Trichinella spp. in Eurasian beaver *(Castor fiber)* in Sweden and Norway – a pilot study

Trikiner hos eurasisk bäver (Castor fiber) i Sverige och Norge – en pilotstudie

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Swedish University of Agricultural Sciences, SLU
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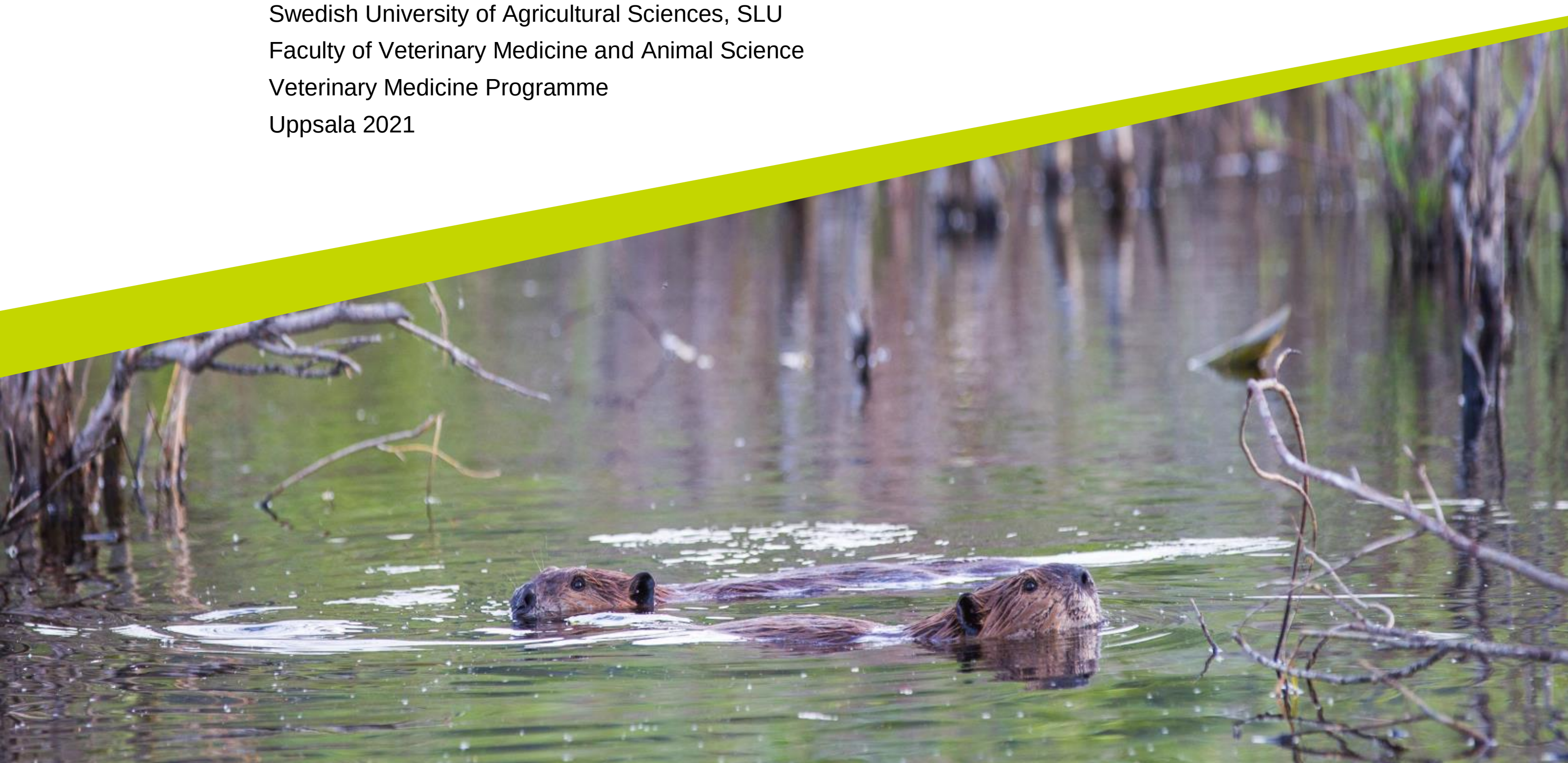

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Abstract

Trichinellosis is a zoonotic disease that can lead to severe illness and even death in humans. Transmission of larvae occurs with consumption of infected meat. The major sources of infection are pork and wildlife such as wild boar (*Sus scrofa*) and carnivores. Horses, considered herbivores, have however been responsible for several human outbreaks throughout history. Other herbivores have also been found to be infected by *Trichinella*.

The Eurasian beaver (*Castor fiber*) is common in Sweden with numbers reaching 130,000 and an estimated 6,000-9,000 or more are hunted annually. Beavers are strict herbivores, but despite that older findings combined with recent studies from Poland and Latvia show that beavers can become infected with *Trichinella*. One case of human trichinellosis has been reported with beaver meat being the probable source of infection.

The purpose of this study was to investigate the presence of *Trichinella* spp. in beavers from Sweden and Norway. A review of current scientific literature revealed no information on similar studies in any of the Nordic countries. In Sweden, beavers are not routinely tested for *Trichinella*. This study may therefore play a role in establishing the potential need for recommendations regarding beaver meat handling in the future, as well as increase the knowledge of beavers and diseases they may carry. It also raises the question of how infection in beavers occur, since they are considered strict herbivores.

Materials used in this study was beaver meat sent in from hunters throughout Sweden. Hunters participated voluntarily. Front legs including shoulder muscles were the asked for samples, most samples received fulfilled these conditions. A few were missing shoulder muscles, and solitary samples consisted of diaphragm muscle tissue. At the end stages of this study, seven samples collected in Norway were received as part of a scientific collaboration and subsequently added to the results from the Swedish samples.

The method used in this study was artificial digestion with the magnetic stirring method (MSM), according to the EU guidelines.

In total, 58 samples were tested, of which the majority (51) were from different parts of Sweden and some (7) from Norway. No *Trichinella* spp. was detected.

The method used in this study is considered adequate for detecting *Trichinella* spp. Despite not finding any *Trichinella*, possible infection amongst beavers in Sweden and Norway cannot be ruled out. More studies and a larger sample size are needed. For future studies, focusing sampling to areas where *Trichinella* has been found in other species and systematically collaborating across the Swedish-Norwegian border might give a more thorough insight into the *Trichinella*-situation amongst beavers and other herbivores.

Keywords: Trichinosis, trichiniasis, parasite, zoonotic, herbivore, magnetic stirrer method, artificial digestion

"To give the Beaver his due, he does things because he has to do them, not because he believes that hard work per se will somehow make him a better Beaver -- the Beaver may be dumb, but he is not that dumb! The Beaver was made to gnaw, and gnaw he does. There you have him in a nutshell." ~ Will Cuppy

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Abbreviations

CDC	Centers of Disease Control and Prevention, Atlanta, USA
CH	Switzerland
CIA	Competitive Inhibition Assay
ECDC	European Centers of Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority, Parma, Italy
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
ICT	International Commission on Trichinella
i.e.	*Id est*, “that is”
iELISA	Indirect ELISA
IETB	Immuno-Electro Transfer Blotting/Blot Assay (Western Blot)
MSM	Magnetic stirrer method
NRL	National Reference Laboratory
OIE	World Organization of Animal Health
SLU	Swedish University of Agricultural Sciences, Uppsala, Sweden
SVA	National Veterinary Institute, Uppsala, Sweden

1. Introduction

Trichinellosis is a zoonotic disease that can lead to severe illness and even death in humans (Touratier 2001; Murrell & Pozio 2011; European Food Safety Authority [EFSA] *et al.* 2019). Transmission of larvae occurs with consumption of infected meat. The major sources of infection are pork and wildlife such as wild boar (*Sus* scrofa) and carnivores (Dupuoy-Camet *et al.* 2007; Wang *et al.* 2007; Murrell & Pozio 2011; European Food Safety Authority [EFSA] *et al.* 2019), but horses, a herbivore, are responsible for large human outbreaks throughout history. Between 1977 and 1989, at least 3200 humans in France and Italy were infected with *Trichinella* larvae from horse meat (Boireau *et al.* 2000; Touratier 2001).

Other herbivores such as cattle, goats, deer, moose (*Alces alces*), camels (*Camelus dromedarus*) and hares (*Lepus* spp.) have also been found to be infected by *Trichinella* (Rausch *et al.* 1956; Holliman & Meade 1980; Darwin Murrell 1994; Kapel 2000; Hall *et al.* 2009; McLaughlin & Castrodale 2015; Wilson *et al.* 2015), although it often concluded that these findings are of rather small significance for human health since such cases are relatively rare (Pozio 2001).

