PREVALENCE OF *TAENIA SAGINATA* CYSTICERCOSIS IN ESTONIAN CATTLE

*TAENIA SAGINATA* TSÜSTITSERKOOSI LEVIMUS EESTI VEISTEL

Graduation Thesis in Veterinary Medicine

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Permission for public examination:

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ag-ELISA</td>
<td>Antigen ELISA (see: ELISA)</td>
</tr>
<tr>
<td>ARIB</td>
<td>Estonian office of agricultural registers and information (<em>Põllumajanduse registrite ja informatsiooni amet</em>, PRIA)</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>CL</td>
<td>confidence limit</td>
</tr>
<tr>
<td>CSBSP6</td>
<td>6th conference of The Scandinavian-Baltic Society of Parasitology</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>e.g.</td>
<td><em>exempli gratia</em> (in Latin; for example)</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>et al.</td>
<td><em>et alii</em> (in Latin; and others)</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>i.e.</td>
<td><em>id est</em> (in Latin; that is)</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>SCAR</td>
<td>Sequence characterized amplified region</td>
</tr>
<tr>
<td>spp.</td>
<td>subspecies</td>
</tr>
<tr>
<td>VFB</td>
<td>The Veterinary and Food Board (<em>Veterinaar- ja Toiduamet</em>, VTA)</td>
</tr>
<tr>
<td>VFL</td>
<td>Estonian Veterinary and Food Laboratory (<em>Veterinaar- ja toidulaboratoorium</em>, VTL)</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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SUMMARY

PREVALENCE OF *TAENIA SAGINATA* CYSTICERCOSIS IN ESTONIAN CATTLE

*Taenia saginata* is a zoonotic helminth that has a worldwide distribution. In cattle the larval stage of this parasite causes cysticercosis, in humans it causes mainly taeniosis. Human cysticercosis is not a notifiable disease in Estonia, but cysticercosis in animals is. Our aim was to investigate the prevalence of *T. saginata* cysticercosis in Estonian domestic cattle using routine meat inspection in slaughterhouses. To available knowledge no previous prevalence studies of *T. saginata* has been conducted.

Sampling spanned from February to April 2014, and was conducted in four abattoirs scattered all over Estonia. These abattoirs slaughter approximately 80% of the cattle in Estonia. Methods for detecting cysts were visual inspection of carcasses, palpation and incisions at chosen predilection sites: external masseter, tongue, heart, and diaphragm, also the internal pterygoid muscle and esophagus.

A total of 564 cattle were examined in the abattoirs. Cattle that were included in this study represented 6.5% of all slaughtered cattle between February and April 2014. From all the examined bovine carcasses two *Taenia*-like cysts were found from two different carcasses: one from a 20-month old bull (sample CH1) and one from 32-month old cow (sample CH2). Cysts were found in different abattoirs. Both of these cysts were found in the tongue and its connective tissues. Cysts were found in 0.36% (95% CL: 0.06-1.17) animals. All found cysts were stored in alcohol before DNA extraction and PCR amplification of the *cox1*-gene for species identification on genus level.

Using PCR we acquired unclear, but about right sized (~550 bp) bands on the gel. From this we believe that the cysts are very likely to be *Taenia* spp. but sequencing can confirm it. Assuming that the cysts found were *T. saginata*, we found the apparent prevalence to be low: 0.36% (95% CL: 0.06-1.17). We also estimated that the true prevalence would be >1% (95% CL: 1.40-1.43).

Only few cysts were detected possibly due to low sensitivity of the applied inspection method used in abattoirs. It should be discussed if the current routine meat inspection is sensitive enough to find all the infected animals. Furthermore, the cooperation between veterinarians and human doctors should be encouraged, as well as emphasizing the importance of notifying the findings of this zoonotic parasite.

Keywords: *Taenia saginata*, cattle, cysticercosis, prevalence, Estonia
**KOKKUVÕTE**

**TAENIA SAGINATA TSÜSTITSERKOOSI LEVIMUS EESTI VEISTEL**


Kokku uuriti 564 veist. Need esindavad 6.5% kõigist proovivõtu ajal tapetud loomadest Eestis. Leiti kokku kaks *Taenia*-moodi tsüsti erinevate loomade rümpadelt, erinevate tapamajadest. Üks tsüst leiti 20-kuuselt pullilt (proov CH1), teine 32-kuuselt lehmalt (proov CH2). Mõlemad tsüstid leiti keelest ja selle sidekoest. Tsüste leiti 0.36% (95% CL: 0.06-1.17) veistest. Leitud tsüstid hoiustati alkoholis enne DNA eraldamist ja *cox1* geeni amplifitseerimist PCR-iìga liigi tõlgendamaks väljaselgjas perekonna tasandil.

Kasutades PCR-i saavutasime geelis mitte väga selged kuid umbes õige pikkusega (~550 bp) piirkonnad. Sellest tulenevalt usume, et leitud tsüstid kuuluvad väga suure tõenäosusega *Taenia* perekonda, seda saab kinnitada sekk- ja sekveneerimisega. Oletades, et leitud tsüst on liigilt *T. saginata*, on ilmne levimus madal: 0.36% (95% CL; 0.06-1.17). Tõeline levimus aga oleks sellisel juhul >1% (95% CL; 1.40-1.43). Ainult mõningate tsüstide leidmine võib tuleneda valituduurimismeetodi madalast sensitivususest. Tuleks arutleda, kas rutinne lihainspektsoon on piisava sensitivususega, et sellega suudaks leida kõiki nakatunud loomi. Lisaks tuleks julgustada looma- ja inimesearste rohkem koostööd tegema, ja rõhutada zoonootiliste parasiitide leidude teavitamise olulisust.

Märksõnad: *Taenia saginata*, veised, tsüstitserkoos, levimus, Eesti
1. INTRODUCTION

Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) list *Taenia saginata* (*T. saginata*) as the most widely distributed human *Taenia* tapeworm worldwide, resulting more than 60 million human cases a year (FAO/WHO 2014). In bovines the larval stage of this tapeworm causes cysticercosis (Geysen *et al.* 2007). Humans can get infected after consuming inadequately cooked beef that contain cysts. In humans *T. saginata* is considered to be clinically less important than *T. solium*, another food-borne parasite from the same genus, since it only causes taeniasis, and not cysticercosis like *T. solium* does (Dorny *et al.* 2009).

The written records of taeniasis go as far back as in antiquity and it must be noted that *Taenia* species were among the earliest recognized helminths in humans (Hoberg 2006). The origin of these human tapeworms was associated with domestication of cattle around 10,000 years ago. Actually the occurrence of taeniids in humans seem to pre-date the domestication of cattle and the development of agriculture and animal husbandry (Hoberg *et al.* 2001; Bobes *et al.* 2014). As a species *Taenia saginata* was mentioned by Goetze in 1782, and the genus *Taenia* was mentioned by Linnaeus in 1758 (Hoberg 2002). In some older studies and case reports *Taenia saginata* cysticercosis has also been referred as ‘finnoss’ (Jõgiste *et al.* 2000).

Today cysticercosis is considered to be an eradicable disease (Bouteille 2014). At the same time this tapeworm can still be found in both industrialised and developing countries (Dorny *et al.* 2009). Taeniasis affects all income classes – the beef can be raw or undercooked due to local practical problems (e.g. not enough firewood to be found) or on purpose (gourmet-foods, like steak tartar, carpaccio). As long as humans eat beef there is a potential infection risk. In many countries the lack of prevalence studies, both human and bovine, creates the illusion of a non-existent problem. Due to globalisation tons of beef and beef-products are imported and exported every year. In the year 2013 Estonia exported 1,956,000 kg of beef, mainly to Finland (40 %) and Holland (26 %), and imported 2,328,000 kg of beef, mainly from Latvia (34 %) and Poland (24 %) (Voog *et al.* 2014). Estonians consumed 68.9 kg of meat per capita in the year 2013, which included seven kilograms of beef, 35.5 kg of pork, 0.5 kg of mutton and goat meat and 23.2 kg of poultry (Rosenberg 2014).

