



ESTONIAN UNIVERSITY OF LIFE SCIENCES

Institute of Veterinary Medicine and Animal Sciences

Tuuli Maria Åhlberg

**PREVALENCE OF *TAENIA SOLIUM* CYSTICERCOSIS
IN ESTONIAN PIGS**

TAENIA SOLIUM TSÜSTITSERKOOSI LEVIMUS EESTI
SIGADEL

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Supervisors: Brian Lassen, MSc, PhD
Maria Vang Johansen, DVM, PhD, Dipl EVPC

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Permission for public examination:

Supervisor

Brian Lassen, MSc, PhD

(Signature)

(Date)

Supervisor

Maria Vang Johansen, DVM, PhD, Dipl EVPC

(Signature)

(Date)

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ABBREVIATIONS

Ab-ELISA	Antibody detection by ELISA
Ag-ELISA	Antigen detection by ELISA
ARIB	Estonian Agricultural Registers and Information Board (Põllumajanduse Registrate ja Informatsiooni Amet; PRIA)
Bp	Base pairs
CI	Confidence limit
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ESA	Eesti Statistika Amet
EU	European Union
NZD	Neglected zoonotic disease
spp.	subspecies
VFB	Veterinary and Food Board (Veterinaar- ja toiduamet; VTA)
VFL	Veterinary and Food Laboratory
WHO	World Health Organization

SHORT SUMMARY

PREVALENCE OF *TAENIA SOLIUM* CYSTICERCOSIS IN ESTONIAN PIGS

Taenia solium is categorized by the World Health Organization as an important neglected zoonotic disease, as well as number one global parasitic disease. It has a complicated life cycle mainly involving pigs and humans, and is endemic in several low to middle income countries. Although human taeniosis rarely causes symptoms, human neurocysticercosis has been recognized as one of the greatest causes of neurological disease in endemic areas.

The aim was to investigate the prevalence of cysticercosis in Estonian pigs through post-mortem meat inspection in Estonia's three largest abattoirs. Found cysts would be analyzed with PCR. The hypothesis was that the prevalence of cysticercosis would be $\geq 0\%$. A total of 1217 pigs were examined from February to April 2014 through visual inspection, palpation as well as incisions of predilection sites to find cysts. One cyst was discovered in the left ventricle of the heart of a finishing pig. PCR analysis showed that the found cyst could not be identified as *Taenia* spp., confirming the hypothesis with an apparent prevalence of 0% (0.00-0.25 95% CI).

Although proof of the appearance of *T. solium* cysticerci in Estonian slaughterhouses was not obtained during this study, it doesn't confirm that Estonia is free of the parasite. Low sensitivity of the applied inspection method is likely to have contributed to low amount of cysts. As cysts appear in slaughterhouses and taeniosis has been diagnosed in humans in Estonia in 2003, further studies are warranted.

LÜHIKOKKUVÕTE

TAENIA SOLIUM TSÜSTITSERKOOSI LEVIMUS EESTI SIGADEL

Taenia solium on liigitatud World Health Organizationi poolt tähtsaks unarusse jäetud zoonootiliseks haiguseks, ning globaalselt number üks parasitaarhaiguseks. Sellel on keeruline elutsükkel mis haarab kaasa peamiselt inimest ja siga, ning on endeemiline mitmetes madala ning keskmise sissetulekuga maades. Siiski kui tavaliselt tenioos ei põhjusta kliinilisi tunnuseid, inimeste neurotsüstitserkoos on tunnustatud üheks kõige harilikumaks neuroloogilise haiguse põhjustajaks endeemilistes maades.

Töö eesmärgiks oli uurida sigade tsüstitserkoosi levimust Eesti sigadel kasutades post-mortem lihainspeksiooni. Leitud tsüstid uuritakse PCR'iga. Hüpoteesiks oli leida levimus mis oleks $\geq 0\%$. Kokku 1217 siga uuriti aastal 2014 Veebruarist Aprillini visuaalselt, palpeerides ning sisselõiked tehes parasiidi eeliskohtadesse. Üks tsüst leiti nuumsea südame vasakust vatsakesest, aga PCR analüüs ei saanud tõestada et tegu oleks *Taenia* perekonnaga, andes näilikuks levimuseks 0% (0.00-0.25 95% CI).

See et *T.soliumi* tsüstitsekide olemasolu ei saadud tõestatud selle uuringu jooksul ei tähenda et Eesti on tsüstitserkoosivaba. Tulemust tõenäoliselt mõjutab näiteks lihainspeksiooni madal sensitiivsus kui diagnoosimismeetod. See, et tsüste ikka leitakse vahepeal tapamajades ning et Eestis on diagnoositud inimestel taenioosit viimati aastal 2003, viitab sellele et lisauuringuid on vaja.

1. INTRODUCTION

The zoonotic tapeworm *Taenia solium* is a parasite of great destructive capability both health wise as well as regarding economic loss. It has been ranked as number one on the global scale of foodborne parasites by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 2014 (FAO & WHO 2014). It resides in its main host the human as a tapeworm causing human taeniosis, and its intermediate host the pig in its larval stage causing cysticercosis. Humans are contaminated by eating raw or insufficiently prepared pork, while pigs are contaminated by consuming proglottids and eggs (Murrell *et al.* 2005). One of its most significant aspects is that it can also cause human cysticercosis and neurocysticercosis. Neurocysticercosis has been evaluated as one of the greatest causes of neurological disease in endemic areas commonly in developing countries, where it is estimated to cause 29% of acquired epilepsy cases (García *et al.* 2003; Ndimubanzi *et al.* 2010).

Taenia solium taeniosis/cysticercosis is described by the WHO as an important neglected zoonotic diseases (NZD), but scientists are now concerned that there is a threat of it becoming an emerging zoonotic disease as well (Dorny *et al.* 2009; Del Brutto 2012). NZD's are an ever growing list of zoonotic diseases that, despite causing severe morbidity and mortality, remain insufficiently addressed mainly due to being a problem of financially restricted countries. NZD's, in contrast to emerging diseases, have been known to cause disease in humans for ages, but commonly have ceased to threaten first world countries while remaining endemic in poorer areas (WHO 2011). *Taenia solium* is however threatening first world countries through increased human immigration and, to a lesser extent, pig import from endemic areas (Gabriël *et al.* 2015; O'Keefe *et al.* 2015).

In Estonia there are records of *T. solium* taeniosis in humans as well as cysticercosis in swine, but no recent assessment of the prevalence of either human or porcine disease have been conducted. The most recent recorded cases of *T. solium* taeniosis in humans were in 2000 and 2003, while cysticercosis in swine was most recently detected in 2006 (Jõgiste 2005; Cliquet *et al.* 2010). This study aims to assess the prevalence of *T. solium* cysticercosis in Estonian pigs through a cross-sectional study in 3 of the largest Estonian abattoirs involving visual inspection, tongue palpation and incisions into predilection sites. Furthermore this study included a literature review.

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2. LITERATURE REVIEW

2.1. Background on *Taenia solium*

2.1.1. Classification, morphology and life cycle

The members of the genus *Taenia* tend to be long, white to yellowish segmented flatworms that have a rostellum with a double row of hooks, a head or scolex with radial symmetry, as well as four suckers for attachment. They lack a mouth and digestive tract, using their tegument for digestion and absorption. *Taenia solium* can reach a length of around 1-5m, and a width of 3-10mm (Flisser 2013; Murrell *et al.* 2005). The neck connects the head to the hermaphroditic segments, collectively termed strobilia, which become more mature the further distal they are relative to the head. The uterus of mature segments contain about $50-60 \times 10^3$ fertile eggs, branching into seven to eleven lateral branches (García *et al.* 2003; Flisser, 2013; Murrell *et al.*, 2005).

Taenia solium belongs to the phylum plathelmintha or flatworms, class cestodes and subclass eucestodes, order cyclophyllida and family Taeniidae (Järvis 2011). It belongs to the same genus as *T. saginata* cysticercosis found in cattle as well as *T. saginata asiatica* cysticercosis in pigs, however these two species are more closely related to each other than they are to *T. solium*. There are two genotypes of *T. solium* currently identified, one strain occurring mainly in Latin America as well as Africa, the other in Asia, but the differences between these two genotypes seem to be mainly genetical (Ito *et al.*, 2003).

