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Survey of Zoonotic Aspects of Some Parasitic Diseases in the Czech Republic

Master Thesis

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Declaration

I declare that I have developed and written the enclosed Master Thesis completely by myself, and have not used sources or means without declaration in the text. Any thoughts from others or literal quotations are clearly marked. The Master Thesis was not used in the same or in a similar version to achieve an academic grading or is being published elsewhere.

In Prague 26.04.2018 B.Sc. Shavkat Azimov

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Abstract

Diseases transmitted from vertebrates to humans are referred to as zoonoses. The aim of this work was focused on evidence and zoonotic aspects of parasitic developmental stages: *Cysticercus bovis* in cattle and *echinococcus* in foxes. A post-mortem inspection of cattle and a laboratory examination of the developmental parasitic stages of both zoonoses were carried out. The prevalence of cysticercosis/taeniosis in the Czech Republic and human alveolar echinococcosis in the Czech Republic and individual EU countries, were also monitored. The informations were obtained from the scientific databases from the domestic (EPIDAT and SVS CR) and European (ECDC) databases. No positive findings in *Cysticercus bovis* inactive (infectious) or inactive (calcified) parasitic stages were recorded in the cattle slaughterhouse during the year 2016. In the years 2010-2016, total amount of 224,185 cattle carcases (chewing muscles and heart) were examined in the same slaughterhouse. Only 24 cases were positive (0.01%) and *Cysticercus bovis* was detected. A higher prevalence of cysticercosis (0.003-0.02%) was reported between 2010-2014 (1-8 cases), the lowest prevalence was confirmed in 2015-2016 (0%). Although the outbreak of taeniosis in the Czech population was reported in 2013 in 30 cases (prevalence of 0.03%), in the following years there was also a gradual decline in positive case reporting in people with this diagnosis. Nowadays, there was no increase in cysticercosis or taeniosis in the Czech Republic. A high prevalence of echinococcosis (33%) was demonstrated in cats and foxes testing in 2011, compared to 2010, when the prevalence of 22% was recorded in the Czech Republic. During the 2011-2012 reporting period, the zero prevalence of human alveolar echinococcosis was reported as well as in the six reported cases from EU databases (2014). The statistical evaluation revealed that the lowest number of positive cases of human alveolar echinococcosis was recorded in Central European countries, in Northern and Western Europe. However, the highest incidence was recorded mainly in southern European countries. In those countries, where the occurrence of cysticercosis and echinococcosis is sporadic, the potential sources of infection and the route of spreading from other regions or countries must still be carefully monitored to ensure biosecurity.

Keywords: cysticercosis, taeniosis, alveolar echinococcosis, *post mortem* examination, prevalence, Czech Republic, EU

Abstrakt

Nemoci přenášené z obratlovců na člověka jsou označovány jako zoonózy. Cílem této práce bylo zaměřit se na průkaz a zoonotické aspekty parazitárních vývojových stadií: *cysticercusbovis*u skotu a echinokoky u lišek. Byla provedena po porážce skotujatečná prohlídka a laboratorní vyšetření vývojových parazitických stadií u obou zoonóz. Byla monitorována prevalence cysticerkózy/taeniózy v České republice a humánní alveolární echinokokóza v České republice a jednotlivých zemích EU. Informace byly získány z vědeckých databází z domácích (EPIDAT a SVS ČR) a evropských (ECDC) databází. V roce 2016 nebyly na jatkách zaznamenány žádné pozitivní nálezy *cysticercusbovis*v aktivní (infekční) nebo neaktivní (kalcifikované) formě parazitárních vývojových stadií. V letech 2010-2016 bylo vyšetřeno celkem 224 185 skotu (žvýkací svaly a srdce), na stejných jatkách. Pouze 24 případů bylo pozitivních (0,01%) a byl zjištěn *cysticercusbovis*. Vyšší prevalence cysticerkózy (0,003-0,02%) byla hlášena v letech 2010-2014 (1-8 případů), nejnižší prevalence byla potvrzena v letech 2015-2016 (0%). Přestože v roce 2013 bylo hlášeno ohnisko taeniózy v české populaci ve 30 případech (prevalence 0,03%), v následujících letech došlo k postupnému poklesu pozitivních hlášení u humánní populace s touto diagnózou. V České republice nedošlo v současné době k nárůstu cysticerkózy nebo teniózy. Vysoká prevalence echinokokózy (33%) byla prokázána při vyšetření koček a lišek v roce 2011, ve srovnání s rokem 2010, kdy byla v České republicezaznamenánaprevalence 22%. Během sledovaného období 2011-2012 byla hlášena nulová prevalence humánní alveolární echinokokózy, stejně jako v šesti hlášených případech z databází EU(2014). Statistické vyhodnocení poukázalo na skutečnost, že nejnižší počet pozitivních případů humánní alveolární echinokokózy byl zaznamenán ve středoevropských zemích, v severní azápadní Evropě. Nejvyšší výskyt byl zaznamenán hlavně v zemích jižní Evropy. V těch zemích, kde je výskyt cysticerkózy a echinokokózy sporadický, je třeba stále pečlivě sledovat možné zdroje infekce a cestu šíření z jiných oblastí nebo zemí, z hlediska zajištění biologické bezpečnosti.