In Estonia there has been reported only one human taeniasis case after the change of the millennium (personal communication with Health Board, Jevgenia Epštein, 2014). At the same time there have been no records of bovine cysticercoses (Veterinary and Food Board: Estonian zoonosis reports 2007-2013). There are also no findable studies published about *T. saginata* cysticercoses in bovines in Estonia. This study was designed to shed light on this subject. The aim
of this study was to assess the prevalence of *T. saginata* cysticercosis in the domestic cattle population by post-mortem meat inspection in Estonian abattoirs.
2. LITERATURE REVIEW

2.1. Taenia saginata – zoonotic parasite

2.1.1. The parasite

*Taenia saginata* is an intestinal zoonotic cestode (tapeworm), which is most commonly found in humans (FAO/WHO 2014). The larval stage of this human tapeworm causes bovine cysticercosis in cattle. Also reindeers can be infected (Hallanvuo and Johansson 2010). Some sources (e.g. EFSA reports) still use the former genus name, *Cysticercus*, in describing the larval forms of *Taenia* even though it is not generally in use anymore. The tapeworm belongs to the genus of *Taenia* with few other zoonotic parasites, including *T. solium* and *T. (saginata) asiatica* that cause porcine cysticercosis. *Taenia asiatica* can also infect cattle. *Taenia solium* can be found worldwide; *Taenia (saginata) asiatica* is considered more relevant to Asia and has not yet been reported in Europe (Dorny et al. 2010). Systematically *T. saginata* is classified and taxonomically represented as following (Järvis 2011; Flisser et al. 2005):

- Kingdom (*Regnum*): Animalia, Eukaryota
- Subkingdom (*Subregnum*): Metazoa, Helminthes
- Phylum (*Phylum*): Plathelmintha
- Class (*Classis*): Cestoda
- Subclass (*Subclassis*): Eucestoda
- Order (*Ordo*): Cyclophyllida
- Family (*Familia*): Taeniidae
- Genus (*Genus*): Taenia
- Species (*Species*): Taenia saginata

Flisser et al. (2005) described these tapeworms as flat in shape, opaque white or yellow in colour and segmented. They are exceptionally long, measuring up to twelve meters in their adulthood. The scolex (the head) has four suckers and an unarmed and sunken rostellum. The scolex is pinhead –sized (diameter 1.5-2.0 mm), following the neck and many segments. Segments are also called proglottids or strobili, and they appear as a long ribbon. Ribbon can consist of more than thousands of segments. Segments get gradually bigger in size when moving towards the posterior end of the tapeworm so that the oldest, broadest and longest segments are located at the end of the parasite. When fertilized, the segments start to resemble sac full of eggs, each containing around 50,000 eggs. In this stage the segments are 6-7 mm wide and 15-20 mm long. Inside the segments there is a longitudinal uterus that has 20-30 lateral dictomous branches. By examining the proglottids microscopically it is possible to differentiate between *T. saginata* and *T. solium* – the latter has less longitudinal branches in the uterus (Cabaret et al. 2002).
According to Flisser et al. (2005) the eggs of *T. saginata* are spherical and the size can vary from 26 to 34 μm. They note that eggs of all taeniids are morphologically indistinguishable from each other by using light and electron microscopy.

*Taenia saginata* cysts in bovines are primarily found in the heart and skeletal muscle. After infection the full development of the cysts take from four to five months (Scandrett et al. 2009), and they become infective to humans after ten weeks (Dorny and Praet 2007). Cysts are roughly oval, translucent and contain fluid-filled fibrous capsule a larvae (scolex) within. Diameter of the cysts varies from 0.5 to 1.0 cm (Scandrett et al. 2009). Cysts can be detected from the muscle as early as two to four weeks post-infection. In that time cyst diameter will be around 2.5 mm (Scandrett et al. 2009). The cysts do have predilection sites; amongst those the heart is more frequently infected (Scandrett et al. 2009).

2.1.2. Life cycle

Humans get infected by eating inadequately prepared beef that is contaminated with viable cysts (Allepuz et al. 2008; Dorny et al. 2010). A single cyst is normally sufficient to establish an infection, but multi-infections do occur (Dorny and Praet 2007). There are estimates that one infected bovine carcass could infect on average 8-20 human individuals (Dupuy et al. 2014). The adult tapeworm will develop in the small intestine of its human host. These tapeworms reach maturity in two to three months and can be quite remarkable in size with a length of 3-12 meters (FAO/WHO, 2014). In the absence of treatment the tapeworm can occupy human intestines for 20-25 years (Hoberg 2002).

Adult tapeworms will release gravid proglottids that contain 30,000-50,000 eggs (Flisser et al. 2005). Between three and seven proglottids are released every day (Dorny et al. 2010). These proglottids will leave the body of the host by active migration through the anus or within the stools. In the stools proglottids are usually locate on the surface of the faeces (Cabaret et al. 2002). The released eggs contain oncosphere (a larva) that is infective immediately after being released by the host. Cattle become infected by grazing on a contaminated pasture. The pasture can become contaminated directly with human faeces containing *Taenia* eggs, or indirectly via sewage sediment or flooding (FAO/WHO 2014).

Eggs hatch in the digestive system of cattle and the oncospheres are released. They penetrate through the intestinal wall and start circulating in lymphatic system and in the blood. After the migration in the body the larvae will develop into cysts. This will take place in eight to ten weeks, after what they are already infective to humans (Flisser et al. 2005). The cysts lodge itself into the smooth muscle tissue, including heart, masseter muscles, tongue and diaphragm (FAO/WHO 2014). Approximately nine months after infection most cysts have died and calcified (Flisser et al. 2005) but some remain viable in the muscles. After eating raw beef that is containing
viable cysts humans get infected and the cycle begins again. Previously described life cycle is illustrated in Figure 1. (picture courtesy of CDC-DPDx).

Figure 1. Life cycle of *Taenia saginata* and *Taenia solium*.

Allepuz *et al.* (2008) found that there is a statistically significant association between the infection status and farm type (dairy versus beef). They found that dairy farms are almost twice more likely to be affected than beef farms. They also found that in comparison with uninfected farms the infected farms had bigger farm size (the number of animals). It has been stated that age of the cattle has significant positive effect on the measured sero-prevalence – older individuals have a significantly higher probability to be infected (Dorny *et al.* 2000; Eichenberger *et al.* 2011; Calvo-Artavia *et al.* 2013). Females seem to be in higher risk than males (Calvo-Artavia *et al.* 2013).

In a study with lightly infected cattle it was found that cattle drinking from the streams that carry effluent from the sewage treatment plants is a major risk factor of bovine cysticercosis (Kyvsgaard *et al.* 1991). Other risk factors include outdoor defecation near the pasture or cattle rearing facilities; non-effective fly and bird control in the cattle facility area; irrigating or fertilizing crops and pastures with sewage water, sludge or untreated human faeces; human carriers that take
part of looking after the cattle; and misplaced faeces deposits on camping grounds and along highways and rail tracks (Murrell 2005). The same source listed important risk factors for transmission from cattle to humans as: consumption of raw or undercooked beef and lack of satisfactory meat inspection (veterinary control).