The life cycle of *T. solium* as seen in **Figure 1**, typically involves pigs the intermediate hosts harboring the larval stage of the porcine tapeworm, and humans as the definitive hosts of tapeworms (CDC-DPDx 2014). Humans can also carry the larval stage, technically making them the intermediate hosts, although this tends to mean the end to the parasites lifecycle (Flisser 2013). Pigs usually contract porcine cysticercosis by consuming human feces containing *T. solium* eggs (Gonzalez *et al.*, 2006). Unlike *Taenia saginata* proglottids, which are able to disperse away from the feces, the proglottids of *T. solium* are not motile (Murrell *et al.* 2005). Infection of the intermediate host is assisted by bad hygiene as well as the coprophagic tendencies of pigs (Gonzalez *et al.* 2006). Humans are infected by eating undercooked or raw meat containing cysticerci after which the tapeworm develops in the intestine (Murrell *et al.* 2005).

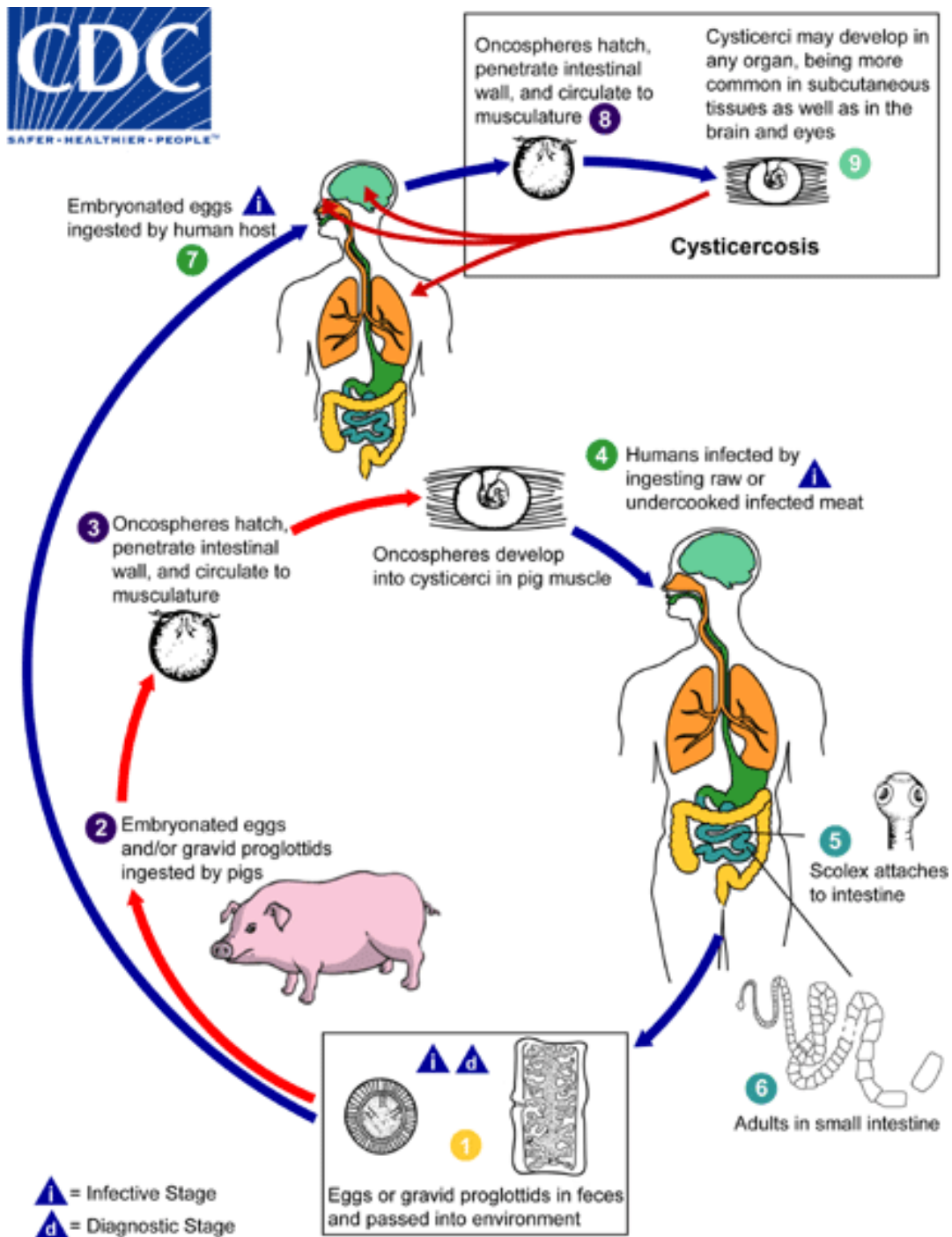


Figure 1. Life cycle of *T. solium*. Source: (CDC-DPDx 2014)

In addition to the human-pig cycle, also human-human and pig-pig contamination is possible. Humans can get cysticercosis by consuming eggs of the tapeworm via the fecal oral route, in which case the oncospheres released will behave similar to how they would in pigs: travel via lymph- and blood vessels to different organs where they can form cysts. In other words humans would act as intermediate hosts (Fabiani & Bruschi 2013). One source suspected that porcine cysticercosis could be transmissible even from pig to pig,

which would be important as this would enable the parasite to spread in a naïve population if a pig with cysticercosis were introduced. (Gonzalez *et al.* 2006) Interestingly also dogs can be an intermediate host of *T. solium* this might carry importance especially in countries where dogs are consumed by people.(Ito *et al.* 2002)

2.1.2. Cysticercosis in pigs

After the consumption of *T. solium* eggs or proglottids by the pig, the oncospheres are released by bile acid and migrate via blood- or lymphatic vessels to various tissues in the body, most commonly skeletal and cardiac muscle as well as the brain (Flisser 2013; Murrell *et al.* 2005). The sites where cysticerci can most commonly be found in pigs have been analyzed in several studies due to the important role it plays in discovery of infected pigs during meat inspection in abattoirs. Boa *et al.* 2002, considered the psoas, triceps brachii, tongue, internal masseter, external masseter, diaphragm and heart muscles as the most important predilection sites (2002). Other sites have been mentioned as the brain and oesophagus (Boa *et al.* 2002; Sciutto *et al.* 1998).

Clinical signs of porcine cysticercosis often go unnoticed, especially in mild cases, and are caused by inflammation around the cysts. Signs can include fever as well as muscle stiffness in limbs and masticatory muscles. Also myocarditis can occur (Järvis 2011; Herenda *et al.* 2000). Cysts are commonly 0.5-1.5 cm, filled with fluid and with a white scolex in the middle (Flisser 2013; García *et al.* 2003). In naturally infected pigs, the number of cysts in each pig tends to be low, normally under 100 cysts, possibly explained by immunity. Also the hierarchical system of pigs allows those with highest status to consume human feces, and those with lower status thus being less likely to be contaminated (Gonzalez *et al.* 2006; Sciutto *et al.* 1998).

2.1.3. Taeniosis and cysticercosis in humans

As discussed in the previous chapter, humans can act as both the definitive host as well as the intermediate host. Taeniosis tends to be asymptomatic, possibly causing mild abdominal distention or pain, nausea and/or diarrhea (García *et al.* 2003). Despite the tapeworm not commonly causing significant clinical signs, its threat to humans lays in its capability of producing eggs which can infect other humans, as well as the host itself, causing cysticercosis (FAO & WHO 2014).

In human cysticercosis/neurocysticercosis, ingested oncospheres can lodge in different kind of organs, the most common site being the central nervous system, creating cysts

ranging from a few mm to 1-2cm over a period of 2-3. Depending on the location of the cysts, humans can have subcutaneous-, intramuscular- ocular- or neurocysticercosis. months (Flisser 2013; García *et al.*2003) Symptoms commonly arise when the cysts degenerate, causing an immune mediated inflammatory response around the cyst. This is why it commonly takes several years before clinical signs occur (Garcia & Brutto 2005; García *et al.* 2003; Mahanty & Garcia 2010).

Neurocysticercosis is, the most common parasitic disease of the central nervous system (CNS) in the world, and is suspected of causing 29% of acquired epilepsy cases in endemic areas (Ndimubanzi *et al.*, 2010). In addition to epilepsy, neurocysticercosis can cause a wide range of CNS symptoms, such as rise in intracranial pressure, meningitis and hydrocephalus, sometimes even leading to death (Garcia & Brutto 2005; Mahanty & Garcia 2010; O'Keefe *et al.* 2015).

2.1.4. Prevention and treatment of taeniosis and cysticercosis

Methods and hygiene of pig husbandry are important factors in the control of *T. solium* (Dorny *et al.* 2009). Access of pigs to human feces increases the risk of pigs being contaminated, and is more common when pigs are allowed to roam freely (Gonzalez *et al.* 2003). The situation is exasperated with absence of human latrines (Krecek *et al.* 2012). Commercially reared pigs tend to be kept under controlled conditions and thus are less likely to come in contact with human feces. Carcasses from commercial farms are commonly sent to slaughterhouses, but those of small scale farms or households tend to slaughter pigs themselves or send them to smaller local slaughterhouses (Dorny *et al.* 2009; Gonzalez *et al.* 2003).