Implementation of stricter rules regarding housing of pork and testing of meat meant for consumption has resulted in decreased numbers of *Trichinella* findings in Europe, although wildlife remains a common source of infection (European Food Safety Authority [EFSA] *et al.* 2019).

The Eurasian beaver (*Castor fiber*) is a common animal in Sweden with numbers reaching 130,000 (Svenska Jägareförbundet 2020) and an estimated 6,000-9,000 or more are hunted annually (Viltdata n.d.). They were hunted to extinction in the late 19th century for their fur, castoreum and meat, but reintroduction to Sweden from Norway in the early 20th century was successful (Wilsson 1971; Müller-Schwarze 1992; Hartman 1994, 1996). Beavers are strict herbivores with a preference for leafy tree-like plants like willow (*Salix* spp.), aspen (*Populus tremula*), birch (*Betula* spp.), various herbs and aquatic plants (Wilsson 1971; Hartman & Gorén 1993; DziČeciołowski & Misiukiewicz 2002; Baskin *et al.* 2011; Sjöberg & Ball 2011; Graf *et al.* 2018; Jackowiak *et al.* 2020). Despite being herbivores, older findings from several countries and recent studies from Poland and Latvia shows that beavers can become infected with *Trichinella* (Rausch *et al.* 1956; Samuel *et* *al.* 2000; Segliņa *et al.* 2015; Różycki *et al.* 2020). As of today, one case of human trichinellosis has been reported where beaver meat was believed to be the source of infection (Bronstein & Lukashev 2019).

The purpose of this study was to investigate the occurrence of *Trichinella* spp. in beavers from Sweden and Norway. A review of current scientific literature revealed no information on similar studies in any of the Nordic countries.

In Sweden, many animal species are routinely tested for *Trichinella* (National Veterinary Institute [SVA] 2019); beavers, however, are not one of them. This study may therefore play a role in establishing the potential need for recommend-dations regarding beaver meat handling in the future, as well as increase the know-ledge of beavers and diseases they may carry. It also raises the question of *how* infection in beavers occur, since they are considered strict herbivores.

2. Literature review

2.1. The Eurasian beaver *(Castor fiber)*

2.1.1. Beavers in the world

Two species of beaver exists in the world: the Eurasian beaver (*Castor fiber*) and the Canadian/American/North American beaver (*Castor canadensis*) (Wilsson 1971; Muller-Schwarze 2011). Both were hunted until near extinction during the 19^{th} and 20^{th} century (Müller-Schwarze 1992; Muller-Schwarze 2011; Halley *et al.* 2012). Today, the Eurasian beaver is spread throughout Northern and Central mainland Europe and parts of Asia (Halley *et al.* 2012), while the Canadian beaver is widespread throughout North America (Muller-Schwarze 2011). The latter has also been introduced in other non-native areas, including Argentina where it has thrived (Lizarralde 1993) and Finland where it is considered an invasive species and a competitor to the Eurasian beaver (Parker *et al.* 2012; Halley *et al.* 2020). In Russia, however, the opposite occurred: *C. fiber* took over an area previously claimed by a population of introduced *C. canadensis* (Parker *et al.* 2012).

2.1.2. Facts about the Eurasian beaver

The Eurasian beaver (*Castor fiber*) is the largest rodent in Sweden, reaching adult weights of 18-30 kg and body lengths of 70-100 cm plus tail (30-40 cm) (Hartman & Gorén 1993). They are nocturnal, semi-aquatic and highly territorial (Wilsson 1971; Graf *et al.* 2018). Life expectancy in beavers is 20-30 years in the wild and 30-50 years in captivity (Naturhistoriska Riksmuseet n.d.).

Beavers are herbivores and eat a wide range of plants including herbs, aquatic and leafy tree-like plants, that they also store underwater in food caches to be used during winter season (Hartman & Gorén 1993; DziČeciołowski & Misiukiewicz 2002; Baskin *et al.* 2011; Graf *et al.* 2018; Jackowiak *et al.* 2020). They are considered central-place foragers, meaning that they focus their foraging close to home, and studies show that most beavers forage no further than 60 meters from the water where they live, usually as little as 20-40 meters (Jackowiak *et al.* 2020).

They build dams with trees felled, thus altering the ecosystem in many aspects (Parker *et al.* 1999; Ecke *et al.* 2017; Graf *et al.* 2018) and during cold winters may be forced spend as long as six months in their huts/lodges due to thick ice, surviving off their underwater food caches (Wilsson 1971).

Mating season is in January and kits are born in May – June (Wilsson 1971; Muller-Schwarze 2011). Normally, one to four kits are born and stay with their parents for 11-23 months, thus creating large families of beavers (Hartman 1997; Muller-Schwarze 2011; Korbelová *et al.* 2016). Yearlings help raise the new youngsters before leaving in search for new territory, usually near their parents' (Muller-Schwarze 2011; Korbelová *et al.* 2016).

2.1.3. Beavers in Sweden

Both species of beaver (*Castor fiber* and *Castor canadensis*) were hunted mainly for its fur and castoreum until near or definite extinction in many countries (Wilsson 1971; Walro & Svendsen 1982; Hartman 1996; Muller-Schwarze 2011). In Sweden, the Eurasian beaver was hunted until extinction in the late 19th century; similar things happened in other countries, and to *C. canadensis* in North America (Wilsson 1971; Hartman 1994, 2011; Muller-Schwarze 2011; Halley *et al.* 2012).

Castoreum is the yellow exudate found in the paired castor sacs located between the pelvis and the base of the tail, and can be found next to the anal glands in both sexes (Walro & Svendsen 1982). It is believed to be used by the beavers to scent mark territory in combination with urine and anal gland secretion (Walro & Svendsen 1982), and was highly sought after for perfumes because of its strong smell and for medical purposes due to its content of salicylic acid (Müller-Schwarze 1992).

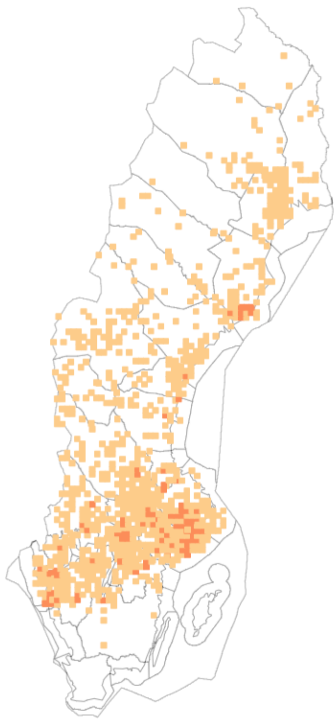

Figure 1. Map of observed beavers in Sweden. SLU Artdatabanken October 7, 2020.

In 1922, a few pairs of mating Eurasian beavers from Norway were introduced in Sweden (Hartman 1994, 1996). Today, the beavers are spread throughout almost the entire country (Figure 1), barring the furthermost north and south parts (Halley *et al.* 2020). In 2005, the population was estimated to 130,000 individuals (Svenska Jägareförbundet 2020).

Since 2005, hunting is allowed between October 1st and May 1st (southern parts of Sweden) or 15th (northern parts of Sweden) (Svenska Jägareförbundet n.d.; Hartman 2011). Roughly 6,000-9,000 beavers are killed during hunting annually, with numbers reaching 12,000 some years (Viltdata n.d.).

2.1.4. Beavers in Norway

In Norway, Eurasian beavers are considered common, and a popular game animal, just like in Sweden. The population is estimated at around 70,000 animals and growing (Parker & Rosell 2012) and approximately 2,000 animals are hunted annually (Statistisk sentralbyrå n.d.). Conditions are similar for beavers in Sweden and Norway, as there are both climatic and habitat similarities.

2.2. *Trichinella* spp.

Trichinella is a nematode that can infect a wide range of hosts, including mammals, birds and reptiles (Pozio 2005, 2020; Gottstein *et al.* 2009). It was considered a single species (*T. spiralis*) for more than 50 years after the initial discovery of the parasite, until the early 1970's when *T. nativa* and *T. nelsonii* were first described as separate species (Britov & Boev 1972). As of today, 13 species of *Trichinella* have been described: the newest species was found in Canadian wolverines (*Gulo gulo*) last year (2019) and is known as T13 with the proposed name *T. chanchalensis* (Sharma *et al.* 2020). The parasite can be found on all continents apart from Antarctica and is divided in two separate groups: encapsulated in host muscle tissue (most species) and non-encapsulated (Pozio 2005, 2020; Gottstein *et al.* 2009). Table 1 shows main features of *Trichinella* species and genotypes, barring *T13*, and comes from Pozio & Murrell (2006) with added data from Gottstein *et al.* (2009).