2.1.3. Bovine cysticercosis

Bovine cysticercosis is a parasitic zoonosis caused by the larval stage of human tapeworm *T. saginata* (Geysen *et al.* 2007). Cattle become infected usually via consumed food, e.g. grazing on pasture, contaminated with human faeces that contains eggs of the parasite (faeces from sewage system or direct pollution). Allepuz *et al.* (2008) found the water supply to be the most likely route of infection for cattle, followed by feed and other routes. In their study personnel and pasture seemed to be less frequent causes of infection. There has been registered outbreaks of bovine cysticercosis, e.g. in Canada, Alberta (Lees *et al.* 2002) and New Zealand (McFadden *et al.* 2011). Humans become infected after ingesting raw or undercooked beef that contains cysts (Dorny and Praet 2007; Scandrett *et al.* 2009). Cysts are not resistant to high temperatures and the transmission is affected by dietary habits and culinary practices that consume beef raw or lightly cooked (Dorny *et al.* 2009).

Generally speaking the clinical expression of cysticercosis is dependent on the number, size and location of the cysts, as well as the host immune response to the parasite (Bouteille 2014). In cattle light infections are more common than heavy infections (Dorny *et al.* 2009). Cattle do not normally show signs of clinical illness (Dorny and Praet 2007; Scandrett *et al.* 2009). Possible clinical signs include mild fever, stiffness in gait, feeding difficulties and inflammation of bowel; with heavy infection death may occur as a result of myocarditis (Järvis 2011b).

The economic consequences of bovine cysticercosis are significant (Yoder *et al.* 1994). It is stated in the European Unions’ Regulation (EC) No 854/2004 (2004): “Meat infected with cysticercus is to be declared unfit for human consumption. However, when the animal is not generally infected with cysticercus, the parts not infected may be declared fit for human consumption after having undergone a cold treatment.” Economic losses are definitely noteworthy and they also occur in Europe (Dorny and Praet 2007). The losses are mainly due to condemnation, cold-treatment (refrigeration) and downgrading of infected carcasses (Dorny and Praet 2007). It has been estimated that these losses go as far as 30-45% of the value of the carcass (Cabaret *et al.* 2002). It has been reported that cattle with cysticercosis also have high prevalences of *Dicrocoelium dentriticum* (60.8%) and *Fasciola hepatica* (13.5%) (Eichenberger *et al.* 2013).
2.1.4. Predilection sites

Previous studies have shown that there are predilection sites where the bovine cysts can be found more often. According to Cabaret et al. 2002 the predilection sites are the head and the heart. In more detailed inspection of those sites it is shown to be 5-50 times higher prevalence rates than what is found upon routine meat inspection (Dorny et al. 2000). Then again Walther and Koske (1980), and McCool (1979) found that 49-51% of the lightly infected animals, cysts are not present in the so-called predilection sites (heart, masseter, oesophagus, diaphragm and tongue).

Scandrett et al. 2009 wrote that traditional inspection sites for Taenia saginata are the heart, masseter and pterygoid muscles, tongue, oesophagus and diaphragm (both membranous and crura). They found that while comparing these sites the heart ranked as the most frequently affected site, although there was no significant difference between the heart and the masseter muscle. The heart had the highest cyst density and frequency of infection. It also had greater visibility of gross lesions due to the cardiac muscles early inflammation response. Scandrett et al. (2009) therefore recommended more extensive examination of the heart so that the detection of infected animals would improve. The recommendation was to perform six evenly spaced deep incisions into the myocardium from the endocardial surface so that all the surfaces would be revealed, also the surfaces should be everted. The least amount of cysts was found from the oesophagus.

2.1.5. Bovine cysticercosis and taeniasis worldwide

2.1.5.1. In Estonia

Unlike many European counties, there are no previously published prevalence studies of T. saginata cysticercosis in Estonia. However, in other countries this parasitosis has been a subject of study. Even though there are no published prevalence studies in Estonia, slaughterhouse reports from 1964 to 1990 include information about the presence of T. saginata in cattle. During the 25-year period 1621 T. saginata cases were reported from slaughterhouses (Jõgiste et al. 2000). The variation through the years can be found in the figure below (Figure 2), findings varying from 167 findings in the year 1978 to only nine findings in years 1987 and 1989. Most of the findings in cattle from 1981-1990 occurred in Harju County with 129 cases out of 248 (52.0 %) (Jõgiste et al. 2000). Unfortunately the data from earlier years is only partial and therefore it was not included in description since it can be misleading. Data from 1991 has been very fragmented and it is not gathered in one document so it was not possible to get a clear overview of the situation following 1990. Because of this it is not possible to evaluate if there has been a drop in the number of cases or if the surveillance data is not gathered systematically and made publicly available.
In the EFSA (European Food Safety Authority) zoonosis reports of 2012 and 2013 it is noted that cysticercosis is a very rare disease in animals in Estonia. According to the report no cases of cysts of *T. saginata* were reported in 2012 or 2013. Estonia has its own monitoring system that VFB (The Veterinary and Food Board; *Veterinaar- ja Toiduamet, VTA*) publishes every year in a national zoonosis report. In 2013 there were no cases of bovine cysticercosis according to VBF’s zoonosis report (2014). VBF reports mention cysticercosis only from reports after 2010, and all the later reports state that there have not been any human or bovine cases. Reports from 2003-2010 that are accessible through their website do not even mention cysticercosis.

All European Union zoonosis reports are gathered together in EFSA website. According to the EFSA report from 2012 and 2013 all the *Cysticerci* spp. (i.e. larval stages of *Taenia*) have a monitoring system in place. It should however be noted that there is no specification in the
monitoring system for different species of animals in the reports. The sampling strategy described by EFSA is that all slaughtered animals are visually (macroscopically) examined at post-mortem inspection. All slaughtered animals that are intended for human consumption are to be examined routinely in slaughterhouses. Liver and carcass are the areas inspected. Cysts are found using visual examination and further analysed using microscopy. In case of detecting of cysts the carcass or organs are declared unfit for human consumption.

Detection of cysts has been notifiable since 2000 according to the Infectious Animal Disease Control Act and the Ministry of Agriculture Regulation No 34 "List of Notifiable Diseases and Diseases subject to Registration". Authorized laboratories that investigate the safety and quality of the products on enterprises that handle food of animal origin are required to notify the VFB about the isolation of these zoonotic agents. Local Veterinary centres notify the local offices of the Health Board (Terviseamet) about isolation of zoonotic agents in food and animals. By law, cysticercosis is subject to notification and it must be registered (Riigiteataja, 2012). Cysticercosis is listed in point B.3 of the Annex I of the Directive 2003/99/EC as an infection to be monitored according to the epidemiological situation.

2.1.5.2. Worldwide distribution of bovine cysticercosis
Distribution of bovine cysticercosis is considered to be related to the distribution of taeniasis in humans and is thus worldwide. The prevalence is very variable. Europe is considered to have low prevalence with a variation from 0.007 to 6.8% (Cabaret et al. 2002). In eastern European countries bovine cysticercosis appears to be more common than in other European regions (Dorny and Praet 2007). In other parts of the world the prevalence has been found to be as high as 18.49% in Ethiopia (Kebebe 2008). Prevalence studies that could be found around the world have been gathered to Table 1.
Table 1. Prevalence studies of bovine cysticercosis (*Taenia saginata*).