Thorough meat inspection at the slaughterhouses as well as preparation and treatment of meat hold quite a large role in the prevention of *T. solium*. During meat inspection, carcasses infected with cysticerci are commonly condemned, which has caused pig owners in several poorer communities to carry out tongue inspection and slaughtering positive pigs at home, consuming the meat themselves or selling it locally (Gonzalez *et al.* 1990; Krecek *et al.* 2012). Barbequing was found to be a risk factor, the meat not always reaching a high enough temperature to kill the larva using this technique (Boa *et al.*, 2006).

In addition to traditional preventative methods, new intervention tools targeting both humans and pigs are being developed and tested for effectiveness. Currently oxfendazol

is considered to be the most promising anthelmintic to be used against porcine cysticercosis, however complete eradication of brain cysts is not always achieved, and there is still work to be done in regards to applying this treatment method to the field (Mkupasi *et al.* 2013). Also a vaccine against porcine cysticercosis called the TSOL 18 vaccine has been under development and research during the recent years and is showing great promise (Flisser *et al.* 2004; Lightowlers 2006).

2.2. *Taenia solium* taeniosis and cysticercosis in Estonia

2.2.1. Background on Estonia

Estonia is a Baltic State with a population of approximately 1 313 271 (Tammur 2015), that has been an EU member since 2004 (Põder & Sahk 2014) Meat holds an important role in Estonian cuisine, 61kg of meat being produced per inhabitant in 2013, pork being the most common. The production of pork accounts for 62% of the 79 800 tons of meat produced in 2013 (Põder & Sahk 2014).

Around Estonia's independence from the Soviet in 1991, the production of pork meat started decreasing, most likely due to rapid decrease of exports to Russia, as can be seen in **Figure 2**. At its height, pork meat production was in 1988, with over 105.2 tons in slaughtered weight, declining to 30.3 tons in 2000. Since then there has been a steady increase in pork production, reaching 49.5 tons in 2013 (PM11 2014).

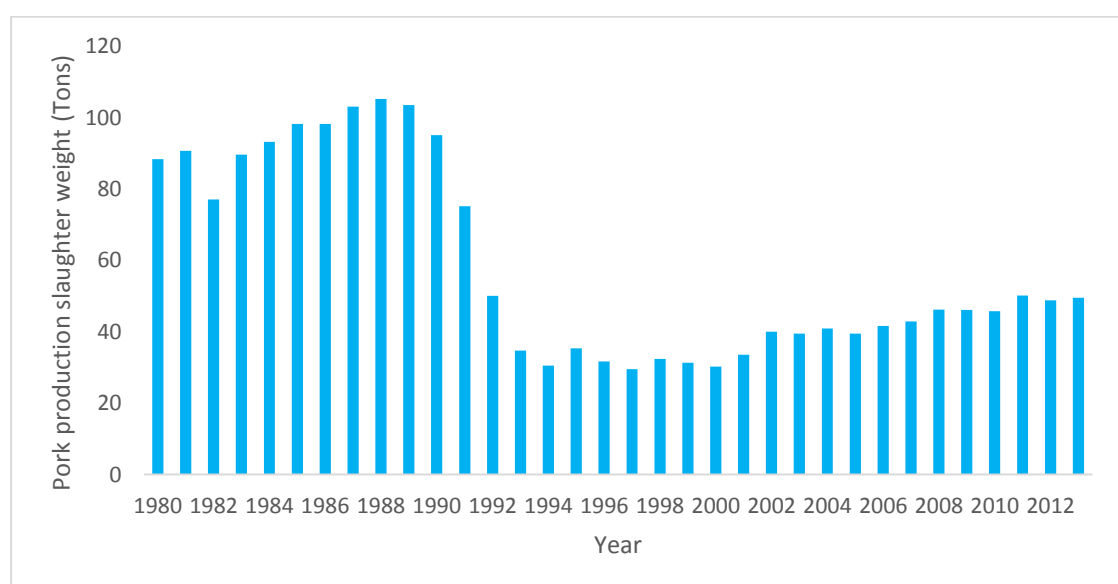


Figure 2. Pork production in tons of dead weight from 1980-2013. *Reference:* (PM11 2014)

While in recent years Estonian pig production has increased, there has been a gradual movement away from small scale pig production to farms with over 20 000 pigs as seen in **Figure 3**. Although the vast majority of pig production is accountable to farms with over 2000 pigs or more, there are still approximately 671 farms with less than 100 pigs according to Statistics Estonia (PMS012 2014).

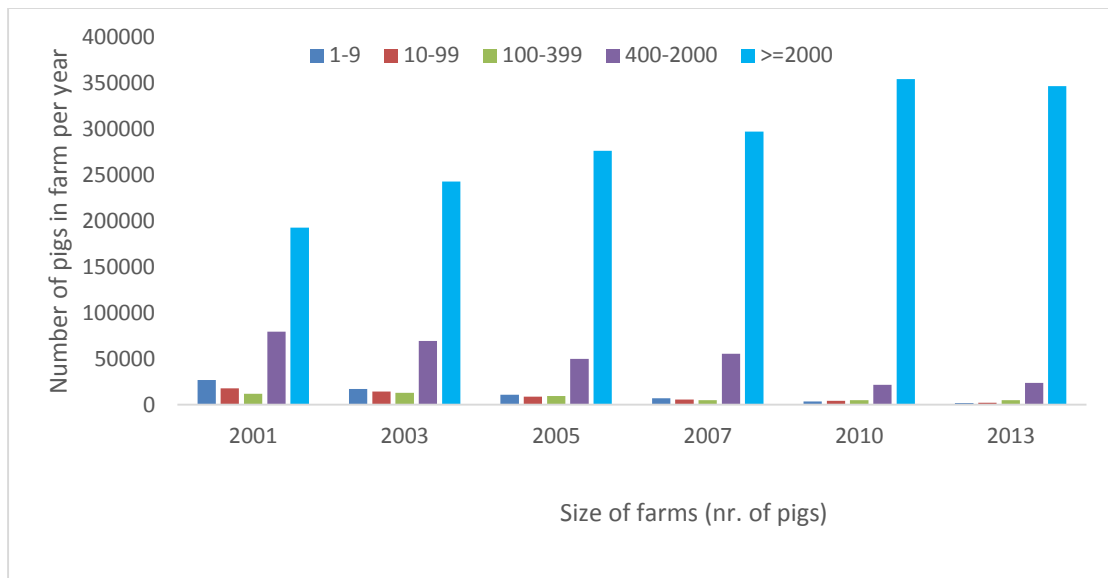


Figure 3. Number of pigs raised in different sized farms from 2001-2013. *Reference:* (PMS012 2014)

According to the Estonian law, *T. solium* cysticercosis in pigs is a reportable disease rather than a notifiable disease. This means that the official who discovered the disease, such as the local veterinarian, the Veterinary and Food Laboratory (VTL) or surveillance officer etc. must note down these cases, which are then annually reported to the Veterinary and Food Board (VFB). In contrast, notifiable diseases must be immediately notified to the VFB. In case of suspicion of disease from the list of reportable and notifiable diseases, samples are to be sent to the VTL, where they are processed accordingly, or are sent abroad to an appropriate laboratory for (further) diagnosis if necessary. Suspected cysticercosis cysts are evaluated using microscopy, PCR methods are not currently available in Estonia (Loomatauditõrje seadus 1999, §38 lg 1-2) (Teatamiskohustuslike ja registreerimiskohustuslike loomataudide loetelu kinnitamine – Määrus 2000).

Due to the public health issues of zoonoses, a system has been created to monitor these diseases on a European Union (EU) level, in addition to the one conducted by each

country. The monitoring of zoonotic diseases in Estonia is controlled by the VFB, which has units in each county (Loomatauditõrje seadus 1999, §5 lg 2). VFB reports to the European Commission in a yearly zoonosis report, which in turn forwards this information to EFSA (EU Directive 2003). According to the Council Directive 92/117/EEC, members of the EU are only required to report on cysticercosis based on their epidemiological situation of that disease (EFSA 2015).

The minimum post-mortem procedures to be performed in EU slaughterhouses to identify carcasses contaminated with cysticercosis are set down by regulation (EC) No 854/2004 and described in further detail in the Commission regulation (EU) No 218/2014. If cysts are found in meat, the carcass should be condemned or, if there are only a few cysts, undergo cold treatment. Finding of *T. solium* cysticercosis must be confirmed preferably by PCR and be reported to EFSA (EFSA 2015).