Table 1. Main features of Trichinella species and genotypes recognized, T13 not included. Data from Pozio & Murrell (2006) and Gottstein et al. (2009).

Clade Species or genotype	Geographical distribution	Host range	Main source of infection for humans	Resistance of larvae in frozen muscles
Encapsulated				
T. spiralis	Cosmopolitan	Domestic and sylvatic mammals	Domestic and sylvatic swine horse	No
T. nativa	Arctic and subarctic areas of the Nearctic and Palearctic regions	Sylvatic carnivores	Bear, walrus	Yes, in carnivore muscles
Trichinella T6	Canada; Alaska, Rocky Mountains and Appalachian in the USA	Sylvatic carnivores	Carnivores	Yes, in carnivore muscles
T. britovi	Temperate areas of the Palearctic region, Northern and Western Africa	Sylvatic mammals, seldom domestic pigs	Wild boar, domestic pig	Yes, in carnivore muscles
Trichinella T8	South Africa and Namibia	sylvatic carnivores	Non-documented	No
T. murrelli	USA and Southern Canada	Sylvatic carnivores	Bear, horse	No
Trichinella T9	Japan	Sylvatic carnivores	Non-documented	No
T. nelsoni	Eastern-Southern Africa	Sylvatic mammals	Warthog, bush pig	No
Trichinella T12	Argentina	Cougars	None documented	Unknown
Non-encapsulated				
T. pseudospiralis	Cosmopolitan	Sylvatic mammals and birds, domestic pigs	Domestic and wild pigs	No
T. papuae	Papua New Guinea	Wild pig, saltwater crocodile	Wild pig	No
T. zimbabwensis	Zimbabwe, Mozambique, Ethiopia	Nile crocodile, monitor lizard	Non-documented	No

2.2.1. Life cycle

Trichinella larvae are infectious in their first stage (L1), whence they can be found in striated muscle cells of many hosts (Dupuoy-Camet *et al.* 2007). When infected meat is ingested by a new host, larvae are released by the digestive process, as demonstrated in Figure 2. L1 larvae enter the small intestine and burrow into the villi in the small intestine (*jejunum, duodenum*). In the *lamina propria* of the villi, the larvae go through four molts before they emerge as adults. Adult female and male worm mate, thus resulting in newborn L1 larvae. While male nematodes die after fecundating the female, females can continue to produce larvae for several weeks before being expelled; an adult female can produce 1,500 L1 larvae during her lifespan, depending on *Trichinella* species and host (Dupuoy-Camet *et al.* 2007; International Commission on Trichinellosis 2020).

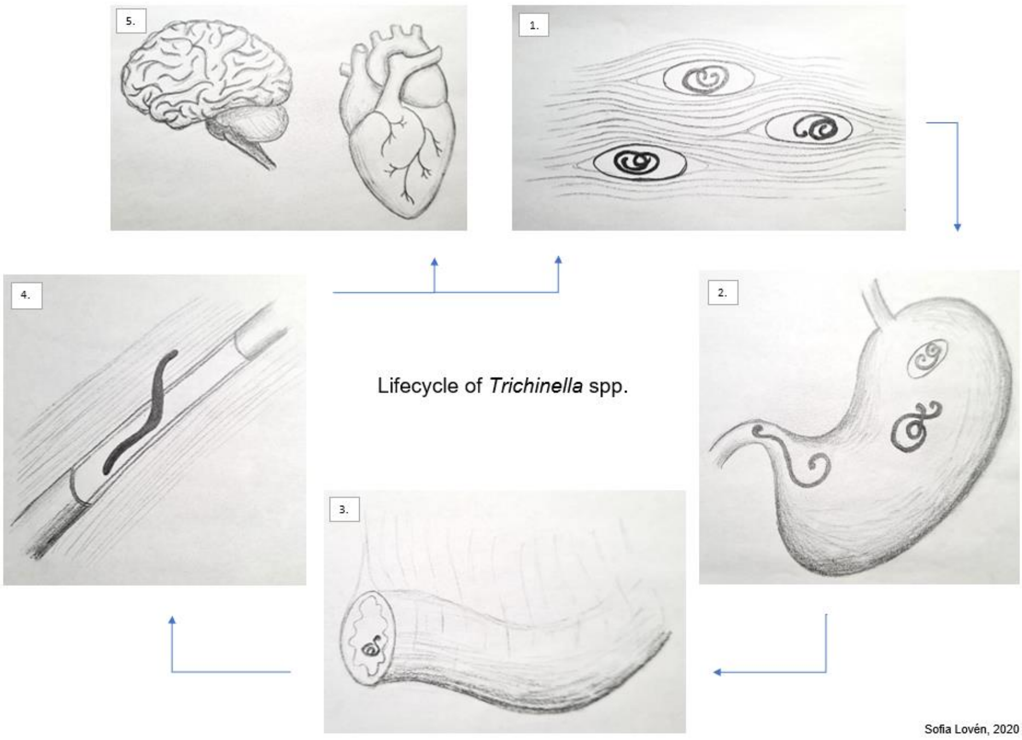

Figure 2. Lifecycle of Trichinella spp. *(1) Cysts with encapsulated larvae in muscle tissue. (2) When infected muscle tissue is ingested, larvae is set free by digestion process. (3) Larvae molts to adults and reproduce in small intestines. (4) Newborn larvae migrate via bloodstream searching for muscle tissue where they create cysts (1). (5) Sometimes, larvae reach other organs, causing disease before they are either destroyed or re-enter the bloodstream.*

Newborn L1 larvae migrate via the bloodstream to striated muscle tissue (Dupuoy-Camet *et al.* 2007). During migration, larvae can travel through many other tissues including myocardium and brain. If reaching non-muscle tissue, larvae are either destroyed or re-enter the circulatory system. Upon reaching striated muscle tissue, the larvae penetrate the sarcolemma of the fibre, where they mature. L1 larvae of some species become encapsulated due to the host's cellular response, and some coil within the muscle fibres, resulting in a characteristic appearance. The host-parasite-complex is known as a nurse cell, and thanks to an increase in vascular supply allowing nutrients and oxygen to reach the encapsulated larvae, the larvae can survive for months or even years. No further increase in number of worms can occur after adults have been expelled by the host and larvae are encysted in muscle tissue. Eventually, the cysts become calcified and the larvae dies (Dupuoy-Camet *et al.* 2007; International Commission on Trichinellosis 2020).

2.2.2. Disease and zoonotic aspect

Infection of humans with *Trichinella* nematodes is fairly uncommon (European Food Safety Authority [EFSA] *et al.* 2019). However, it is a serious disease that can lead to severe illness and death: 65,818 human cases of and 42 deaths by trichinellosis were reported from 41 countries throughout the world from 1986 through 2009 according to Murrell & Pozio (2011). The majority (87%) of cases were found in Europe, and half of these occurred in Romania. In general, men and women were equally infected, and median age of the cases was 33.1 years. The study comprised of 5,377 cases which were clinically examined and characterised. Major symptoms were defined as fever, diarrhoea, myalgia, facial edema and headache. The major source of infection turned out to be pork, but wild game as a source was also common (Murrell & Pozio 2011).

According to the European One Health 2018 Zoonoses Report (European Food Safety Authority [EFSA] *et al.* 2019), 85 suspected human cases of food-borne trichinellosis were reported in 2018 and 66 of them were confirmed. Sources of infection varied with pig meat being the main source (Figure 3). Bulgaria had the highest reported notification rate (0.64 cases per 100,000 population), Romania was second (0.05 cases per 100,000 population). In 2018, 10 food-borne outbreaks (FBO) were reported. Most FBOs are caused by pig meat infected with *Trichinella spiralis*, but one outbreak in Romania was reported to be caused by *Trichinella britovi* in wild boar meat (European Food Safety Authority [EFSA] *et al.* 2019). As shown by Figure 4, the number of cases of trichinellosis tend to vary throughout the year.