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Prevalence</th>
<th>Method used</th>
<th>Cattle (n)</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EUROPE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>1997-1998</td>
<td>3.09%</td>
<td>Antigen ELISA (and routine meat inspection)</td>
<td>1,164</td>
<td>Prevalence with routine meat inspection 0.26%</td>
<td>Dorny <em>et al.</em> 2000</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>0.28%</td>
<td>EU legislation</td>
<td>-</td>
<td></td>
<td>Boone <em>et al.</em> 2007</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>0.15%</td>
<td>-</td>
<td>824,511</td>
<td></td>
<td>EFSA and ECDC 2014</td>
</tr>
<tr>
<td>Catalonia (Spain)</td>
<td>2007</td>
<td>0.022%</td>
<td>EU legislation (+/- histopathology)</td>
<td>498,351</td>
<td></td>
<td>Allepz <em>et al.</em> 2008</td>
</tr>
<tr>
<td>Denmark</td>
<td>2011</td>
<td>0.06%</td>
<td>Routine meat inspection database</td>
<td>4,090,661</td>
<td>True prevalence; animal prevalence at slaughter 0.009%</td>
<td>Calvo-Artavia <em>et al.</em> 2013</td>
</tr>
<tr>
<td>France</td>
<td>2010</td>
<td>0.142%</td>
<td>EU legislation</td>
<td>4,564,065</td>
<td>Apparent prevalence 0.142%, true overall prevalence 1.23%, true prevalence with at least one viable cysticerci 0.113%</td>
<td>Dupuy <em>et al.</em> 2014</td>
</tr>
<tr>
<td>Lublin (Poland)</td>
<td>2009-2012</td>
<td>0.19%</td>
<td>Routine meat inspection database</td>
<td>542,963</td>
<td>Prevalence ranged from 0.15% to 0.23% during inspected years; Mean prevalence is 0.19%</td>
<td>Kozłowska-Łój and Łój-Maczulska 2014</td>
</tr>
<tr>
<td>Sweden</td>
<td>2012</td>
<td>0.0002%</td>
<td>-</td>
<td>419,939</td>
<td>No calves or feeder cattle examined. With routine EU legislation inspection the prevalence was 1.8%; when additional incisions added the prevalence was 4.5%</td>
<td>EFSA and ECDC 2014</td>
</tr>
<tr>
<td>Switzerland</td>
<td>2010</td>
<td>1.8 - 4.5%</td>
<td>EU legislation and additional incisions</td>
<td>1,088</td>
<td>No calves or feeder cattle examined. With routine EU legislation inspection the prevalence was 1.8%; when additional incisions added the prevalence was 4.5%</td>
<td>Eichenberger <em>et al.</em> 2011</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>16.5%</td>
<td>Antibody ELISA</td>
<td>793</td>
<td>Modeled prevalence in dairy cows</td>
<td>Eichenberger <em>et al.</em> 2013</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>2011</td>
<td>0.032%</td>
<td>Routine meat inspection</td>
<td>8,484,371</td>
<td>Adult cattle</td>
<td>Hill <em>et al.</em> 2014</td>
</tr>
</tbody>
</table>
### OTHER PARTS OF THE WORLD

#### Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Prevalence</th>
<th>Methodology</th>
<th>Number</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>2008</td>
<td>18.49%</td>
<td>Routine meat inspection and cyst morphology</td>
<td>4,456</td>
<td>-</td>
</tr>
<tr>
<td>Northern Turkana District (Kenya)</td>
<td>2009</td>
<td>16.7%</td>
<td>Antigen ELISA</td>
<td>792</td>
<td>Human taeniasis estimate 2.5%; The prevalence from the same area through routine meat inspection 0.87%; True seroprevalence 20.0% Asaava et al. 2009</td>
</tr>
</tbody>
</table>

#### Middle East

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Prevalence</th>
<th>Methodology</th>
<th>Number</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iran</td>
<td>2005-2007</td>
<td>0.25%</td>
<td>Routine meat inspection</td>
<td>4,534,105</td>
<td>-</td>
</tr>
</tbody>
</table>

#### America

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Prevalence</th>
<th>Methodology</th>
<th>Number</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecuador</td>
<td>2000-2001</td>
<td>0.37%</td>
<td>Routine meat inspection and Antigen ELISA</td>
<td>806/869</td>
<td>Prevalence from routine meat inspection 0.37%; prevalence from Antigen ELISA 4.03% Rodriguez-Hidalgo et al. 2003</td>
</tr>
</tbody>
</table>
In addition to national reports EFSA also publishes European Union Summary Reports together with ECDC. To these reports there are gathered information from 30 countries, three of which are not members of European Union (Iceland, Norway and Switzerland). In the newest published reports of ‘The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012’ and ‘The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013’ you can find that Belgium and Sweden have reported information on Cysticercus findings in slaughtered animals 2011-2013. In Belgium in 2011 they inspected 859,390 animals and 1,347 (0.16%) carcasses were found positive for T. saginata cysts in cattle. Eleven of those 1,347 were heavily contaminated. One year later 824,511 animals were inspected of which 1,214 (0.15%) we found positive. Nine of those were found to be heavily contaminated. In 2013 808,075 cattle was examined, 994 (0.12%) was found positive and from those 16 were heavily contaminated. In Sweden there were 456,120 inspected animals and one positive finding (0.0002%) in 2011. Year later 419,939 carcasses were inspected with one positive finding (0.0002%). In 2013 417,384 carcasses were inspected and one positive was found. In Finland two cases of bovine cysticercosis are registered; one in 1996, the latest in 2002 (Hallanvuo and Johansson 2010).

2.1.6. Human taeniasis

2.1.6.1. Symptoms, diagnostics and treatment

Usually humans are infected by a single T. saginata tapeworm (Dorny and Praet 2007). In humans the adult T. saginata tapeworm lives in the digestive system, in small intestine. Most frequently infected people are asymptomatic or suffer from anal pruritis and discharge of faecal proglottids (FAO/WHO 2014). With voluntary self-infection experiments with T. saginata the researchers found that shedding of the proglottids happen spontaneously and actively, and humans shed five to fifteen proglottids per day (Craig and Ito 2007). They also found that this shedding can start as early as ten weeks after infection and by that time the tapeworm was found to be approximately three meters long. Humans can shed Taenia eggs with faeces even without any symptoms present. Sometimes there are non-specific symptoms present, such as vomiting, nausea, diarrhea, epigastric pain and weight loss. Rarely this tapeworm can cause ileus, pancreatitis, cholecystitis or cholangitis (FAO/WHO 2014). Even more rarely T. saginata can cause bowel obstruction (Karanikas et al. 2007). There is one case report published with T. saginata causing acute cholangitis – this is not normally in the nature of this parasite (Uygur-Bayramiçli et al. 2012).

In humans Taenia saginata is usually diagnosed via faecal samples using copro-ELISA (Enzyme-linked immunosorbent assay) to detect Taenia spp. antigens. This method can
differentiate between subspecies of *Taenia* (Nunes *et al*. 2003; Nunes *et al*. 2005). Treatment for *Taenia saginata* taeniasis in humans is based on anthelmintics – niclosamide or praziquantel (Dorny *et al*. 2010). It should be noted that these drugs eliminate the intestinal parasite but does not affect the viability of eggs, thus infective eggs can be found in the sewage sludge (Cabaret *et al*. 2002).

2.1.6.2. Distribution

Even though *T. saginata* has a worldwide distribution, it should be noted that distribution of human taeniasis is a cultural matter among other differentials. For example Hindus do not commonly suffer from taeniasis since they do not usually consume beef (Macpherson 2005). The prevalence of human taeniasis in Europe varies in between <0.01-10% (Cabaret *et al*. 2002). There is a report of an outbreak of human taeniasis caused by *T. saginata* with weak evidence in Czech Republic that involved 24 individuals in 2013 (EFSA and EDCD, 2015).

It is important to acknowledge that human taeniasis is not a notifiable disease in European Union (Dorny *et al*. 2010) and reported cases do not give a full picture of the spread of the parasite. Thus, the data on the prevalence of human taeniasis is lacking. Estimates are derived from the sale of the drugs used to treat taeniasis in EU (Dorny *et al*. 2010).