2.2.2. Cysticercosis in Estonian pigs

Although currently a seemingly rare disease, porcine cysticercosis used to be a prevalent disease in Estonia. As can be seen in **Figure 4**, from 1964 to 1990 cysticercosis was found almost yearly. There are generally less than four cases per year, however two peaks occur, one in 1970 with 132 cases, and the other in 1981 with 8 cases. Unfortunately the only additional information to these peaks is that all of the 1981 cases occurred in north-eastern Estonia, six of the cases occurred in Lääne-Virumaa and two in Ida-Virumaa (Jõgiste *et al.* 2000).

The most recent cases according to an EFSA report made in 2010, in 2006 cysticercosis was diagnosed in 10 Estonian pigs using macroscopic examination by the Food and Veterinary Service in Latvia. It should be noted that no mention whether PCR or other final diagnostic methods were used, and as to why cysts were sent to Latvia remains unclear (Cliquet *et al.* 2010). It is also notable that the reporting of cysticercosis ceased after one case diagnosed in 1986 in Harjumaa County of northern Estonia, where the Estonian capital Tallinn is situated (Jõgiste *et al.* 2000). Whether or not this is due to lack of reporting or lack of cysticercosis in Estonia is debatable.

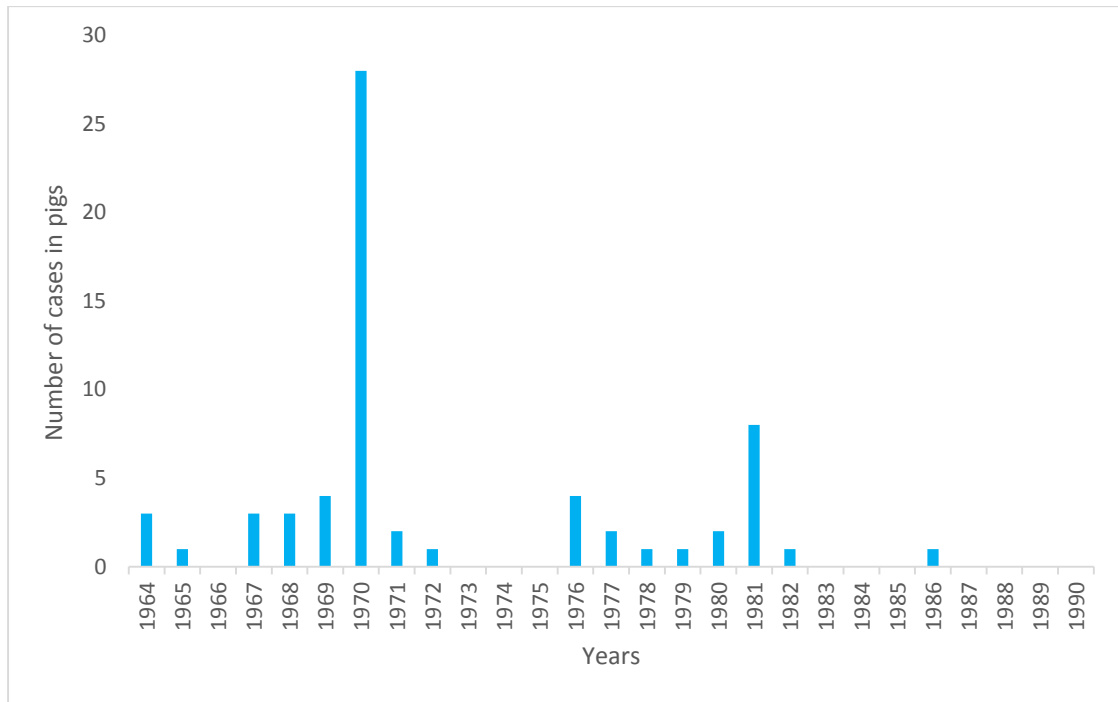


Figure 4. Number of porcine cysticercosis cases in Estonia pigs during the years 1964-1990. *Reference:* (Jõgiste et al, 2000)

2.2.3. Taeniosis in the Estonian human population

Human taeniosis cases in Estonia diminished rapidly from 178 cases in 1959, not exceeding three cases per year after 1976 as seen in **Figure 5**. Last recorded human cases of *T. solium* occurred in Pärnumaa, two cases in 2000 (one 65-69 year old female and one 70-74 year old male) and one in 2003 (15-19 year old female). Interestingly the aforementioned cases are tapeworm infections, human cysticercosis or neurocysticercosis cases are not mentioned. (Jõgiste *et al.* 2000, 2005).

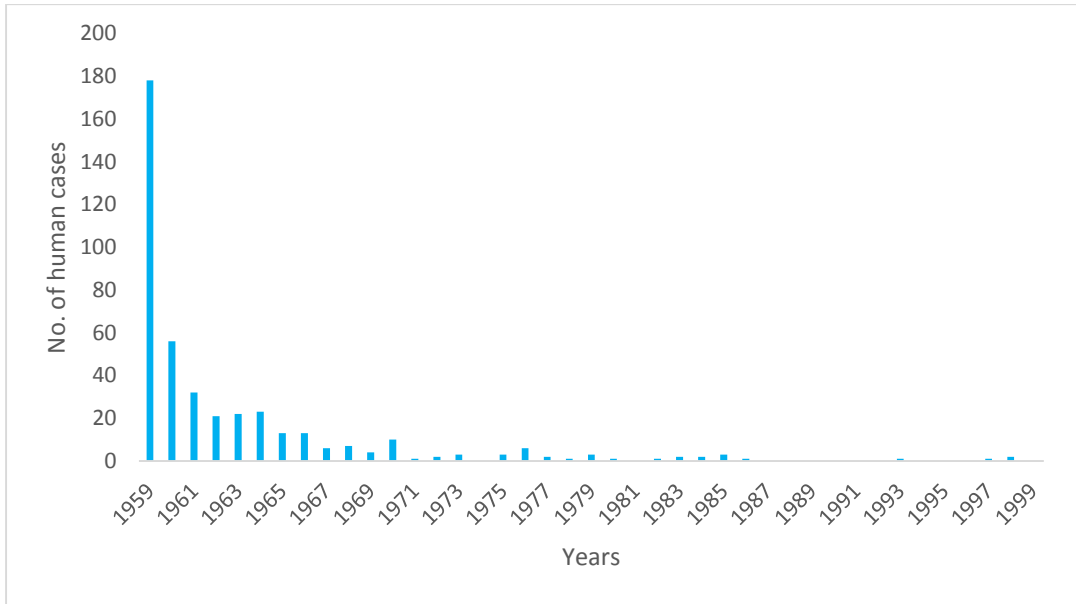


Figure 5. Number of human *T. solium* cases in Estonia during the years 1959-1999. Reference: (Jõgiste et al, 2000)

As can be seen in **Figure 6**, most of the cases occurred in Ida-Virumaa (68 cases), out of which at least 20 cases were in Narva, the third largest city in Estonia. The second biggest prevalence was in Harjumaa (42 cases), 30 of which occurred in the largest city and capital Tallinn (Jõgiste *et al.* 2000).

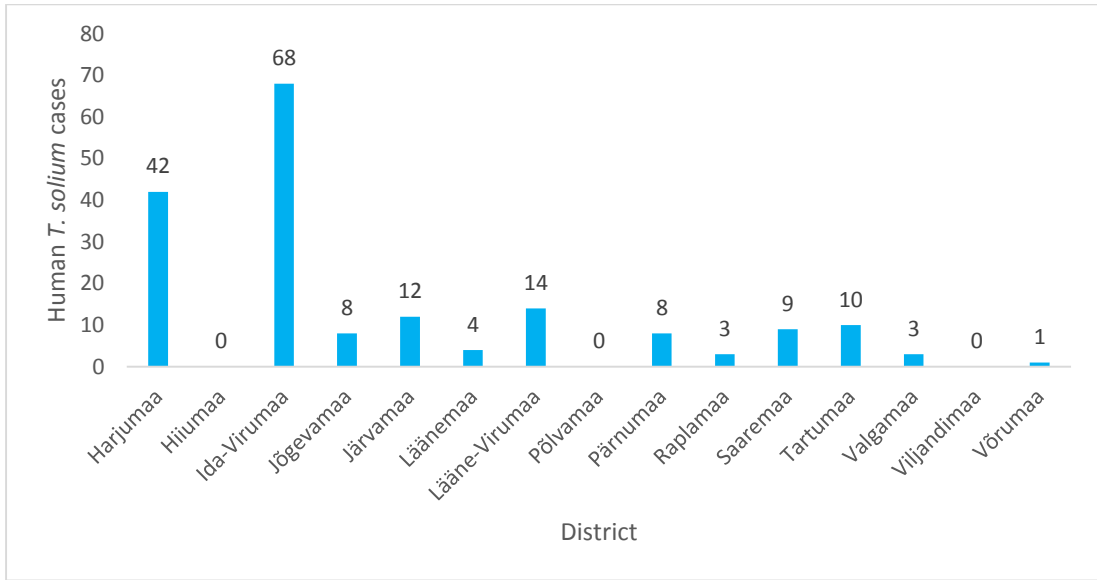


Figure 6. Number of human *T. solium* cases in the Estonian districts during the years 1959-1989. Source: (Jõgiste et al 2000)

