | 0.00 [b] | 0.00 [b] | 0.00 | 1.00 [b] | 1.00 [b] | 1.00 | 0.33 | 1.33 | 0.00 | 0.00 | 0.00 |
| LI | 0.00 [a] | 0.00 [b] | 0.00 [b] | 0.00 | 0.00 [b] | 0.00 [b] | 3.33 | 0.00 | 3.33 | 0.00 | 0.00 | 0.00 |
| Bile Duct | 0.00 [a] | 0.00 [b] | 0.00 [b] | 0.00 | 0.00 [b] | 0.00 [b] | 0.67 | 0.33 | 1.00 | 0.00 | 0.33 | 0.33 |

After 28 days of ensilation recovery from all organs was zero.

Different superscripts within same column indicate significant difference ($\alpha$=0.05).

Cal: Calcified,          N.Cal: Non Calcified        SE: Standard Error        DI: Diaphragm

ME: Mesenteries      PC: Peritoneal Cavity      SI: Small Intestine        LI: large Intestine

**Table 3.2 Average weight (±SE) of lambs infected with *T. hydatigena* eggs ensiled for various time periods. Average daily weight gains of lambs did not vary significantly with length of ensilation (α=0.05).**

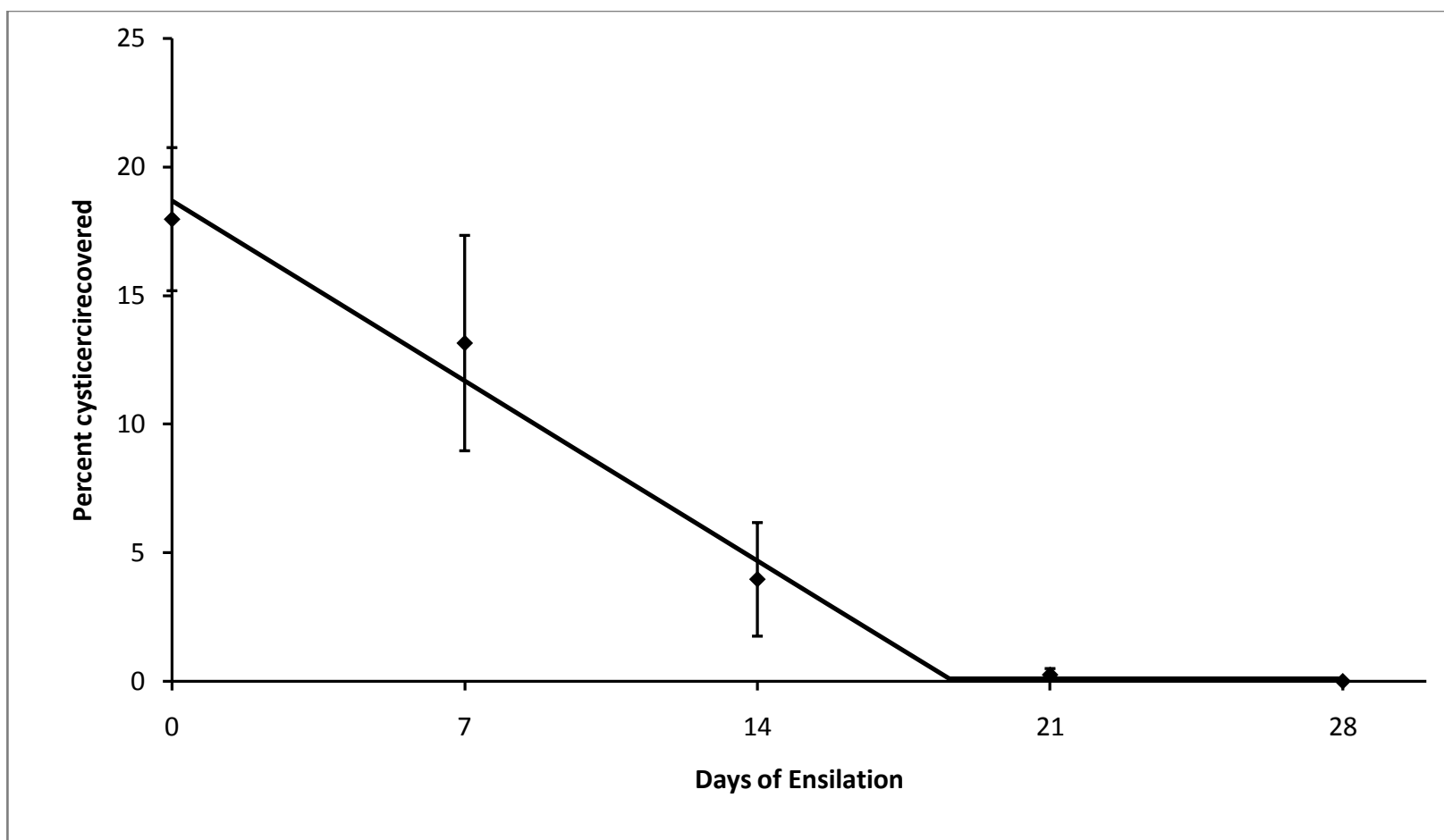| Ensilation length | Day 0 (kg) | Day 13 (kg) | Day 27 (kg) | Day 36 (kg) | Day 49 (kg) |
|---|---|---|---|---|---|
| 0 days (control) | 32.50 ± 1.17 | 37.27 ± 2.45 | 40.53 ± 1.97 | 45.00 ± 1.85 | 46.06 ± 1.9 |
| 7 days | 31.74 ± 1.35 | 36.14 ± 1.27 | 39.02 ± 2.14 | 42.42 ± 1.84 | 43.64 ± 2.06 |
| 14 days | 30.53 ± 0.46 | 34.62 ± 0.40 | 34.47 ± 0.72 | 38.64 ± 0.69 | 40.53 ± 0.67 |
| 21 days | 29.02 ± 1.22 | 32.65 ± 1.35 | 33.94 ± 1.65 | 37.2 ± 1.51 | 38.94 ± 1.19 |
| 28 days | 25.68 ± 1.14 | 29.55 ± 1.14 | 30.23 ± 1.94 | 34.32 ± 1.96 | 38.71 ± 1.93 |