2.1.6.3. In Estonia

Historically speaking there has been numerous reports on human taeniasis in Estonia. According to Jõgiste *et al*. (2000) there were 165 human cases in between 1951-1960. In 1961-1970 there were 395 human cases, during that period the county with most cases was reported to be in Harju County with 229 cases. Of those 229 cases most of them (219 cases) occurred in the capital of Estonia, Tallinn. From 1971 to 1980 there were 106 human taeniasis cases with the most infections again in Harju County (50 cases in total; 44 cases in Tallinn). From 1981 to 1990 twenty-five cases were registered. Eleven of those cases occurred in Tallinn, making Harju County the area with most infections again. From 1991 to 1999 there were 19 cases of human taeniasis, the most cases (5 cases) occurring in Tartu County. You can find previously described numbers in a table (Table 2) below. The only case from 1999 occurred in Ida-Viru County on a 40 year-old man living in the urban area (Jõgiste *et al*. 2005). From 2000 to 2012 there were no registered human cases with *Taenia saginata* (Jõgiste *et al*. 2001; Jõgiste *et al*. 2002; Jõgiste *et al*. 2005).

In the previously mentioned EFSA summary reports of 2012 and 2013 there were no mentioning of human cases in Estonia. Also you cannot find any information about human taeniasis on the website of the Health Board (*Terviseamet*) where statistical reports from 1999 onwards of communicable diseases can be found. Even so the information from 2000-2012, that the writer received from the Chief Specialist of Department of Communicable Disease
Surveillance and Control, Health Board (*Terviseamet*), suggested that there was one registered positive human case of *T. saginata* taeniasis in 2012. It occurred in a over sixty-year-old female patient from Viljandi County. Why this human case is not shown in any published reports is unknown.

Table 2. Human taeniasis caused by *Taenia saginata* by decades and most infected counties with total case numbers (Jõgiste *et al.* 2000; Jõgiste *et al.* 2001; Jõgiste *et al.* 2002; Jõgiste *et al.* 2005; personal communication with Health Board, Jevgenia Epštein, 2014)

<table>
<thead>
<tr>
<th>Years</th>
<th>Human cases in total</th>
<th>Most infected county (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961-1970</td>
<td>395</td>
<td>Harju (229)</td>
</tr>
<tr>
<td>1971-1980</td>
<td>106</td>
<td>Harju (50)</td>
</tr>
<tr>
<td>1991-1999</td>
<td>19</td>
<td>Tartu (5)</td>
</tr>
<tr>
<td>2000-2009</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>2010-2012</td>
<td>1</td>
<td>Viljandi (1)</td>
</tr>
</tbody>
</table>

From the year 1962 the human cases have been registered by gender and age but data is not available for all years (Jõgiste *et al.* 2000). For the years the data is available (from 1962 to 2012) it indicates that women dominate in the reported cases (61%) as seen in the Figure 3.

![Figure 3. Human taeniosis in Estonia 1962-2012 by gender (Jõgiste *et al.* 2000).](image)
Between 1966 to 1999 most of the infections happened in the urban area (73 %; 57 cases out of total of 78) as seen in the Figure 4.

![Figure 4. Human taeniosis in Estonia 1966-1999 by living area (Jõgiste et al. 2000).](image)

When it comes to age groups the highest prevalence was found to be in 30-39 year-old individuals (24%), following 40-49 year-olds (19%) and 20-29 year-olds (17%) (Figure 5). Age group with the lowest prevalence was the group with 0-4 year-olds (2%).

![Figure 5. Human taeniosis in Estonia 1962-2012 by age groups (Jõgiste et al. 2000).](image)

The reported human and bovine cases are compared in Figure 6. There can be seen that reporting of human cases have been on a steady decline regardless of what is found in the cattle. Also, after the 1980’ies less bovine cases has been reported.

![Figure 6. Human taeniosis and bovine taeniosis in Estonia 1962-2012.](image)
Figure 6. *Taenia saginata* cases in humans and bovine by year, 1964-1990 (Jõgiste et al. 2000).
2.2. Possible methods of studying bovine cysticercosis

2.2.1. The knife and eye –method

The classical post-mortem study method that includes visual examination, palpation and incisions has also been called ‘the knife and eye’-method. It is the most commonly used method in slaughterhouses to exam the carcasses that are meant for human consumption. When it comes to finding *T. saginata* caused cysts, the method is not effective enough. It has been estimated that with this method only 15% of lightly infested animals can be found and so the animal level sensitivity of this method is estimated to be 15% (Kyvsgaard *et al.* 1990). Also Eichenberger *et al.* (2013) found that current European Union meat inspection procedure has a diagnostic sensitivity of 15.6 % but by adding additional cuts into the heart it can be increased up to 24.2 percent. There are studies reporting that the low sensitivity of routine meat inspection leads to underestimation the prevalence of bovine cysticercosis with a factor 3-10 (Dorny *et al.* 2000). It has been found that the specificity of meat inspection is high (Geysen *et al.* 2007).

Immunodiagnostic tests, such as the ante-mortem detection of antigens and antibodies, have been reported to be three to ten times more sensitive than the knife and eye-method (Geysen *et al.* 2007). In their study comparing the samples found with visual examination to be *T. saginata* cysts and the PCR result of the same samples, Geysen *et al.* (2007) found that only three percent of studied samples were wrongly attributed as cysticercus lesion (false postives). How many positives were unnoticed by meat inspectors (false negatives), could not be determined in the study. It should be noted that other methods besides routine meat inspection are currently not yet available for a routine diagnostic measure in the slaughterhouses (Dorny *et al.* 2010).

2.2.2. Serology

Infected cattle do not present antibodies against *T. saginata* before 51 days post-infection when using ELISA to detect antibodies from blood serum (Abuseir *et al.* 2007). Abuseir and colleagues also reported that only 91.7 % of the animals with cysts gave positive results with ELISA. It has been found that the sensitivity of Ag-ELISA is close to 100 % when the infected carcasses have 50 or more viable cysts, but only 65% when 1-49 cysts are found (Dorny *et al.* 2010). It was also stated that accurate measurements for specificity and sensitivity of sero-diagnostic techniques is possible only when the carcasses are thoroughly dissected (Dorny *et al.* 2010). It should be noted that Dorny *et al.* (2000) found that the sero-prevalence they found in their study was ten times higher than the annual reported prevalence. Thus the research suggests that the prevalence of bovine cysticercosis is underestimated in EU since it is mostly determined through routine meat inspection (Dorny and Praet 2007).
2.2.3. Molecular methods

Molecular methods use parasitic DNA extracted from the cysts to identify the organism. The DNA is amplified using PCR. This method can also be used in degenerated and calcified cysts since even upon calcification the parasitic material can be isolated (Geysen et al. 2007). Macroscopically calcified cysts can be difficult to identify.

According to McManus (2006) for identifying *Taenia* species via DNA the most commonly used approach is to target the nucleotide sequence of fragments of selected genes using pairs of conserved PCR primers. PCR amplification is used on the segments between primers in the sample and then it is directly sequenced. Sequences can then be compared to already published sequences of different *Taenia* species. The most valuable gene markers for this approach are found to be the mt *cox1, nad1, cob* and 12S rDNA genes; also nuclear 28S rDNA and ITS1/ITS2 rRNA genes have been used.

McManus (2006) also describes a method of multiplex PCR. This method uses primers of species-specific and interspecies-conserved sequences for the simultaneous differential diagnosis of *Taenia* species. With this approach DNA sequencing is not needed. Instead the combination of different primer pairs in the same amplification reaction is used. The aim in this is to create specific PCR products that can be distinguished after using electrophoresis on agarose gel.

In another study it was found that SCAR (sequence characterized amplified region) markers could be potential tools to differentiate *T. saginata* and *T. solium* in epidemiological studies (Dias et al. 2007).