2.3. Prevalence of *T. solium* and cysticercosis around the world

Taenia solium taeniosis and cysticercosis cause massive public health problems that are not only restricted to developing countries (Del Brutto 2012). It is endemic in several low- and middle income countries particularly in Africa, Asia and South America, however due to increased travel and immigration, human taeniosis and cysticercosis are increasingly being identified as an issue all over the world (García *et al.* 2003; FAO & WHO 2014).

As Del Brutto discussed in his review “Neurocysticercosis in Western Europe: a re-emerging disease?”, countries with sufficient meat inspection and hygiene could be facing an increase in cases of *T. solium* neurocysticercosis due to spread by tapeworm carriers. However thanks to intensive rearing of pigs in hygienic facilities, it is highly unlikely to cause an increase in porcine cysticercosis. (Del Brutto 2012; Fabiani & Bruschi 2013). This being said, a systematic review of available literature on cysticercosis in Europe during the years 1990 to 2011 made by Zammarchi *et al.* in 2013 estimated that human cysticercosis and taeniosis are still circulating diseases in Europe (Zammarchi *et al.* 2013).

A report made in 2010 for the European Food Safety Authority (EFSA) mentioned Austria, Estonia, Lithuania, Poland and Romania as countries where porcine cysticercosis have recently been diagnosed (Cliquet *et al.* 2010). In this report, Lithuania estimated a prevalence of 0,01 with 113 positive cases (Year N/A), while in 2007 Austria reported 34 positive cases and Romania around 50 cases (Cliquet *et al.* 2010).

A study made by Kozłowska-łój and Łój-maczulska in 2014 looked into the prevalence of cysticercosis in pigs from 2009 to 2012 in the Lublin province in Poland, and discovered a prevalence of 0.002 to 0.007%. The sample group was very large, consisting of 3 367 444 pigs, out of which 150 pigs carcasses were found to contain *T. solium* cysticerci using post-mortem meat inspection (Cliquet *et al.* 2010; Kozłowska-łój & Łój-maczulska 2014).

Similar results are also shown in other areas around the world. A recent study into human cysticercosis by O’Keefe *et al.* in 2015 was made by analyzing hospital records in the United States (US) from 1998 to 2011. They found an estimated 33 060 (95% CI [12 952.8-15 776.3]) Hispanics formed a majority of cases, and estimated that the majority of cases could be accountable to immigration and travel from *T. solium* cysticercosis

endemic areas. The study highlighted the importance of identification and control of the disease in endemic areas as a method of decreasing human cysticercosis cases in the US (O’Keefe *et al.* 2015).

2.4. Diagnosis of *T. solium* cysticercosis

2.4.1. Tongue palpation and post-mortem meat inspection

The classical method for detecting cysticercosis in swine has been tongue palpation, a popular method as it can be performed relatively easily ante-mortem. The tongue of a firmly restrained pig is visually inspected especially under the tongue (Ngowi *et al.* 2004) and is palpated thoroughly from tip to base (Gonzalez 1990). During meat inspection, the tongue is removed from the hyoid bone, visually inspected and palpated and at least one longitudinal incision should be made, starting from the base covering three quarters of its thickness (Boa *et al.* 2002).

Post-mortem meat inspection is commonly carried out in slaughterhouses in order to detect cysts. This is done by visual control as well as by making incisions into predilection sites. Predilection sites are described by Boa *et al.* to be “organs or muscle groups that harbour a high proportion of cysts have the highest cyst density and are parasitized in the vast majority of animals examined” (Boa *et al.* 2002). Predilection sites commonly include the tongue, heart, internal and external masseter, triceps brachii, heart as well as the diaphragm. In addition to various muscles, other organs where cysts can be found are the brain and oesophagus, and unlikely organs include the liver, kidney, spleen and lungs (Boa *et al.* 2002). However it should be emphasized that the usefulness of those predilection sites might vary depending on extent of infestation. Although meat inspection is a useful method in diagnosing cases with a high degree of infection, it might not be sufficient to detect lighter infections (Boa *et al.* 2002; Dorny *et al.* 2004; Sciutto *et al.* 1998). These methods have however been reported as having very low sensitivity and high specificity. Boa *et al.* evaluated that, using recommended meat inspection regulations in ideal conditions, only 10,6% of the total amount of cysts could be found from a pig carcass (Boa *et al.* 2002). Dorny *et al.* expected the sensitivity of this method to be at 22.1% [CI: 15–27%], using Bayesian estimation (Dorny *et al.* 2004). Sciutto *et al.* infected 47 pigs, and discovered that a maximum of 64% of pigs with cysticercosis

could be detected in slaughterhouses and 74% during tongue dissection (Sciutto *et al.*, 1998).

Tongue inspection is estimated to have a similarly high specificity as meat inspection (100%) but a poorer sensitivity. Dorny *et al.* using Bayesian estimation, estimated the sensitivity to be at 21% [CI: 14–26%], however they found that none of the lightly infected pigs and about half of the moderately to heavily infected pigs were detected during their experiment (Dorny *et al.* 2004). Ngowi *et al.* described the sensitivity of tongue palpation to vary from 14,9% to 70% however mentioned sources varied greatly in year, region and extent of infection (Ngowi *et al.*, 2004). In a study carried out by Sato *et al.*, 34% of tongue inspection negative pigs were seropositive by ag-ELISA (Sato *et al.*, 2003).

2.4.2. Serological method

Two serological methods for the detection of pigs with *T. solium* cysticercosis using Enzyme-linked immunosorbent assay (ELISA) are the antibody (Ab-ELISA) and antigen (Ag-ELISA) methods. Ab-ELISA measures exposure to *T. solium* cysticercosis, which might lead to false positive results. Ag-ELISA in turn only detects active infection, or pigs with viable cysticerci. Ag-ELISA is also not species specific, and cross reaction with for example *T. hydatigena* may occur (Dorny *et al.*, 2004; Sato *et al.* 2003; Sciutto *et al.* 1998). The sensitivity and specificity of ELISA tests of pig blood samples varies between studies, depending on the population and used methods. However most sources mention that the tests tend to be less sensitive if the cyst burden is lower (Sato *et al.* 2003; Sciutto *et al.* 1998). Dorny *et al.*, using Bayesian estimation, evaluated the Ab-ELISA test sensitivity to be at 35.8% and specificity at 91.7%, while expecting Ag-ELISA to be at a sensitivity of 86.7% and specificity of 94.7% (Dorny *et al.*, 2004).

3. AIM, OBJECTIVES AND HYPOTHESIS

3.1. Aim

The aim of this thesis is was to assess the prevalence of *T. solium* cysticercosis in pigs slaughtered at the three main slaughterhouses in Estonia

3.2. Objectives

The objective of this thesis was to study the prevalence of cysticercosis caused by *T. solium* in the Estonian domestic pig population by post-mortem meat inspection in three of the largest slaughterhouses in Estonia. Found cysts were to be analyzed by PCR, amplifying the *cox1*-gene and identifying whether it belonged to *Taenia* spp.

3.3. Hypothesis

Through research of available literature in Estonia and Europe, it was suspected that the prevalence of *Taenia solium* in Estonian domestic pigs is low, and that it would be very unlikely to find *T.solium* cysticerci in the slaughterhouses. Hypothesis was that the prevalence of *T. solium* cysticerci in Estonian slaughterhouses was $\geq 0\%$.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Sample group

According to the Estonian Agricultural Registers and Information Board (ARIB) the total amount of pigs registered in Estonia on the 1st of May 2013 was a total of 343 969 pigs. Pigs in Estonia are not registered individually, each pig farm must announce the amount of pigs kept in their facilities once a year on the 15th of May to ARIB. It has to be noted that a proportion of pigs are exported, which was 96115 pigs during 26.10.2012-25.10.2013. Unfortunately there is no record of the number of pigs imported specifically to slaughterhouses in Estonia (Personal correspondence with Ahti Bleive from ARIB, 2013).