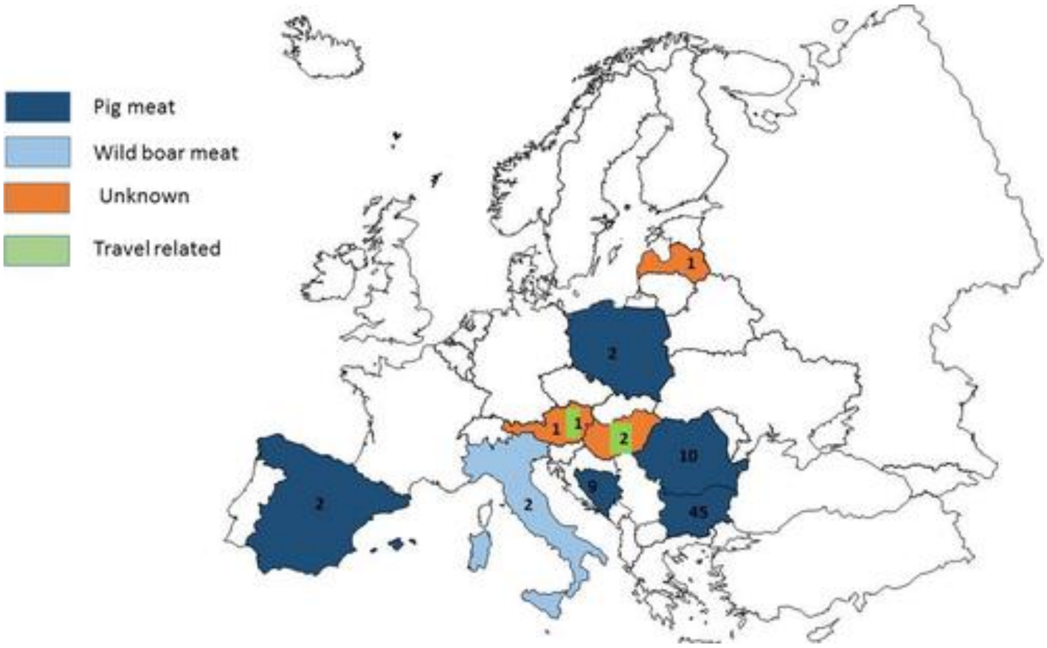

Figure 3. Total human cases in EU, EEA and CH (ECDC data) and in Bosnia and Herzegovina 2018 (European Food Safety Authority [EFSA] et al. 2019)

According to Commission Regulation (EU) No. 1375/2015 all animals susceptible for *Trichinella* infection that are intended for human consumption should be tested for presence of larvae in muscle tissue (European Food Safety Authority [EFSA] *et al.* 2019). Animals included are domestic pigs, farmed wild boar and equids (solipeds). Member states are also recommended to monitor *Trichinella* in main natural reservoir hosts (carnivores and omnivores), although no mandatory reporting requirements exist.

In most member states of the European Union (EU), notification of *Trichinella* infections in humans is mandatory (European Food Safety Authority [EFSA] *et al.* 2019). A few countries have voluntary surveillance systems, and one (Denmark) lacks a notification system. Diagnosis in humans is mainly based on clinical signs and symptoms, and serology (indirect enzyme-linked immunosorbent assay, iELISA) and western blot, whereas histopathology of muscle biopsies is rarely performed (European Food Safety Authority [EFSA] *et al.* 2019).

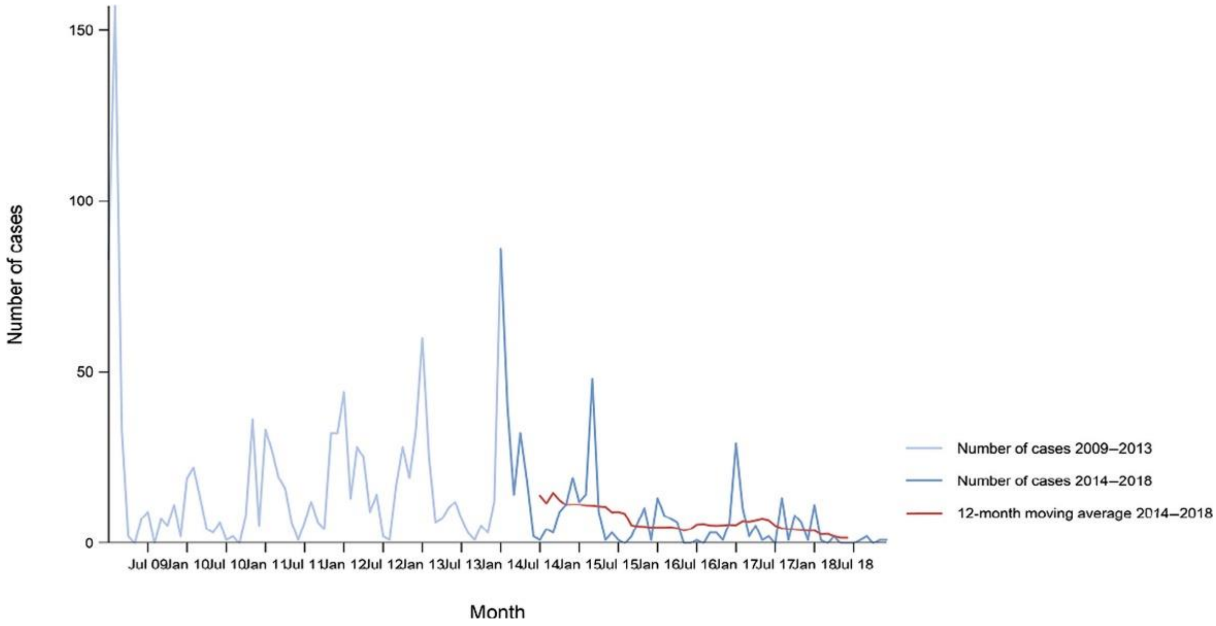

Figure 4. Trend in reported confirmed human cases of trichinellosis in the EU/EEA by month, 2009–2018. (European Food Safety Authority [EFSA] et al. 2019)

Pork is generally considered a major source of infection (Murrell & Pozio 2011; European Food Safety Authority [EFSA] *et al.* 2019); for example, in China, pork was the cause for 522 out of 548 human cases of trichinellosis between 1964 and 1999 (Wang *et al.* 2007). However, data from EFSA's 2018-report shows that farming conditions of pigs is a key factor to prevent infection (European Food Safety Authority [EFSA] *et al.* 2019). No *Trichinella* infections were reported in tested fattening pigs (76.6 million) and breeding pigs (0.5 million) kept in controlled housing conditions. In contrast, 0.0002% of fattening pigs and 0.0003% of breeding pigs not kept in controlled housing were found positive for *Trichinella*. Earlier reports from 2014-2018 also show that free-range and backyard pigs in rural parts of EU regions were the main source of *Trichinella* infections (European Food Safety Authority [EFSA] *et al.* 2019).

Wild boar is another commonly infected animal, and EFSA reported a prevalence of *Trichinella*-infections of 0.09% during 2018 (European Food Safety Authority [EFSA] *et al.* 2019). The overall proportion of infected wild boars fluctuated from one year to another, never exceeding 0.13% (European Food Safety Authority [EFSA] *et al.* 2019).

Red foxes are considered indicator animals for *Trichinella* infection, although not tested to the same extent as wild boar, and in 2018 the amount of foxes tested positive was 1.6 % (out of 6612) according to EFSA (European Food Safety Authority [EFSA] *et al.* 2019). Earlier data shows that the prevalence fluctuates between years but has not exceeded 1.6 % as of 2018 (European Food Safety Authority [EFSA] *et al.* 2019).

2.2.3. *Trichinella* in Sweden

Trichinellosis in Sweden

According to the Communicable Disease Act (SFS 2004:168 with the amendments of SFS 2013:634), trichinellosis in humans is notifiable and mandatory in Sweden.

Human infection with *Trichinella* (trichinellosis) is uncommon in Sweden (Folkhälsomyndigheten n.d.). During the last 15 years, 47 suspected cases have been reported: seven of them were confirmed but only one patient had presumably been infected in Sweden, the other six were most likely infected abroad. Despite a clear clinical presentation and compatible history, the etiology of the most recent Swedish case from 2013 was never confirmed as the European Union Reference Laboratory for Parasites could not detect the parasite or traces of it, and no more wild boar meat was available for confirmatory testing (Folkhälsomyndigheten n.d.). Before this, no human cases of native infection had been confirmed since 1978 in Sweden. Between 1919 and 1969, 10 outbreaks of trichinosis involving 504 humans was reported, and between 1970 and 1978 only sporadic cases were seen (Pozio *et al.* 2004).

Trichinella in meat in Sweden

According to SJVFS 2013:23, *Trichinella* is notifiable in animals. Official controls of *Trichinella* in meat is regulated by Commission Implementing Regulation EU 2015/1375 of 10 August 2015.

Routine controls for *Trichinella* is performed on many species in Sweden (National Veterinary Institute [SVA] 2019). For domestic pigs kept in holdings officially recognized to apply controlled housing conditions (EU 2015/2013), Sweden applies reduced testing since 2014. Breeding sows and boars are examined for *Trichinella*, and recommendations are that all pigs from production sites without controlled housing should be tested. Fattening pigs from controlled holdings are exempted from testing. All horses meant for human consumption, as well as all wild boars and brown bears sent to game handling establishments for slaughter, are tested for *Trichinella* in Sweden. Most hunters choose to test wild boars and brown bears meant for private consumption as well.