2.2.4. Other methods

Other methods to diagnose bovine cysticercosis are via histopathology and/or morphological identification. Histopathology requires facilities suitable for histology and is quite costly and time consuming. Ogunremi et al. 2004 described immunohistochemical method that helps to differentiate between viable and degenerated *T. saginata* cysts as well as it helps to distinguish them from other cyst-forming infections, non-cysts and normal bovine structures. Morphological identification requires a microscope and specific knowledge of morphology. This also takes more time than routine meat inspection and is not considered to be usable in everyday slaughterhouse practice.
3. AIM, OBJECTIVES AND HYPOTHESIS

3.1. Aim
Aim of the study was to assess the prevalence of *Taenia saginata* cysticercoses in Estonian domestic cattle population.

3.2. Objectives
To assess the prevalence of *T. saginata* cysticercoses by using post-mortem study method in abattoirs. The study consists of a practical study period in selected slaughterhouses and analysing the collected data. Found cysts were recorded and preserved, and later analysed with a PCR method to potentially identify the *Taenia* species using *cox1*-gene.

3.3. Hypothesis
After preliminary research and enquiries it was suspected that it is a small chance that there will be any *Taenia saginata* caused cysts found in the larger slaughterhouses. If however cysts were to be found, there is a chance that they would not be caused by *Taenia*. If so, the cysts could be caused by *Echinococcus* for example and required verification using PCR. Our hypothesis was that the presence of *T. saginata* cysts in cattle slaughtered in Estonian slaughterhouses would to be >0%.
4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Sample size calculation

The study sample size was calculated using online sample size calculator (Sampsize, 2013). There our assumptions were: precision = 1.00%; prevalence = 1.00% and population size = infinite. The precision of the prediction using a 95% confidence interval were set to 0-2.00%. With those numbers in mind the estimated sample size was calculated to be 381 animals.

Using the slaughterhouse categories the sample size was found to be stratified as: 305 cattle from large slaughterhouses (80%), 69 cattle from medium slaughterhouses (18%), and 8 cattle from small slaughterhouses (2%).

4.1.2. Cattle

In Estonia ARIB (Estonian office of agricultural registers and information; Põllumajanduse registrite ja informatsiooni amet, PRIA) keeps a register of all animals and slaughterhouses. There are also other registries (e.g. Statistics Estonia, Statistikaamet) that keep records about animals and other associated information. According to Põder (2014) there were 261,400 cattle in Estonia in the year 2013, including 97,900 dairy cows. In 2013 there were 31,800 cattle slaughtered in registered abattoirs (Voog et al. 2014).

Cattle included in this study (n=564) thus represented 6.5% of all slaughtered cattle between February and April 2014 (n=8,700; Statistikaamet 2015).

4.1.3. Abattoirs

There were 53 registered (licenced) abattoirs in Estonia in 2013. Of those 31 slaughter cattle (58%) (VFB 2013). These abattoirs are scattered all over Estonia.

In this study Estonian slaughterhouses were categorized by dividing the number of slaughtered cattle per slaughterhouse in a year into quartiles: 25% quartile was 0-201 cattle, 50% quartile was 202-385 cattle, 75% quartile was 386-811 cattle. Categories according to these numbers were: 1) large slaughterhouses: >811 cattle/year (80% of all slaughtered cattle), 2) medium slaughterhouses: 201-811 cattle/year (18% of all slaughtered cattle), and 3) small slaughterhouses: 0-201 cattle/year (2% of all slaughtered cattle).

In total four different abattoirs were chosen for the sample collection. Abattoirs were chosen by convenience and accessibility. They are all in different parts of Estonia, in different counties. Abattoirs number one, two and four were categorised as “large”, and abattoir number three was categorised as “medium” sized. Categorization was based on previously described criteria. These abattoirs represent 13% of all the slaughterhouses that slaughter cattle in Estonia.
1. visual inspection of the head and throat; incision and examination of the sub-maxillary, retropharyngeal and parotid lymph nodes (*Lnn retropharyngiales, mandibulares and parotidei*); examination of the external masseters, in which two incisions must be made parallel to the mandible, and the internal masseters (internal pterygoid muscles), which must be incised along one plane. The tongue must be freed to permit a detailed visual inspection of the mouth and the fauces and must itself be visually inspected and palpated. The tonsils must be removed;

2. inspection of the trachea and oesophagus; visual examination and palpation of the lungs; incision and examination of the bronchial and mediastinal lymph nodes (*Lnn. bifucationes, eparteriales and mediastinales*). The trachea and the main branches of the bronchi must be opened lengthways 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;

3. visual inspection of the pericardium and heart, the latter being incised lengthways so as to open the ventricles and cut through the interventricular septum;
   a. If possible (if the slaughterhouse allows), then do six evenly spaced deep incisions made into the myocardium from the endocardial surface.

4. visual inspection of the diaphragm (membranous and crura);

5. visual inspection and palpation of the liver and the hepatic and pancreatic lymph nodes, (*Lnn portales*); incision of the gastric surface of the liver and at the base of the caudate lobe to examine the bile ducts;

6. visual inspection of the gastro-intestinal 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;

7. visual inspection and, if necessary, palpation of the spleen;

8. visual inspection of the kidneys and incision, if necessary, of the kidneys and the renal lymph nodes (*Lnn. renales*);

9. visual inspection of the pleura and the peritoneum;

10. visual inspection of the genital organs (except for the penis, if already discarded);

11. visual inspection and, if necessary, palpation and incision of the udder and its lymph nodes (*Lnn. supramammarii*).
a. In cows, each half of the udder must be opened by a long, deep incision as far as the lactiferous sinuses (*sinus lactiferes*) and the lymph nodes of the udder must be incised, except when the udder is excluded from human consumption.

If there are positive findings during the meat inspection, depending on parasite burden following procedures should be applied. In case of heavy burden the carcasses must be condemned. Carcasses with light burden are frozen to inactivate the cysts before human consumption.

**4.2. Methods of sampling**

Cattle in different slaughterhouses were included in the study to get more accurate oversight of the geographical differences in *Taenia* caused cyst prevalence in Estonia. The practical study was performed in the three largest slaughterhouses in Estonia combined with mandatory slaughterhouse practice in 5th year of veterinary studies. Also a short period was spent in a mid-size abattoir to sample carcasses. No cattle were killed for the purpose of this study. All samples were discarded meat unfit for human consumption collected post-mortem and with the oral consent of the slaughterhouses. Cysts found were preserved in 96% alcohol and placed in freezer (-20 °C) for later species identification with PCR.

In more detailed description of sampling, we concentrated on the previously described predilection sites. In our study the predilection sites chosen were: external masseters, internal pterygoid muscles, tongue, oesophagus, heart and the diaphragm (membranous and crura). Incisions were made in to the heart and masticatory muscles (external masseters and internal pterygoid muscles), other predilection sites were inspected visually and they were also palpated. For every inspected animal the following data was recorded:

1. Date of sampling
2. Slaughterhouse number
3. Sample number
4. Animal ID number (earmark)
5. Examined sites
6. Cysts found
7. Comments – cysts location and looks, possible picture etc.

Depending on the placement of the person sampling in the slaughterhouse the examined sites in animals vary. In most of the samples all except the masticatory muscles were examined.

Criteria for cysts collected for further identification were formed based to the literature and after a meeting with a fellow researcher from University of Tartu, Epp Moks, who has done a lot of practical studies identifying cysts. Criteria for searching for the cysts were the following. Bovine cysts are found primarily in the cardiac and skeletal muscles. The cysts are oval shaped and circa
0.5-1.0 cm long. They consist of a translucent fluid-filled fibrous capsule that contains a larval tapeworm hence the milky colour. Cysts are firm but not hard (compared to Echinococcus cysts that have a hard capsule and irregular shape).