The sample size was calculated using an online sample size calculator Sampsiz (Glaziou, 2013), using a precision of 1.00; Prevalence of 1.00% and an infinite population size. With a confidence interval of 98%. With these parameters we got an estimated sample size of $n = 536$.

The study was a cross-sectional study, pigs included were those that were brought to the slaughterhouse within the given time frame. The timeframe in each slaughterhouse was two weeks, and pigs that entered the slaughterhouse came according to a scheme fixed by the slaughterhouses. Two of the main groups that were investigated were finishing pigs, which are generally around the age of 6 months, as well as sows, which vary in age.

4.1.2. Abattoirs

According to VFB, there was a total of 51 registered abattoirs in Estonia during 2012, and out of these 50 slaughtered pigs (VFB 2013). The slaughterhouses were categorized according to amounts slaughtered yearly. When amount slaughtered was over 4000 pigs an abattoir was considered large, medium when the amount was 300-3999 pigs and small when it slaughtered less than 300 pigs.

Three abattoirs were chosen which were the three largest in Estonia, each in different counties. Abattoir 1 slaughtered the greatest amount of pigs in 2012: 234 539 pigs in total. During the same year, abattoir 2 slaughtered 70 862 pigs, and abattoir 3 slaughtered 35 495 pigs, making them the second and third largest abattoirs. The total amount of pigs

slaughtered in abattoirs in 2012 was 436 431, which means that these three abattoirs slaughter around one third of the pigs in Estonia (Personal correspondence with Aivar Alt VFB 2013).

4.2. Methods

3.2.1. Protocol in slaughterhouses

The specific rules for the post-mortem inspection of swine has been laid down in Regulation (EC) No 854/2004 of the European Parliament and of the Council (29 April 2004) and described in further detail in the Commission regulation (EU) No 218/2014. The predilection sites further discussed in the literature review, are the internal and external masseter, heart, diaphragm, triceps brachii and tongue. The following includes minimal inspection procedures to be done in slaughterhouses.

- a. Head: visual inspection of the head and throat; incision and examination of the submaxillary lymph nodes (*Lnn mandibulares*); visual inspection of the mouth, fauces and tongue. Further examinations we carried out were palpation of the tongue and palpation of internal and external masseter when possible.
- b. Trachea, oesophagus, mediastinum, lungs and associated lymph nodes: Visual inspection of the lungs, trachea and oesophagus; palpation of the lungs and bronchial and mediastinal lymph nodes (*Lnn. bifurcationes, eparteriales and mediastinales*). The trachea and the main branches of the bronchi must be opened lengthwise and the lungs must be incised in their posterior third, perpendicular to their main axes; these incisions are not necessary where the lungs are excluded from human consumption;
- c. Pericardium and heart: visual inspection of the pericardium and heart, the latter being incised lengthways so as to open the ventricles and cut through the interventricular septum;
- d. Diaphragm: visual inspection of the diaphragm; Additional examination: thorough examination.
- e. Liver and associated lymph nodes: Minimal inspection procedures: visual inspection of the liver and the hepatic and pancreatic lymph nodes, (*Lnn portales*); palpation of the liver and its lymph nodes
- f. GI-tract, mesentery and associated lymph nodes: visual inspection of the gastrointestinal tract, the mesentery, the gastric and mesenteric lymph nodes (*Lnn. gastrici*,

- mesenterici, craniales* and *caudales*); palpation and, if necessary, incision of the gastric and mesenteric lymph nodes;
- g. Spleen: visual inspection and, if necessary, palpation of the spleen;
 - h. Kidneys and associated lymph nodes: visual inspection of the kidneys and incision, if necessary, of the kidneys and the renal lymph nodes (*Lnn. renales*);
 - i. Pleura and peritoneum: visual inspection of the pleura and the peritoneum;
 - j. Genital organs: visual inspection of the genital organs (except for the penis, if already discarded);
 - k. Udder and associated lymph nodes: visual inspection of the udder and its lymph nodes (*Lnn. supramammarii*) incision of the supramammary lymph nodes in sows;
 - l. Procedures for young animals: visual inspection and palpation of the umbilical region and joints of young animals; in the event of doubt, the umbilical region must be incised and the joints opened.

3.2.2. Sampling

The sampling was conducted during February to April 2014 at the three largest slaughterhouses. Two weeks were spent at each slaughterhouse, the timeframe set by the mandatory slaughterhouse practice completed by veterinary students in their 5th year of studies. Sampling took place on the slaughter-lines by two students simultaneously, one student evaluating organs (including tongue, diaphragm and heart), while the other evaluated the meat carcass (including masseter and triceps brachii). Incisions were made into the heart, all other organs were visually inspected and palpated.

Protocol for collection of information included the following:

1. Date of sampling
2. Slaughterhouse number
3. Carcass number
4. Farm number
5. Examined sites
6. Number of found cysts
7. Comments

In addition to the protocol papers, equipment carried at the slaughterhouse included re-sealable minigrip® bags, lables and a pencil. When the cyst was found, we had permission from the slaughterhouse to remove the organ for further incisions. Found cyst

was appropriately incised with margins, placed in a bag and labeled appropriately. After sampling, the found cyst was photographed with a ruler for size, placed in a sample tube, submerged in 96% rectified alcohol and placed in a polystyrene box for transportation. The cyst was taken to Estonian University of Life Sciences, Office of Veterinary Research and Population Medicine to be stored for analysis..

3.2.3. Analysis of cysts

The cyst was stored in the refrigerator at a temperature of 4°C submerged in 96% rectified alcohol until further processing, PCR amplification and analysis. Disposable powder-free gloves were worn throughout handling of the cyst. During initial processing, the cyst was placed in a sterile crucible, cut into pieces using a scalpel and forceps and ground thoroughly using a pestle to obtain possible DNA. It was noted that the cyst was quite tough, possibly calcified. Tissue sample was sealed into a 2ml centrifuge tube with a safety lock (≤ 25 mg), and the remains of the crucible sealed into a 50 ml falcon tube. 0.2 g of cyst material was lysed with proteinase K for 20 hours. The DNA extraction was performed using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions. The entire used laboratory plastic was RNase and DNase free.

PCR for the detection of the *cox1*-gene was performed in order to identify whether the DNA extracted from the cyst belongs to *Taenia* spp. It was performed using a commercial premix 5x FIREPol® Master Mix (MgCl₂ concentration 12,5mM) (Solis Biodyne, Tartu, Estonia) containing all reagents required for PCR except for templates and primers. Primers for the *Taenia cox1*-gene were designed by Primer3 program using sequences of *Taenia solium* acquired from pigs in China, described by Nakao et al. (2003) with the Gene Bank ref number: AB086256.

Forward: 5'-TTG ATC CAT TAG GTG GTG GAG – 3'

Reverse: 5'-TCC AGT AAT TAA AGG TCA CCA TC – 3'

Every reaction contained around 200 nanograms of extracted DNA sample. The PCR program was as follows: Initial denaturation to separate complementary DNA strands was performed at 94 °C for 1 minute, followed by 30 cycles of denaturation at 94°C lasting 30 seconds. Annealing of primers was performed at a temperature of 55 °C for 30 seconds. Extension of DNA strands was completed at an increased temperature of 72°C

for 60 seconds. The final extension step was carried out at a temperature of 72°C for 10 minute. Possible amplicons achieved were expected to be ~550 base pairs (bp) implying *Taenia* spp. The agarose gel was stained using ethidium bromide staining, and electrophoresis carried out.

5. RESULTS

In total, 1217 pig carcasses and their organs were inspected, and a total of one cyst was found in abattoir 1 in the left ventricle of the heart of a finishing pig (**Figure 7.**) The cyst was excised with 5 mm margins, the rest of the heart was examined and excised with care in case of further cysts could be found. Cyst was placed in a labeled bag for further storage. After photographs, cyst was placed in a sampling tube submerged in rectified 96% alcohol and placed in a freezer until further processing.



Figure 7. Cyst found in the left ventricle of the heart of a finishing pig.

The PCR and gel electrophoresis was carried out and results from the agarose gel showed that the sample was negative for *Taenia* spp. This can be seen in **Figure 8**, where the band for *cox1*-gene at 500bp does not appear for the pig sample. As a result the cyst could not be confirmed to be *T. solium* cysticercosis. Thus the apparent prevalence was found to be 0% (0.00-0.25; 95% CI).