In 2019, 22,928 breeding sows, 269 boars and 1,459,867 fattening pigs from controlled holdings, 503,367 slaughtered pigs (all categories) from uncontrolled holdings, and 1,749 slaughtered horses were tested - *Trichinella* was not detected in any horse or pig (National Veterinary Institute [SVA] 2019). Between 1970 and

1999, 127 *Trichinella* positive pigs were reported in Sweden; 52 cases in 1970-79, 67 cases in 1980-89, four cases in the early 1990's, and no positive pigs have been reported since 1994 (Pozio *et al.* 2004; Airas *et al.* 2010; National Veterinary Institute [SVA] 2019).

Trichinella in Swedish wildlife

The Swedish national disease surveillance program includes, beside domestic pigs, species such as wild boar, large carnivores (brown bear, grey wolf, lynx, wolverine), some raptors, and medium-sized carnivores (red fox, mustelids) (National Veterinary Institute [SVA] 2019). Wildlife is tested for surveillance purposes and to keep track of environmental infection (National Veterinary Institute [SVA] 2019).

Species of *Trichinella* found in Swedish wildlife are *T. britovi, T. spiralis, T. nativa*, and *T. pseudospiralis* (Pozio *et al.* 2004). The most commonly detected *Trichinella* species is *T. nativa* (37 instances) followed by *T. pseudospiralis* (19 instances) according to Table 2. Table 2 is based on data from SVA's national disease surveillance program (2014-2019) and personal communication with staff at SVA[1].

Table 2. Findings of Trichinella spp. in Swedish wild animals 2014-2019. SVA, National Veterinary Institute (SVA) (2014, 2015, 2016, 2017, 2018, 2019)

Animal species	**No. samples**	**No. Positives***	**%**	***T. britovi***	***T. nativa***	***T. pseudo-spiralis***	***T. spiralis.***
Brown bear	1325	3	0.2%	-	3	-	.
Lynx	437	27	6.2%	5	21**	-	1
Raccoon dog	41	2	4.9%	-	2	-	-
Red fox	122	1	0.8%	1	-	-	-
Wild boars	607 334	30	0.005%	9	-	19	-
Wolves	197	11	5.6%	3	8**	-	
Wolverine	27	3	4.9%	-	3	-	-
Total	**-**	**609 483**	**-**	**18**	**37**	**19**	**1**

*** In two cases, two wild boars, the species of the larvae could not be determined**
**** In two cases, one lynx and one wolf, infection with both *T. britovi* and *T. nativa* was found**

In 2019, *Trichinella* was found in five (out of 138,374) wild boars, six lynxes and two grey wolves (National Veterinary Institute [SVA] 2019). A total of 33 beavers,

[1] Email conversation with Eva Osterman-Lind and Anna Lundén, SVA, 2020-11-20

mostly sent in from a game handling establishment and the rest from hunters[2,3], were also tested, with no *Trichinella* larvae found.

2.2.4. *Trichinella* in Norway

In Norway, a domestic case of human infection has not been detected since 1980 (Folkehelseinstituttet 2019). Two human cases were reported in 1996 and were found to have been infected in the Balkans. As in Sweden, trichinellosis is a notifiable disease, according to Norwegian legislation (Folkehelseinstituttet 2019), and domestic pigs, horses, and wild boar are regularly tested. The last reported finding in domestic pigs was in 1994 (Hofshafen *et al.* 2011). The parasite is more common in carnivores such as the red fox, and Davidson *et al.* (2006) found a prevalence of 4.8% (n=19) in 393 tested animals in 1994-95 and 2002-2005).

2.2.5. *Trichinella* in beavers

Segliņa *et al.* (2015) conducted a study in Latvia in 2015 and found *Trichinella britovi* in one beaver. The study was the first conducted on beavers in Europe and shows the need for testing beaver meat before consumption. Samples (at least 25 grams muscle tissue per beaver) was collected from different parts of the country between 2010 and 2014, and gender and age were recorded. Samples were taken from diaphragm, tongue, or foreleg (or all three muscles). Muscle tissue were stored at -20°C until analysis. Analysis was conducted separately for each animal. The employed method was Magnetic stirrer method (MSM) for artificial digestion (according to European Communities Commission Regulation (EC) No. 2075/ 2005). A total of 182 beavers were analyzed and five *Trichinella* larvae were found in one animal (foreleg muscle). Thus, the prevalence was 0.5%, and subsequent species identification through PCR analysis confirmed that the beaver was infected by *Trichinella britovi*. The source of infection is hitherto unknown (Segliņa *et al.* 2015).

In 2019 a study was conducted in Poland (Różycki *et al.* 2020) where 69 beavers were tested and one infected individual was found. 50-100 g of muscle tissue were sampled from each animals' left rear leg and digested separately according to the EU reference method (MSM). *Trichinella* larvae in the digested material were found through examination with a trichinoscope, and subsequent analysis of larval DNA by PCR confirmed the presence of *Trichinella spiralis* (Różycki *et al.* 2020).

A study in the North American Arctic found one out of 29 beavers *(Castor canadensis)* infected with *Trichinella* spp. using artificial digestion of tissue from

[2] Email conversation with JP Hästveda, 2020-12-04
[3] Email conversation with Vidilab, 2020-12-14

diaphragm (Rausch *et al.* 1956). At that time, no molecular analysis was available and *Trichinella spiralis* was the only known species. *Trichinella* spp. has also been found in *Castor canadensis* in Alaska and Iowa, USA (Samuel *et al.* 2000).

In 2019, 33 beavers were tested for *Trichinella* in Sweden; none were infected (National Veterinary Institute [SVA] 2019). Most were submitted for testing by a game handling establishment[4] and three by civilians[5].

As of today only one possible case of human trichinellosis associated with beaver meat consumption has been published (Bronstein & Lukashev 2019). The case report states that a woman in Russia became ill and hospitalized after consuming beaver her father had hunted. According to the woman her father developed similar symptoms but refused care. The woman was tested for antibodies against *Trichinella* and was found positive. She recovered after treatment, and claimed her father recovered without treatment at home (Bronstein & Lukashev 2019). According to Centers of Disease Control and Prevention (CDC) in USA, an estimated 10,000 humans worldwide are infected by *Trichinella* annually after consumption of poorly cooked, infected meat (Centers for Disease Control and Prevention 2019).

Uncertainty remains as to how beavers become infected due to the fact that they are strict herbivores (Wilsson 1971; Pozio 2001; Segliņa *et al.* 2015; Jackowiak *et al.* 2020; Różycki *et al.* 2020). It has been discussed that herbivores may be consuming meat residues by accident, thus becoming infected with pathogens normally associated with carnivores or omnivores (Pozio 2001).

2.2.6. *Trichinella* in other herbivores

In the late 1970s, several outbreaks of human trichinellosis occurred due to consumption of horse meat in France and Italy (Touratier 2001). According to Boireau *et al.* (2000), at least 3,200 people were involved in at least 13 outbreaks of trichinellosis in the subsequent 25 years after the initial outbreaks in France, all due to horse meat and all located in Italy and France. Several infected horses were found at slaughterhouses between 1984 and 1999 with origination from several different countries: Poland, Mexico, former Yugoslavia, Romania, Serbia, USA, and Canada (Boireau *et al.* 2000; Touratier 2001). A culinary preference for eating raw horse meat is believed to be the explanation of why outbreaks took place only in Italy and France, despite the fact that other countries such as Belgium has an average higher consumption of horsemeat *per capita* (Boireau *et al.* 2000).

[4] Email conversation with JP Hästveda, 2020-12-04
[5] Email conversation with Vidilab, 2020-12-14

In 2002 a horse in Serbia was found infected with *Trichinella spiralis* (Murrell *et al.* 2004). The infected horse was traced back to the farmer, where interviews revealed that the horse had been fed food waste prior to sale to condition it. Based on interviews with other horse owners, it was discovered that feeding animal by-products to horses were common. Horses in poor condition were known to readily consume meat: a subsequent study found that 32% of 219 horses ate meat (Murrell *et al.* 2004).

It is thought that some species of *Trichinella* (*T. spiralis, T. murrelli,* and *T. britovi*) may infect horses without causing classical symptoms, but also that horses may be more susceptible for *T. spiralis* than other species (Boireau *et al.* 2000). It is estimated that horse meat production constitutes roughly 0.25% of the total worldwide meat production; horse meat is responsible for 16% of reported human cases of *Trichinella* in literature (Rostami *et al.* 2017). As such, horse meat is considered a low frequency infection source with high human risks, and natural infection is believed to occur all over the world (Boireau *et al.* 2000).