Cysts found were recorded in the protocol as mentioned. For preserving the cyst they were cut out of the carcass without cutting in to it (circa 1 cm margins were left). Cysts were placed into sample tubes and 96% alcohol was added for better preservation. Sample tubes were marked with cyst identification numbers, animal ID numbers, date and the slaughterhouse number. Same identification markers were added to the zipper bag (inside and on the bag) the sample tube was placed. Cysts were placed into the freezer (-20 °C) prior to transport into laboratory.

4.3. Sample analysis
In the laboratory of Estonian University of Life Sciences the cysts were taken out of the freezer and individual cyst was poured into a sterile crucible. With a scalpel and forceps the tissue was cut to pieces. A pestle was used to further grind the tissue. Tissue sample from the cyst was put in a 2 ml centrifuge tube with a safety lock (≤25 mg) and sealed with stretchable tape. The remains in the crucible were added to a 50 ml falcon tube and sealed with stretchable tape.

The cysts were stored at +4°C in the 96% EtOH until analysis. Prior to DNA extraction, 0.2 g of cyst material was dissected and 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 and disposable powder-free gloves were changed between cysts.

To identify, whether the DNA from the cysts belongs to Taenia spp., cox1-gene specific PCR was performed. Primers for the PCR test were designed using Primer3. Conserved regions of the Taenia cox1-gene were identified using sequences from GenBank (Accession numbers FJ518620.1, JN831298.1, JN831307.1, JN831305.1). The primer sequences are as follows:
Forward: 5’-TTG ATC CAT TAG GTG GTG GAG – 3’
Reverse: 5’-TCC AGT AAT TAA AGG TCA CCA TC – 3’

PCR was performed by using 5x FIREPol® Master Mix (MgCl2 concentration 12.5mM) (Solis Biodyne, Tartu, Estonia). Every reaction contained ~200 ng of extracted DNA sample. Conditions for PCR were as follows. Initial denaturation was performed in 94 °C for one minute, it was followed by 30 cycles of denaturation (in 94°C for 30 seconds). Annealing was performed in 55 °C for 30 seconds and extension was carried out in 72°C in 60 seconds. The final extension step was performed in 72°C for ten minutes. Amplicons were expected to be ~550 bp on agarose gel that was stained with EtBr. In the case of positive signals of PCR, the product can be sequenced in order to determine of exact species.
5. RESULTS

All the cattle included in this study were not previously decided; they were included in the study based on their date of slaughter in a current slaughterhouse where sampling took place. No selection between sex, age or production type was performed. In total 564 cattle was examined. For 14 of these animals we do not have complete individual data on them. For others the individual data was analysed using STATA (Statistics/Data Analysis) tool. Most of the included cattle (n=550) were found to be female (n=372, 67.6%), less were males (n=178, 32.3%). The median age of slaughter was 32 months (n=551) with a variation between the sexes; median age for females slaughtered was 52 months (n=373), and for males 19 months (n=178). The youngest slaughtered was one month old, the oldest 181 months – both were females.

Cattle included were from all over Estonia; there were samples (n=550) from every county except Ida-Viru county. Biggest portion of the cattle examined came from Lääne-Viru county (n=103, 18.7%) and the least samples from Võru county (n=4, 0.7%). The breeds represented in this study (n=550) were: Aberdeen angus, Blonde d’Aquitane, Belgian blue, Charolais, Estonian Holstein, Estonian Red, Hereford, Limousin, Piedmontese and Simmental. Most of the cattle were Estonian Holsteins (n=307, 55.8%), the least were Piedmontese (n=2, 0.4%). Dairy cattle was representing 76.7% (n=422) of all cattle included in this study (n=550), beef cattle was representing 23.3% (n=128).

Abattoirs number one, two and four was categorised as “large”, and abattoir number three was categorised as “medium” sized. In the first abattoir 87 cattle was examined which 15% of all examined cattle in the study. In the second abattoir 314 cattle was examined (56% of all examined), in the third seven cattle was examined (1% of all examined) and in the fourth 156 cattle was examined (28% of all examined). Cattle that were included in this study (n=546) represented 6.5% of all slaughtered cattle in Estonia between February and April 2014 (n=8,700).

From previously described predilection sites we were able to study external masseter and internal pterygoid muscles only in three abattoirs out of four, and from 493 animals out of 564 (87.4%). The tongue was examined in all 546 animals, oesophagus in 549 animals (97.3%), and heart and diaphragm in 563 animals (99.8%).

From 564 examined bovine carcasses two Taenia-like cysts were found from two different carcasses; one from a 20-month old bull (hereafter sample CH1) and one from 32-month old cow (hereafter sample CH2). The cyst in a bull was found in the abattoir number one; cyst from the cow was found in abattoir number four. Both cysts were found in the tongue and its connective tissues. Cysts found were recorded in the protocol and preserved as mentioned earlier.
The results of the PCR analysis are presented in Figure 7. We acquired not very clear, but about right sized (~50 bp) bands on the gel. From this we could presume that the cysts may belong to *Taenia spp*.

![Figure 7. PCR results 07.04.2015. On the left is the marker (100 bp ladder; legend on the right side), CH1 and CH2 are cattle samples number one and two.](image)

Results should be considered as preliminary since the subsequent sequencing (services by Macrogen Ltd.) using the given PCR products was not successful. Specific results from Macrogen sequencing studies of the cysts can be found in the appendix IV-V. Assuming that the signals from unoptimized PCR analysis show the cysts to be *Taenia* and possibly *saginata*, we found the apparent prevalence to be very low: 0.36% (95% CL; 0.06-1.17; Mid-P Exact). We estimated that the true prevalence would be >1% (95% CL; 1.40-1.43; Rogan-Gladen CL).
6. DISCUSSION

Despite the known global distribution of *T. saginata* the true prevalence in cattle and in humans is underestimated due to imperfect diagnostics and imperfect reporting systems. We found in our study that *T. saginata* cysticercosis is likely to be present in Estonian cattle and at the same time there are no official reports that would state the same. The apparent level of prevalence that we found is considered to be very low (0.36%), and calculated true prevalence to be >1%. With this we are accepting our hypothesis that was that the presence of *T. saginata* cysts in Estonian cattle slaughtered in slaughterhouses would to be >0%.

When comparing to other similar studies, using EU legislation/routine meat inspection to assess prevalence, conducted in European countries we see that Belgium and Denmark have lower prevalences than Estonia; 0.15% in Belgium (EFSA and ECDC 2014) and 0.06% in Denmark (Calvo-Artavia *et al.* 2013). It should be noted that Switzerland has higher prevalence of 1.8% and even higher when additional incisions are added (Eichenberger *et al.* 2011). Even though Estonia seems to have low to very low prevalence of cysticercosis it could be argued that the prevalence is underestimated due imperfect diagnostics and reporting.

It should be discussed if the current methods of meat inspection (e.g. visual inspection, palpation and limited amount of incisions) are effective enough to detect cysts caused by *T. saginata*. As mentioned earlier, it has been estimated that with current method of meat inspection only 15% of lightly infested animals can be found (Kyvsgaard *et al.* 1990). At the same time slaughterhouses encounter a problem since routine meat inspection is the only routine in-house diagnostic tool that slaughterhouses have; other method such as serological and molecular methods are still under development. Since routine meat inspection is currently the only accessible method for slaughterhouse staff, they should be trained to better identify the cysts. Also the importance of notifying the findings and sending cysts to further identification should be emphasized.