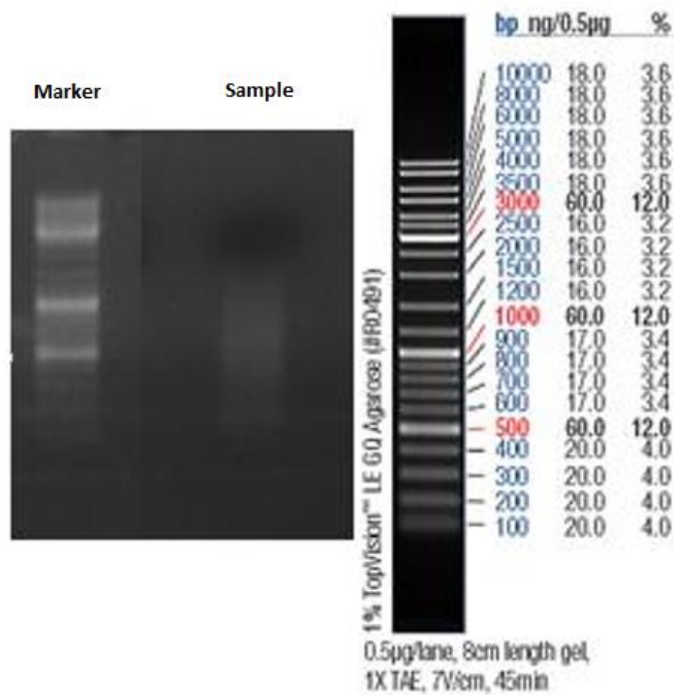


Figure 8. PCR analysis of found a cyst found in the left ventricle of the heart of a finishing pig the 07.04.2015. Marker appears on the left, sample of the pig cyst is in the middle and legend furthest on the right.

Using the application EpiTool, the true prevalence of *T. solium* was estimated with an imperfect test (AusVet, 2015). Using the parameters: sample size of n=1217, number of positives 0, test sensitivity of 0,15, specificity of 0,9 and a confidence level of 0,95 the estimated true prevalence was estimated to be ≥ 0 .

6. DISCUSSION

Taenia solium cysticercosis is a disease that could be eradicable by using sufficient preventative hygiene measures. Estonia has had several cases in the past, but now cysticercosis has fallen under the radar either because of very low prevalence or due to lack of sufficient monitoring and/or recording. This investigation was one of the first attempts to map the apparent prevalence of cysticercosis, and despite lack of hard evidence of its existence, a need for further evaluation was established.

During this study, 1217 pigs were examined during two weeks at each of the three largest abattoirs, amply covering the minimum sample size of 536 pigs. One cyst was found in abattoir 1 in the left ventricle of a finishing pig, but analysis with PCR could not confirm the cyst to be a *T. solium* cysticercus. This result agrees with the original hypothesis, which expected the amount of cysts to be found at the slaughterhouses to be very low, with an apparent prevalence of 0% (0.00-0.25 95% CI) and a true prevalence of $\geq 0\%$.

The results, although in accordance to what was expected, should be considered in light of similar studies in Europe. The EFSA report from 2010 showed that Lithuania had had 113 positive cases with an estimated prevalence of 0,01 (Cliquet *et al.* 2010). A study made in Poland found 150 positive cases from 2009 to 2012, with a prevalence of 0.002-0.007% (Cliquet *et al.* 2010; Kozłowska-lój & Lój-maczulska 2014). Both studies found an extremely low prevalence, but positive cases were found nonetheless. It is probable that Estonia could have a similar prevalence to these two countries.

Our sample group of 1217 pigs was perhaps not sufficiently large to find cysticercosis with the investigation method. For example the study made in Poland had conducted post-mortem inspection on 3 367 444 pigs, and found 150 pig carcasses with cysticerci (Kozłowska-lój & Lój-maczulska, 2014). The three largest abattoirs in Estonia generally receive carcasses from large commercial farms which tend to rear their pigs under controlled conditions. The pigs brought up in households with up to 3 pigs as well as those of small scale farms tend to be slaughtered either at home or in smaller local slaughterhouses. The pigs in these farms are more likely to be free ranging and thus are more likely to come into contact with human feces (Gonzalez *et al.* 2003).

The amount of pig meat being produced in Estonia has increased during recent years, and to meet demand, methods of pig rearing has changed from a large portion of

Estonia's pigs being reared in households or small-scale farms, to large establishments containing a majority of all of Estonia's pigs. This might be a contributing factor to the decrease in cases of cysticercosis and taeniosis.

Porcine cysticercosis and human taeniosis caused by *T. solium* used to be relatively common in diseases in Estonia, but the number of cases has diminished rapidly. Cysticercosis in pigs was frequently diagnosed in Estonia during the years 1964-1986, however with a decreasing tendency and the last recorded case being in 2006. Also the amount of human cases showed a decreasing tendency from 1959 to 1999, last recorded cases being in 2000 and 2003. It should also be mentioned that human cysticercosis or neurocysticercosis cases have not been studied. (Jõgiste *et al.* 2000, 2005).

The method of palpation, visual inspection and incisions into predilection sites has been evaluated as having very low sensitivity and high specificity by many authors (Dorny *et al.* 2004; Sato *et al.* 2003; Sciutto *et al.* 1998). Also most sources mention that the tests tend to be less sensitive if the cyst burden is lower (Dorny *et al.* 2004; Sato *et al.* 2003). It should be seriously considered that using techniques with higher sensitivity, such as ag-ELISA from blood samples, as this method would make it more likely to catch any possible pigs with *T. solium* cysticercosis. The serological approach could be a method for future studies in Estonia.

During the examination of the pig head only visual examination of these organs needs to be carried out by law. Further examinations that were carried out in our study included palpation of the tongue and, where possible, palpation of the internal and external masseter. Additional procedures that are required for higher method sensitivity include freeing the tongue from the hyoid bone for proper visual examination as well as one deep incision from base to tip. Also incisions into the internal and external pterygoid muscles would need to be carried out. When the heart is examined in the slaughterhouses, the ventricles are opened and the heart visualized, further incisions would be recommended but would be difficult to carry out due to fast pace of the line. Inspection of the diaphragm was carried out at the slaughterhouse, although as a predilection site, special attention was paid during the investigation which normally is not carried out (Boa *et al.* 2002).

Even if cysts are found in slaughterhouses and sent for further diagnostics, reliable diagnostic methods for zoonotic *Taenia* spp. are not necessarily available. Currently the only laboratory in Estonia certified by the Estonian government to analyze cysts found

in pigs is the Veterinary and Food Laboratory, where currently only morphology is used for species identification of cysts. Macroscopic and microscopic study of the cysts is carried out, along with histology if needed. PCR is not considered cost effective, as the annual number of cysts they receive is very low (personal communication with Age Kärssin from VFL 2015).

For future studies of *T. solium* porcine cysticercosis in Estonia, several improvements could be made. It would be recommended to use a different kind of diagnostic tool, for example serology, preferably using a sample group from small farms or households. If the chosen method of detecting cysts is meat inspection, it would be advisable to study pigs in small abattoirs instead of large ones, since they are more likely to receive pigs from smaller farms. It would also be interesting to investigate taeniosis and cysticercosis in human patients in Estonia, as the amount of immigration and travel has increased in and from Estonia as well, making human cases an issue to be investigated.

7. CONCLUSION

A total of 1217 pigs were sampled in three of Estonia's largest abattoirs and one cyst was found in the left ventricle of a finishing pig. PCR identification could not confirm the cyst to be *Taenia* spp. The apparent prevalence was 0%, 0.00-0.25% 95% CI and the true prevalence $\geq 0\%$. The prevalence of *T. solium* cysticercosis in pigs slaughtered in large abattoirs seems to be very low, either due to lack of cysticercosis in Estonia, or because we do not have the tools to find it.

SUMMARY

Taenia solium is a zoonotic parasite which has a complicated life cycle involving pigs and humans. It is categorized by the WHO as an important neglected zoonotic disease, as well as by FAO and WHO as number one global parasitic disease. It is endemic in several low to middle income countries in Africa, South America and Asia, but due to immigration and travel most countries are affected. Although human taeniosis is commonly asymptomatic, neurocysticercosis can cause neurological disease, and has been recognized as one of the greatest causes of acquired epilepsy in endemic areas. In Estonia *T. solium* cysticercosis is a reportable disease, not a notifiable disease. Porcine cysticercosis and human taeniosis have been diagnosed in the past, last confirmed cases occurring in 2006 and 2003 respectively.