According to the 2018 report from EFSA, no *Trichinella* positive solipeds were accounted; only four horses have been found positive in the last 11 years of testing more than one million animals (European Food Safety Authority [EFSA] *et al.* 2019).

Experimental infections with *Trichinella* in herbivores such as sheep, goats and cattle showed that they can get infected (Smith *et al.* 1990; Reina *et al.* 1996; Theodoropoulos *et al.* 2000; Kořínková *et al.* 2006), although proof of natural infection remains absent (Pozio 2001). Despite this, occasional suspected cases of human trichinellosis with sources of cattle (Darwin Murrell 1994), deer (Hall *et al.* 2009; Wilson *et al.* 2015), moose (McLaughlin & Castrodale 2015), beaver (Bronstein & Lukashev 2019) sheep and goat (Takahashi *et al.* 2000) has been reported. *Trichinella* infections has also been reported in reindeer (Pozio 2001), camel, hippopotamus, hare and several species of rodents, amongst others (Rausch *et al.* 1956; Holliman & Meade 1980; Kapel 2000).

A recently published study investigated the prevalence of *Trichinella* spp. in 463 wild moose (*Alces alces*) in Estonia (Kärssin *et al.* 2020). No *Trichinella* spp. was found using artificial digestion, 12 were positive using ELISA but negative using Western Blot (WB). The authors of the study mean that being WB-negative should not be considered evidence of absence of the parasite or *Trichinella*-antibodies, since the method has no reference serum for moose available (Kärssin *et al.* 2020).

In general, routes of how natural transmission for *Trichinella* in herbivores can occur remains uncertain, and is mainly considered to be incidental (Pozio 2001). Experimental studies show that transmission of *Trichinella* may occur if infected rat carcasses are mixed and stored with feed: infection is still possible after four weeks (Oivanen *et al.* 2002), which may be of importance regarding livestock infections. In horses, it has been discussed that two routes of transmission may be possible: the first being grazing in pastures contaminated with rodent carcasses or even pork scraps, the second ingestion of meat from pork and wild carnivores (Pozio 2001). The first is in line with Oivanen *et al.* (2002) study on rat carcasses in feed mentioned above, and the second is further supported by the practises of using carcasses of carnivores hunted or farmed for fur in mashes to fatten horses before slaughter (Pozio 2001). Using animal proteins to fatten herbivorous animals such as horses is common in many countries, as also shown in the example of the Serbian horse mentioned earlier in this section (Murrell *et al.* 2004).

2.2.7. Testing and diagnostic methods for *Trichinella* detection

The magnetic stirrer method (MSM) for pooled sample digestion of muscle tissue

The Magnetic stirrer method (MSM) for pooled sample digestion is the reference method according to the (Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for Trichinella in meat [Text with EEA relevance] 2015), it is also recommended by the European Union Reference Laboratory for Parasites (Mayer-Scholl *et al.* 2017). Raw muscle tissue is collected and subjected to an artificial digestion that mimics the mammalian digestion, thus breaking down the muscle tissue but leaving potential larvae intact. The sediment acquired through this process is manually examined using a trichinoscope or stereomicroscope. If larvae are found, they can be extracted with a pipette and stored in 90% ethanol to be sent for identification and sequencing (Mayer-Scholl *et al.* 2017).

Several versions of digestion-methods exists, and many are considered equivalent to the reference method: the Mechanically assisted pooled sample digestion method/sedimentation technique, the Mechanically assisted pooled sample digestion method/on filter isolation technique, the Automatic digestion method for pooled samples of up to 35 grams (g), the Magnetic stirrer method for pooled sample digestion/on filter isolation and larva detection by a latex agglutination test and the Artificial digestion test for *in vitro* detection of *Trichinella* spp. larvae in meat samples, PrioCHECK® *Trichinella* AAD Kit (Commission Implementing Regulation [EU] 2015/1375 of 10 August 2015 laying down specific rules on official controls for Trichinella in meat [Text with EEA relevance] 2015). The two later are only considered equivalent if performed on pig meat (Commission Implementing Regulation [EU] 2015/1375 of 10 August 2015 laying down specific rules on official controls for Trichinella in meat [Text with EEA relevance] 2015).

Trichinoscopy (compression method) of animal muscle tissue

Trichinoscopy is a simple method that can be used in various situations so long as an ordinary light microscope (stereomicroscope) is available (Gamble *et al.* 2000; Gajadhar *et al.* 2009). Very small pieces of muscle tissue (28 pieces should correspond to one gram of muscle tissue) are compressed individually between two glass plates or slides until they become translucent. Thereafter they are examined using trichinoscope or a stereomicroscope at 15-40x magnification, looking for *Trichinella* larvae (Gamble *et al.* 2000). The method is time-consuming and, according to Forbes *et al.* (2003), has a lower sensitivity than digestion methods. Examination with stereomicroscope is considered more sensitive than trichina-scope. Larvae of non-encapsulated species of *Trichinella* (*T. pseudospiralis, T. zimbabwensis* and *T. papuae*) are harder to detect with this method; the otherwise present thick collagen capsule is more easily seen (Forbes *et al.* 2003; Gajadhar *et al.* 2009). The International Commission on Trichinella (ICT), World Organization for Animal Health (OIE) and EU does not recommend the use of this method for routine examination of meat meant for human consumption (Gajadhar *et al.* 2009).

Serology – indirect diagnosis in animals

Enzyme-linked immunosorbent assay (ELISA) is the most commonly used method for serological testing in both animals and humans, and is generally considered the most sensitive and reliable method, as well as economic; ELISA is recommended for use in both domestic pigs and wild boar (Gamble *et al.* 2004; Gajadhar *et al.* 2009). A study from Vietnam investigated ELISA (PrioCheck® *Trichinella* Ab ELISA) in pigs: 206/1035 (19.9%) pigs had antibodies for *Trichinella* (i.e. were positive) (Vu Thi *et al.* 2010). Muscle samples from 76 of these were tested with artificial digestion, only 11 were found to be positive (14.5%). The authors state that these differences may be due to the digestion method being insufficient for detecting larvae in muscles with low worm burden (LPG) and/or that the specificity of the ELISA kit could be lower than expected (Vu Thi *et al.* 2010). Even if not taken into account by the authors, there is also a possibility that some pigs had been exposed to the parasite (therefore having antibodies in their blood) but no longer having active infections (hence the negative result at the artificial digestion).

ELISA is not, however, considered a good testing method in all animals: difficulties were observed when trying to develop an ELISA method for detection of *Trichinella zimbabwensis* in experimentally infected Nile crocodiles (*Crocodylus niloticus*) (Ludovisi *et al.* 2013). Results indicated that *T. zimbabwensis* did not induce a large immune response, neither in levels of antibodies nor in the persistence of them, and thus the method was considered unreliable. However, it was also discussed that several factors influence immune responses in crocodiles, including age, temperature, hormones and season (Brown *et al.* 2001).

Another study also concluded that circulating antibodies cannot be used in diagnosing *Trichinella* infection or estimating the prevalence of infection in horses, since the larvae remain infective longer than the IgG-antibodies produced by the immune response (Pozio *et al.* 2002). The study aimed to evaluate the methods of using ELISA and Western Blot to find *Trichinella* antibodies in horses, but despite still having infective larvae, no antibodies could be detected 4-5 months after experimental infection occurred. Out of four horses with muscle larvae, only one was detected by ELISA and Western Blot. The study also showed inconsistencies between ELISA and Western blot: in some cases the optical density of serum samples used in ELISA were higher than the cut-off value but when subjected to Western Blot showed negative (Pozio *et al.* 2002). Similar conclusions regarding circulating antibodies and infective larvae have also been seen in other studies on horses, both on naturally (Pozio *et al.* 1997) and experimentally infected horses (Soule *et al.* 1989; Sofronic-Milosavljevic *et al.* 2001).

Diagnosis of trichinellosis in humans

In humans, diagnosis is mainly clinical or based on serology (detection of antibodies by indirect enzyme-linked immunosorbent assay, iELISA, and Western Blot, IETB), whereas histopathology of muscle biopsies (trichinoscopy) is rarely performed (European Food Safety Authority [EFSA] *et al.* 2019). Competitive inhibition assay (CIA) and IETB acts as confirmatory tests rather than primary screening tests when used on humans (Gamble *et al.* 2004).