There has been a discussion of the need to implement new regulations for beef inspection. EFSA has presented three different scenarios (strategies) to EU Commission; they should be decided on soon. In strategy 0 nothing changes. Strategy 1 describes general loosening of meat inspection, meaning no more incisions to masseter and pterygoid muscles for example. Strategy 2 covers only visual inspection of low-risk cattle. This would mean that the risk factors, that divide cattle into low- and high-risk cattle, need to be well identified. Before anything can be decided all the strategies need to be evaluated from public health point of view. Also the risk level between different countries in EU should be carefully weighed. It could be speculated that if strategy one or two are implemented, the sensitivity of meat inspection will decline even further. It could also be argued if the risk factors found are specific enough to convincingly divide the cattle into low- and high-risk categories. When there are big differences in the prevalence of cysticercosis between
the member countries it could be argued whether loosening of regulations even in some low-risk countries is the right way to go.

It has been suggested that with current meat inspection methods the prevalence of *Taenias* are underestimated and finding cysts is highly dependent on the skill and motivation of the meat inspector. Also the prevalence is underestimated in cases where animals are lightly infested and/or the cysts are not located at the predilection sites (Dorny and Praet 2007). In this current study we sampled carcasses only in the large and medium sized abattoirs. It remains a question whether the prevalence we found would be the same if the smaller slaughterhouses and animals from organic farms would be included. Danish study from 2013 found that cattle that spent most of their lives in organic herds had higher risk of bovine cysticercosis than cattle in conventional herds (Calvo-Artavia et al. 2013). Since year 2000 there has been a trend to even larger cattle farms in Estonia and they are mainly indoor farms. It could be speculated that since there is now less grazing animals the risk of getting in contact with human faeces is now diminished. At the same time the popularity of organic farming is constantly growing so the risk of bovine cysticercosis is still eminent.

Also it should be discussed if the current methods for species identification are effective enough. For example, in Estonian Veterinary and Food Laboratory (VFL), which is the only certified laboratory in Estonia for analysing samples from slaughter animals, uses only morphology for species identification. They use routinely macroscopic and microscopic measures; histology is performed if needed. When there is little to no cysts to investigate, it is not considered to be cost-effective to keep the PCR up and running (personal communication with VFL, Age Kärsin, 2015). Thus it can be argued if Estonia even has a proper diagnostic measures in place that can reliably identify the cysts to be *T. saginata*.

In humans asymptomatic disease character complicates finding the prevalence in humans even further. The last recorded (and at the same time unreported) case of human *T. saginata* taeniosis in Estonia was in 2012, the last before that was in 1999. Now the question is, are there really so few cases or are there problems in diagnosing or reporting the found cases? As seen in the Figure 6. human cases have been declining for decades. The same cannot be said about the bovine cases; fewer reports start only from 1980’s. This leads to speculation why this is – is it because of better managing practice on the farm level or is it because of poor reporting? Also, are the control measures in Estonia good enough to be able to identify cysts to be *T. saginata* so they can be reported? For the declining human cases we can speculate that nowadays people do not eat so much undercooked beef, or as said earlier, the disease can be underdiagnosed.

When it comes to limitations of our study it should be noted that the sample size studied exceeded our initial expectations. At the same time sampling design was not ideal since the smaller
abattoirs were not included in the study due to lack of time and other resources. Despite the fact that we were unable to cover all sizes of abattoirs, the number of samples in total was high and with this we almost doubled the planned sample size. Smaller abattoirs may slaughter just one cattle a day so it was evaluated to be unfeasible to sample a representative sample within the projects time frame. Also, mid-sized and smaller abattoirs were harder to get in contact with. Problematic was the fact that not all predilection sites could be examined in all the animals due to different layouts of the slaughterhouses (logistics). Also, using many people in cyst detection in different slaughterhouses can be a problem since finding and recognizing cysts is very subjective. That is apparent even with sample guidelines how to detect cysts due to human error that can be minimized but not removed completely. For data analysis we did not have complete background information for all the examined cattle. For some reason not all of the examined animals were listed in the registers of Estonian Livestock Performance Records Ltd or the records could not be found.

The subsequent sequencing (Macrogen Ltd.) using the PCR products of choice was not successful. This shortage could be ascribed to the time-pressure that did not allow us to properly optimize the PCR conditions in our laboratory. There was no working Taenia PCR test introduced in our lab before and the testing just started when the cysts arrived. Not all the possible solutions to address the unclear signal of the PCR product have been tested yet. The chances were quite low that sequencing of the available PCR products at the time would be successful.

For future similar studies we would suggest even larger sample size and samples from abattoirs of all sizes. With this it would be possible to have more information about how the abattoir size, organic herds etc. affect the prevalence. It should be considered to also include serological methods since it has higher sensitivity than routine meat inspection. The blood sample collection for serology could be collected in the slaughterhouse anti-mortem, and then the bovine carcasses could be examined using routine meat inspection also. This combination of study methods would give a better picture of the actual prevalence of cysticercosis in Estonia and in the same study these two methods could be compared.

It cannot be stressed enough that in our globalizing world cysticercosis is not just a local problem. Beef and live animals are imported and exported every day to various countries all over the world – it is unclear how we can be sure about the safety of beef products if the diagnostics and reporting systems are imperfect. Also, more cooperation between medical doctors and veterinarians would be needed, e.g. in form of educational seminars about zoonotic parasites. That would insure that if traditional meat inspection fails to recognize all the infected carcasses and human cases do occur, the medical doctors would recognize these cases and the cases would be reported on national level. This would also be in the concept the One Health initiative – their goal is to improve human and animal lives through the integration of veterinary and human medicine.
and environmental sciences. Still, educating medical doctors about the hazards of zoonotic parasites is not enough. General public should also be educated about the potential risks of eating raw or undercooked beef. This is more than just a problem of lack of knowledge since many people consider undercooked meat to be a delicacy (e.g. Italian bresaola and carpaccio, French steak tartar).

It can be argued if the presence or absence of *Taenia saginata* represents the real situation since many countries, including Estonia, do not have effective control methods for it. Also, to our knowledge, before this study there were no previous prevalence studies conducted in this field in Estonia that could be found. Prevalence studies from different countries can be hard to find and in many cases they do not even exist. As Carl Sagan has said, we must remember that “absence of evidence is not evidence of absence”.
7. CONCLUSION

Sampling 564 cattle in four abattoirs in Estonia in total two cysts were found. PCR identification of cox-1 gene specific regions showed possibly both of the cysts belong to *Taenia*. Sequencing of the PCR product could reveal the exact species and strain. In the case of cattle, most probably *T. saginata*. Hence, there is fair evidence of the presence of *T. saginata* in large sized abattoirs in Estonia. The apparent prevalence of bovine cysticercosis found in this study was 0.36% (95% CL; 0.06-1.17) and true prevalence was >1% (95% CL; 1.40-1.43). The few cysts detected were possibly due to low sensitivity of the inspection method.
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APPENDICES

Appendix I
The abstract of prevalence study of cysticercoses in Estonian pigs and cattle. This 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
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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

The abstract about the prevalence study of cysticercosis in Estonian pigs and cattle. It was presented as a poster in 6th conference of The Scandinavian-Baltic Society of Parasitology, in Uppsala Sweden, 23.-24.04.2015.

PREVALENCE STUDY OF CYSTICERCOSIS IN ESTONIAN PIGS AND CATTLE

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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

The poster about the prevalence study of cysticercosis in Estonian pigs and cattle. It was presented in the 6th conference of The Scandinavian-Baltic Society of Parasitology, in Uppsala Sweden, 23.-24.04.2015.
Appendix IV

Raw sequence data processed by Macrogen, using the primers described in Material and Methods for cattle sample nr. 1, forward (-F) and reverse (-R).
Appendix V

Raw sequence data processed by Macrogen, using the primers described in Material and Methods for cattle sample nr. 1, forward (-F) and reverse (-R).