**Figure 3.1** *In vivo* **response of percent recovery of** *T. hydatigena* **cysticerci recovered as a percent eggs administered after ensilation in minced potato for 0, 7, 14, 21 and 28 days. The data points are average for the treatment with standard error bars. The regression equation was Y= –1.00 X + 18.69 with plateau starting at 18.59±3.65 days.**

# Chapter 4

## CONCLUSION

Beef cysticercosis, caused by intermediate life stages of *T. saginata*, has relatively high prevalence in the Pacific Northwest USA probably due to widespread feeding of potato co-products. To control the disease one of the alternatives is to adequately treat potato co-products before feeding at feedlots. In the present study the efficacies of heat treatment and ensilation of potato were studied as control measures to reduce the viabilities of *Taenia* spp. eggs. A *T. hydatigena* model was used as a surrogate species and life cycle was maintained under laboratory conditions.

Five minutes of heat treatment of *T. hydatigena* eggs at 60°C resulted in 100% reduction of viabilities of eggs both *in vitro* and *in vivo*. Similar heat treatment of potato co-products may significantly reduce or completely eliminate the threat of *T. saginata* without causing significant gelatinization of potato starch. Statistical analysis of the data revealed 99.47 and 100.00% reduction was obtained after five minutes of heat treatment at 60.00 and 57.38°C under *in vitro* and *in vivo* conditions, respectively. At the industry level, heat treatment of potato co-products is possible at potato processing plants just before transportation to feedlots. Heat treated potato co-products may be immediately transferred to transport trucks where they may retain heat for a sufficient time period to significantly reduce the number of viable *T. saginata* eggs.

Exposure of *T. hydatigena* eggs to 28 days of ensilation in potatoes caused 100% reduced in viability of the eggs. While ensilation is less resource intensive than heat treatment, it requires much longer time to effectively eliminate the *T.*

*saginata* threat from potato co-products. The ensiling process may be carried out at feedlots provided that large pits are available for prolonged ensilation. Since environmental conditions also play role in survivability of *T. saginata* eggs, the survivability of the eggs in ensiled potato co-products may vary significantly with season and region and may be explored further.

Both pasteurization and ensilation may have application at the industry level as techniques to effectively control beef cysticercosis. Epidemiologically, it will be interesting to observe the changes in prevalence rates of beef cysticercosis after application of either or a combination of the two complementary treatments mentioned in the study at industry level. It is not known, however, if a combination of the two treatments may require milder heat and/or shorter ensilation times to effectively eliminate *T. saginata* eggs.