A large study on humans found that the sensitivity and specificity of the ELISA-test used was 99.2% and 90.6% respectively, which showed that the test had good sensitivity, repeatability and reproducibility, but limited specificity (Gómez-Morales *et al.* 2008). In conclusion, the study found that this test was suitable for diagnostic purposes but perhaps not in epidemiological surveys (Gómez-Morales *et al.* 2008).

3. Material and Methods

3.1. Literature review

Web of Science, PubMed, Scopus and Google Scholar, SLU Primo and books have been used to access relevant literature. Citations used in articles have also been used to find other articles, as well as direct contact mainly via email. Searches have been made using combinations and versions of the words “beaver”, “trichinella”, “herbivore”, “trichinosis”, “trichinellosis”, “trichiniasis”, “horse”, “human”, “zoonotic”, “magnetic stirrer method”, “trichinoscopy”, “detection method”, “muscle biopsy”, “ELISA”, “serology”, “PCR”.

3.2. Material

The material used in this study was raw muscle tissue from 58 beavers harvested during hunting in spring and fall of 2020. The meat samples collected in Sweden (n=51) were sent voluntarily by hunters throughout the country. The Norwegian samples (n=7) were originally collected by Norwegian hunters within a research project on beaver reproduction. All the samples were stored frozen at -20℃ until the time of processing.

3.3. Method

The samples were processed with the EU reference method: artificial digestion with magnetic stirrer method (MSM), at SVA, the national reference laboratory (NRL) for *Trichinella* in Sweden.

Samples were prepared by being thawed, skinned and with the removing of bones, tendons and fat until pure muscle remained. From every beaver, approximately 20 grams of muscle tissue was collected; five samples were analyzed as a batch weighing approximately 100 g (the maximum amount of muscle tissue per batch allowed is 115 g). Due to an uneven number of samples, the last run also contained

40 g wild boar meat that had previously been confirmed negative for *Trichinella*. The meat was mixed until finely minced and placed in a solution of 2 L of warm water (48℃), 16 mL HCl 25% and 30 mL of liquid pepsin (at least 600 units/mL). The mixture was whisked to ensure no clumps of meat remained, thereafter placed on a heated (48℃) magnetic stirrer machine for 30 minutes (500-510 rpm).

The solution containing the digested tissues was poured through an 180µm sieve to ensure that remaining pieces of undigested tissue (non-muscular) was removed, but still allowing potential larvae to pass through. The solution was poured into a separation glass funnel and left to sediment for 30 minutes. The first 50 mL of the sediment was collected in a 50 mL measuring glass and left to sediment for another 10 minutes. To wash the sample and increase readability, the supernatant (40 mL) was carefully removed, and 40 mL of pure water was added to the 10 mL of sediment left in the measuring glass. Thereafter the measuring glass was left to sediment for 10 further minutes.

The supernatant (40 mL) was removed again, and the remaining 10 mL of sediment was poured in a larval counting basin with one cm^2 grids. 10 mL of water was used to rinse the measuring glass to ensure that all the sediment was included and then also poured into the larval counting basin. The samples were immediately examined with a trichinoscope. All samples were examined once again afterwards by laboratory staff at the SVA to control the results.

If L1 larvae were to be detected in pool samples, each of the five samples from the positive batch would be analyzed once again individually, to identify which animal was infected. Found larvae would have been sent to the European Union Reference Laboratory for Parasites (EURLP, Rome, Italy) to obtain identification by molecular methods (multiplex PCR).

4. Results

In total, 58 beaver meat samples were tested for presence of *Trichinella* larvae using artificial digestion with MSM. All samples were negative. All samples were examined once again and thus controlled by laboratory staff at SVA.

5. Discussion

The purpose of this study was to investigate the presence of *Trichinella* spp. in Eurasian beavers (*Castor fiber*) using artificial digestion with the magnetic stirrer method (MSM). Samples from Norway were received as part of a scientific collaboration, but were fewer than expected, and arrived late in the project. Therefore, there was limited time to perform further investigations of the possible occurrence of *Trichinella* in Norwegian beavers, which consequently lead to this study being mainly focused on Sweden.

The lack of *Trichinella* findings in this study could be explained by one or more of the following hypotheses:

- beavers in Sweden and Norway are uninfected
- the sample size was too small
- the chosen and performed method could have some limitations when aimed at detecting low numbers of larvae

The method, MSM, used in this study followed recommendations and was performed according to the EU guidelines, and as such should be optimal and reliable since they are designed and validated to protect people from getting infected with the parasite. However, according to Mayer-Scholl *et al.* (2017), sedimentation time should be doubled to 60 minutes if using frozen meat or examining meat with expected low infection rate. In our case, all samples had been frozen, and we did not suspect high presence of *Trichinella*. Therefore, it could be argued that we should have lengthened sedimentation time, but we chose to follow the EU guidelines that state that with the MSM 30 minutes is considered an acceptable sedimentation time even when using frozen meat.

Pooling samples together could be another factor leading to higher risk of missing larvae, but again, the EU recommendations for artificial digestion with MSM state that the threshold for minimum amount of tissue needed to have reliable results depend on species; in domestic pigs 1 g of muscle tissue is needed per individual (Mayer-Scholl *et al.* 2017). When searching for *Trichinella* in wild boar, 5 g of muscle tissue is used per animal and analyses are run in batches of 20 individuals to reach a total of 100 g[6]. To increase the reliability of the method with the expected low presence of *Trichinella* in this study, the proportions were reversed, i.e., each batch contained only five individuals with larger amount of tissue per individual.

Segliņa *et al.* (2015) and Różycki *et al.* (2020) found Trichinella in beavers: in both studies artificial digestion with magnetic stirring was the method used. Różycki *et al.* (2020) used 50 grams of muscle tissue per beaver from a hind leg and analyzed them separately. The recipe was modified to keep the proportions of the ingredients despite using less than 100 g of muscle tissue per batch, to ensure proper digestion. They also used 30 min sedimentation time and found one larvae of *T. spiralis* (prevalence 1.4%). Segliņa *et al.* (2015) used at least 25 g of muscle tissue from foreleg per beaver, and also analyzed them separately with proper modifications of the recipe. They found 142 larvae in tissue from one beaver (prevalence 0.5%), of which 5 were identified as *T. britovi* through DNA sequencing.

Sedimentation time of 30 minutes, as used in this study, is considered sufficient even when using frozen samples with an expected low presence of larvae. As far as could be discerned when conducting this study, no official consensus on preferred muscles for *Trichinella*-infection in beavers exists. The use of forelimb in this study is supported by the fact that front leg muscles are one of the preferred muscles by *Trichinella* in wild boar, and the findings of Segliņa *et al.* (2015) supports the use in beavers too. The forelimb is also a part of the beaver that is rarely used for cooking or smoking. The use of a limb instead of tongue or diaphragm, which is other normally used sample sites in some animal species, also has practical purposes: in small animals, it is easier to retrieve larger amounts of muscle tissue from a leg than from the tongue or diaphragm. It is also easier to convince hunters to voluntarily help submit samples if the collection is simple and require little effort. Removing a forelimb is easy, instructions are straight-forward, and extra muscle tissue might be available if the shoulder is included.

It has been discussed that herbivores might get infected from contaminated feed, or perhaps even from gnawing on bones and carcasses in times of starvation to supplement themselves (Oivanen *et al.* 2002). One could hypothesize that chances of finding *Trichinella* spp. in beavers would be higher for animals harvested early spring, after a potentially harsh winter where food supplies easily run low. Most samples tested in this study were harvested in April and May 2020, and with this theory in mind one would expect to find potential parasites in these samples. None of the spring-harvested beavers were infected with *Trichinella* spp., which could imply the opposite, although the sample size was rather small. However, since no other beavers, collected from June to December 2020, were infected, it is hard to

[6] Personal communication with laboratory staff at SVA, 2020

draw conclusions regarding this. A seasonal variation in presence of parasites ought to be considered in future studies.

In this study, 51 Swedish beavers were tested, which constitutes 0.04% of the estimated population of 130,000 beavers (Svenska Jägareförbundet 2020). That is a small proportion of the population, regardless of what one aims to investigate. An expected prevalence for *Trichinella* spp. in beavers in Sweden is unavailable since it has not been investigated prior, so estimating how many beavers are needed to find one infected individual is difficult. Segliņa *et al.* (2015) found a prevalence of 0.5% in their study. Latvia reportedly have a higher prevalence of *Trichinella* spp. in the most common hosts (carnivores and omnivores) than Sweden. Różycki *et al.* (2020) found a prevalence of 1.4% and the infected beaver came from an area where *Trichinella* spp. had previously been found in other animal species. If we assume that the prevalence of *Trichinella* spp. in Swedish beavers is similar to that of their findings (*P*=0.5-1.4%), 650-1820 beavers would theoretically be infected with *Trichinella* in Sweden. Using the formula explained by Pourhoseingholi *et al.* (2013), we can calculate a theoretical sample size (*n*) needed to find infected beavers using the prevalence shown by Segliņa *et al.* (2015) and Różycki *et al.* (2020). We set our confidence level (*Z*) to 95%, and precision (*d*) to 0.001 (for prevalence P=0.5%) or 0.0035 (for prevalence P=1.4%), according to the suggestions of Pourhoseingholi *et al.* (2013) regarding precision setting in cases with low prevalence.