The aim was to investigate the prevalence of cysticercosis in Estonian pigs through post-mortem meat inspection in Estonia's three largest abattoirs. A total of 1217 pigs were examined to find cysts in the spring of 2014 from February to April through visual inspection, palpation as well as incisions of predilection sites to find cysts. Found cyst were to be analyzed whether they belonged to *Taenia* spp. using PCR amplification of the *cox1*-gene. The hypothesis expected the prevalence of cysticercosis to be $\geq 0\%$. One cyst was discovered in the left ventricle of the heart of a finishing pig. PCR analysis showed that the found cyst could not be identified as *Taenia* spp., confirming the hypothesis with an apparent prevalence of 0% (0.00-0.25 95% CI) and a true prevalence of $\geq 0\%$.

The fact that *T. solium* cysticerci could not be found during this study doesn't confirm that Estonia is free of the parasite. The result was most likely affected by the low sensitivity of the used method of meat inspection, as well as the fact that the used sample group came from large farms which were less likely to be in contact with human feces. As cysts do appear in slaughterhouses and taeniosis has been diagnosed in Estonia in 2003, it is likely that further studies are warranted. Improvements include using methods with higher sensitivity, such as serological methods, or consider a sample group from smaller farms which are allowed to go outside, making contact with *T. solium* eggs more likely.

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APPENDICES

Appendix I

Abstract on the prevalence study of cysticercoses in Estonian pigs and cattle which was submitted to COST Action TD1302 CYSTINET: 1st Working group meeting & 2nd Management Committee meeting CYSTINET, 06.-07.05.2014, Evora, Portugal. Our work was presented as a power point presentation by our supervisor, Maria Vang Johansen.

Prevalence study of cysticercoses in Estonian pigs and cattle

Åhlberg, T.,¹ Dorbek-Kolin, E.,¹ Johansen, M. V.,² Lassen, B.¹

¹ Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia

² Faculty of Health and Medical Sciences, University of Copenhagen

The last recorded Estonian case of human taeniosis with *Taenia solium* was in 2001, and 2012 for *T. saginata*, while preliminary results indicate that 2.2% of the population has antibodies against *T. solium*. Human cystercercosis is not a notifiable disease in Estonia, but all animal cases must be registered at the county Veterinary Centre.

Our aim was to investigate the prevalence of cysticercosis caused by *T. solium* and *T. saginata* in Estonian domestic pigs and cattle, respectively.

The abattoirs included were the three largest in Estonia, slaughtering approximately 80% of pigs and cattle. Sampling spanned February to March 2014. Visual inspection, palpation and incisions at predilection sites were used to detect cysts. The sites for both species were: external masseter, tongue, heart, and diaphragm. In addition, the internal masseter was examined in pigs, and the internal pterygoid muscle and esophagus in cattle. All cysts were stored in alcohol for species identification based on morphology and PCR.

A total of 408 cattle and 1217 pigs were examined, and 2 cysts were found: in a finishing pig and a 20 months old bull. The cysts are currently awaiting identification. The low number of cases found in this study may reflect the true prevalence of cysticercosis in large abattoirs but could also be a result of low sensitivity of the detection methods used. Moreover, it does not exclude the possibility that the situation is different in animals slaughtered in smaller abattoirs or privately.

Appendix II

Abstract presented in Uppsala, Sweden 24.04.2015 as a poster at the 6th conference of The Scandinavian-Baltic Society of Parasitology.

PREVALENCE STUDY OF CYSTICERCOSIS IN ESTONIAN PIGS AND CATTLE

Tuuli Ählberg¹, Elisabeth Dorbek-Kolin¹, Lea Tummeleht¹, Maria Vang Johansen², Brian Lassen¹

¹ *Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia, email: brian.lassen@gmail.com*

² *Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*

Introduction

Serological results indicate 2.2% of the Estonian population carry antibodies against *T. solium*. Taeniosis was last reported in humans in 2001 for *T. solium*, and 2012 for *T. saginata*. Human cysticercosis is not a notifiable disease in Estonia, but cysticercosis in animals is. Our aim was to investigate the prevalence of *T. solium* and *T. saginata* cysticercosis in Estonian domestic pigs and cattle, respectively.

Materials and methods

Sampling spanned from February to April 2014, and was conducted in four abattoirs slaughtering approximately 80% of pigs and cattle in Estonia. Methods for detecting cysts were visual inspection of carcasses, palpation and incisions at predilection sites: external masseter, tongue, heart, and diaphragm. In addition, the internal masseter was examined in pigs, and the internal pterygoid muscle and esophagus in cattle. All cysts were stored in alcohol before DNA extraction and PCR amplification of the *cox1*-gene for species identification.

Results


A total of 564 cattle and 1217 pigs were examined, and three cysts were found: one in a finishing pig, one in a yearling bull, and one in a dairy cow. Cysts were found in 0.08% (95% CL: 0.00-0.40) of the pigs. For cattle cysts were found in 0.36% (95% CL: 0.06-1.17) animals. The cysts are currently awaiting the final DNA based results.

Conclusion

Few cysts were detected possibly due to low sensitivity of the applied inspection method. Molecular analysis will determine whether the found cysts are *Taenia* species.

Appendix III

Poster on prevalence study of cysticercosis in Estonian pigs and cattle, presented in Uppsala 24.04.2015 at the 6th conference of The Scandinavian-Baltic Society of Parasitology.



Prevalence study of cysticercosis in Estonian pigs and cattle

Tuuli Ählberg¹, Elisabeth Dorbek-Kolin¹, Lea Tummeleht¹, Maria Vang Johansen², Brian Lassen^{1,*}

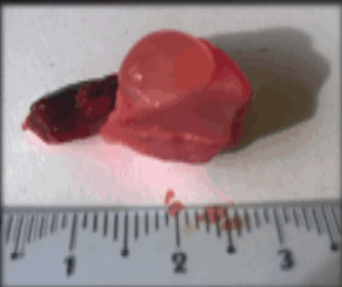
¹ Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences
² University of Copenhagen, Faculty of Health and Medical Science
 * brian.lassen@gmail.com

Introduction

FAO and WHO list *Taenia solium* as the most important food borne parasite and *Taenia saginata* as the most widely distributed human *Taenia* tapeworm worldwide.

Preliminary serological results indicate 2.2% of the Estonian population carry antibodies against *T. solium*. Taeniosis in Estonia was last reported in humans in 2001 for *T. solium*, and 2012 for *T. saginata*.

Human cysticercosis is not a notifiable disease in Estonia, but cysticercosis in animals is. Our aim was to investigate the prevalence of *T. solium* and *T. saginata* cysticercosis in Estonian domestic pigs and cattle, respectively.



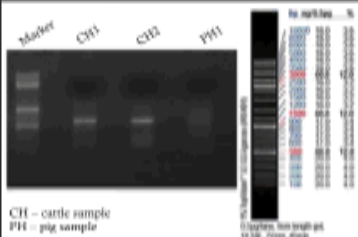
The pig cyst after sample collection.

Material and methods

- Sampling was conducted from February to April 2014 in four abattoirs slaughtering approximately 80% of pigs and cattle in Estonia.
- Methods for detecting cysts were visual inspection of carcasses, palpation and incisions at predilection sites: external masseter, tongue, heart, and diaphragm. In addition, the internal masseter was examined in pigs, and the internal pterygoid muscle and esophagus in cattle.
- Cysts were stored in alcohol before DNA extraction and PCR amplification of the *cox1*-gene for species identification.

Conclusions

Few cysts were detected possibly due to low sensitivity of the applied inspection method. Molecular analysis will determine whether the found cysts are *Taenia* species.



CH – cattle sample
PH – pig sample

The optimization of PCR conditions is still in process. Quality of the PCR signal is not sufficient for sequencing yet. Hereby the results of most recent test (7.04.2015).
 Amplifications were expected to be ~380 bp if positive for *Taenia* *cox1* gene amplified with the primers pair:
 Forward: 3'-TTG ATC CAT TAG GTG GTG GAG -3' & Reverse:
 5'-TCC AGT AAT TAA AGG TCA CCA TC -3'

Results

Pigs	Cattle
1213 pigs were examined in three abattoirs.	564 cattle were examined in four abattoirs.
Cysts (n=1, left ventricle) were found in 0.08% (95% CL: 0.00-0.40) of the pigs.	Cysts (n=2 animals, both tongue) were found in 0.36% (95% CL: 0.06-1.17) of the cattle.

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Eesti Maaülikool
Estonian University of Life Sciences
Veterinaarmeditsiini ja loomakasvatuse instituut
Institute of Veterinary Medicine and Animal Sciences



Eesti Teadusagentuur
Estonian Research Council

TerVE



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Eesti Loomakasvatuse Instituut