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

$$n_1 = \frac{0.95^2 \times 0.005(1-0.005)}{0.001^2} = 4{,}489.9$$

$$n_2 = \frac{0.95^2 \times 0.014(1-0.014)}{0.0035^2} = 1{,}016.9$$

The sample size would have needed to be higher in order to find the parasite, especially considering that Sweden in general has a low prevalence amongst wildlife, and no presence in domestic pigs. For example, prevalence of *Trichinella* spp. in wild boar was reported to 0.08% in Sweden, 0.98% in Romania, 0.7% in Latvia, and 1.8% in Republic of North Macedonia in 2018 (European Food Safety Authority [EFSA] *et al.* 2019). In brown bears, prevalence was 0% in Sweden, 25% in Romania and 0.9% in Slovenia in 2018. In red foxes, prevalence was 8% in Slovakia and 0.9% in Hungary. No red foxes were tested in Sweden in 2018, however, the combined prevalence of *Trichinella* spp. in red foxes in Sweden during 2014-2019 was 0.8% (National Veterinary Institute [SVA] 2014, 2015, 2016, 2017, 2018, 2019; European Food Safety Authority [EFSA] *et al.* 2019). This generally rather low prevalence of *Trichinella* spp. in Sweden should result in lower risk of contamination to feed and incidental infection to herbivores. Subsequently, the prevalence of *Trichinella* in beavers in Sweden overall is likely lower than that shown by Segliņa *et al.* (2015) and Różycki *et al.* (2020), which also increases the number of beavers needed to be tested to properly search for the parasite.

The beavers examined in this study were collected randomly by volunteers throughout Sweden and Norway. For further studies, it may also be interesting to target the harvesting to areas where *Trichinella* spp. has been found in other species, as the presence of the parasite in any given region should increase the risk of contamination of feed and of finding infected carcasses from various species. For example, red fox and wild boar have been found in Sweden with prevalence of *Trichinella* reaching 0.8% and 0.005% respectively in the last six years (National Veterinary Institute [SVA] 2014, 2015, 2016, 2017, 2018, 2019).

If *Trichinella* spp. were to be found in future investigations, the question regarding how infection occurred is an interesting and important point of discussion. It has already been hypothesized that wild animals may gnaw on bones and carcasses in time of nutritional deficiency and starvation or even by accident, which of course could be a source of infection (Pozio 2001). Thanks to *Trichinella*'s mostly aerobic metabolism in their larvae-form, they can survive rather long time in carcasses and even in putrefying flesh (Despommier 1990; Pozio & Darwin Murrell 2006). Combined with some species' ability to survive for one or several years when frozen (0°C to - 18°C) (Pozio & Murrell 2006), carcasses can surely pose a source of infection even for extended periods of time. Some studies also show that invertebrates can become infected from eating infected carcasses for a limited period of time (Merkushev (1955), as referred to by (Pozio & Murrell 2006)), and thus accidentally ingesting infective insects while eating plants is not unthinkable although perhaps not likely. Infected rat carcasses has been shown to contaminate feed, which goes along the same line of thoughts (Oivanen *et al.* 2002), and other micromammals may contribute in a similar way although uncertainty as to how big a role this play remains (Pozio & Murrell 2006).

Another potential source of infection is through territorial defense or deflecting predators. Beavers are highly territorial and are known to attack intruders, be they other beavers, humans, or predators. They use their sharp claws and teeth for defense, and it is not impossible for them to incidentally ingest raw meat while attacking an infected intruder. Although seemingly farfetched, beavers could in theory also consume live small rodents or critters if nutritionally deficient or starved; records show horses and sheep, also herbivores, eating small rodents or birds whole.

Despite finding no *Trichinella* in this study, the possibility of beavers from Sweden and Norway being infected cannot be dismissed. More studies are needed before conclusions can be drawn, and several more beaver samples need to be analyzed. This pilot study provides baseline information that was not available before and has hopefully awoken curiosity amongst hunters and others interested in wildlife and food security. With more information regarding possible *Trichinella* infection reaching those that hunt and consume beaver meat, the possibility of attaining more samples increase, which hopefully makes further studies easier to perform.

A future study could involve identifying 'hot spots' of *Trichinella* infection in Sweden by using information from the large numbers of tested wild boar, and in some instances also large and medium-sized carnivores. Thus, one or more areas of interest would be targeted, and sampling of different herbivores (if possible) can be performed, including moose, beavers and small rodents. Since Sweden and Norway share a long border and wildlife move freely across it, a synchronized coordinated collection of samples from both countries would increase the level of accuracy and reliability on performed investigations.

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Popular Science Summary

Trichinellosis is a disease that can be transmitted between animals and humans and can lead to severe illness and even death in humans. The disease is caused by small roundworms, known as *Trichinella*. Today, 13 different species of *Trichinella* are known. Transmission of larvae occurs with consumption of infected meat. The major sources of infection are pork and wildlife such as wild boar and carnivores. Horses, a herbivore, have however been responsible for large human outbreaks throughout history. Other herbivores have also been found to be infected by *Trichinella*, but it is thought to have low significance for human health as the cases are few.

The Eurasian beaver (*Castor fiber*) is a common herbivore in Sweden with numbers reaching 130,000 and an estimated 6,000-9,000 or more are hunted annually. They were hunted to extinction in the late 19th century but were successfully reintroduced from Norway in the early 20th century. Recent studies from Poland and Latvia shows that beavers can become infected with *Trichinella* spp. As of today, one case of human trichinellosis has been reported with beaver meat being the suspected source of infection.

The purpose of this study was to investigate the presence of *Trichinella* spp. in beavers from Sweden. A review of current scientific literature revealed no previous studies in any of the Nordic countries. In Sweden, beavers are not routinely tested for *Trichinella* although many other wildlife species are part of the surveillance against *Trichinella*. This study may therefore play a role in establishing the potential need for recommendations regarding beaver meat handling in the future.

Materials used in this study was raw beaver meat (primarily forelimb) sent in from hunters throughout Sweden, and a few samples sent from Norway.

The method used in this study was the artificial digestion with magnetic stirring method (MSM), as per the EU guidelines. This method is aimed at separating *Trichinella* larvae, if present, by reproducing *in vitro* the functions of the mammal stomach, and at the end the digested material is examined under the microscope to search for parasitic larvae.

No *Trichinella* spp. was detected in this study after testing 58 samples from Sweden and Norway.

Despite not finding any *Trichinella*, possible infection amongst beavers in Sweden and Norway cannot be ruled out. More studies and a more beavers tested are needed. For future studies, focusing sampling to areas where *Trichinella* has been found in other species and systematically collaborating across the Swedish-Norwegian border might give a more thorough insight into the *Trichinella*-situation amongst beavers and other herbivores.

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(I would also like to thank my friends and family for your unending support. Lastly, thank you to me. Well done!)

Appendix 1

Form sent to hunters wanting to participate by collecting and submitting beaver meat samples to this study.

Projekt: **Trikiner hos svensk bäver**

Kontaktpersoner: Sofia Lovén, veterinärstudent, tel [redacted] epost [redacted]
Jonas Malmsten, veterinär, Institutionen för vilt, fisk & miljö, SLU, tel [redacted], epost [redacted]

Provsamlare (kontaktperson):________________________________telefon/epost:__________________________

Län, Kommun, ort: Län:_______________________Kommun:____________________Ort:___________________

Insamling: Spara **ett helt flått framben** (inklusive underarm, överarm, bog) i en påse i frys. För att förenkla provhanteringen får benet gärna styckas fritt från skelettet, men det är inte nödvändigt.
Märk med nummerlapp enligt protokoll nedan – VIKTIGT att alla prover märks med nummer & insamlingsplats!
Alla prover läggs i ytterligare en märkt påse (namn på provtagare), förvara i -20 frys tills de hämtas/skickas!

FRÅGOR – Telefon och epost ovan, tveka aldrig att ringa! Lycka till och stort tack för hjälpen!

Provnr	Insamlingsplats	Datum	Kön	Ålder (vuxen/unge)
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				