Keywords: cysticerkóza, taenióza, alveolární echinokokóza, vyšetření *post mortem*, prevalence, Czech Republic, EU

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CDFA	California Department of Food and Agriculture
CDC	Centre for Diseases Control and Prevention
CFSPH	The Centre for Food Security and Public Health
CT	Computer tomography
ECDC	European Centre for Disease Prevention and Control
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
EPIDAT	Epidemiological date base (owner National Institute of Public Health in Prague)
EU	European Union
FAO	Food and Agriculture Organization
GLP	Good Laboratory Practise
Ig	Immunoglobulin
LAMP	Loop-mediated isothermal amplification
MRI	Magnetic resonance imaging
OIE	World Organization for Animal Health
SVA	State Veterinary Administration
SVI	State Veterinary Institute
SZU Praha	National Institute of Public Health in Prague (Statni Zdravotni Ustav)
WHO	World Health Organization

1. INTRODUCTION

Illnesses transferred between animals and humans are called zoonotic diseases. There are various factors that cause such diseases, such as viruses, bacteria, parasites, and fungi. Some of these conditions are quite widespread. Concerning zoonotic diseases which are caused by parasites, the kind of clinical signs and symptoms may vary depending on the type of the parasite and individual characteristics of the person. Sometimes people who contracted zoonotic infections can be very sick, but at the same time some other people display no signs at all and do not ever get sick. Some other people may have symptoms like muscle aches, diarrhea and fever. Zoonotic infections can be transferred to humans through foods can be the source for some zoonotic infection when animals such as cows and pigs are infected with parasites such as cysticerci *or* echinococci [12].

Bovine cysticercosis is a zoonotic disease which is spread in different regions across the globe and which affects both people and cattle. It is caused by the tapeworm *Taenia saginata (T. saginata)*, whose definitive hosts are humans while cattle are the most common intermediate host. Cattle contract the infection through ingesting eggs of the tapeworm that were shed in human feces. The eggs attach to grass, leaves and other vegetation, where they can find shelter to survive for several weeks to months. After being ingested by cattle, they evolve and turn into larvae and afterwards migrate to muscle tissue, most frequently they travel to the heart or around the jaw, diaphragm and tongue. Each larvae creates a fluid cyst inside a fibrous capsule, which is known as a cysticerci or *Cysticercus bovis*. The occurrence of cysticerci in muscle is generally referred to as “beef measles”. One animal may have one to hundreds of cysts in its muscle tissues [12].

T. saginata is listed as the most widely spread human Taenia tapeworm around the world by Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO). 60 million human cases of infections a year [29] are caused by this tapeworm. The larval stage of this tapeworm causes cysticercosis [32] in bovine. Humans generally may contract the infection after eating improperly cooked beef that contain cysts [17].

Echinococcosis is a disease caused by a parasite. This disease occurs in two main forms in humans: cystic echinococcosis (also known as hydatidosis) and alveolar echinococcosis, caused by the tapeworms *E. granulosus* and *E. multilocularis*, respectively. Adult worms find shelter in dogs', foxes' and other carnivores' intestines. The eggs of the parasite are released with the faeces of the animals. If a human ingests the eggs, they develop into larvae in several different organs, especially in the liver and lungs. A typical characteristic of both cystic and alveolar echinococcosis is their asymptomatic incubation periods that can last many years until the parasite larvae develops and starts causing clinical signs. Both diseases can cause serious morbidity and death [41].

2 LITERATURE REVIEW

2.1 Bovine cysticercosis

Bovine cysticercosis is a parasitic zoonosis, the cause of which is known to be the larvae of human tapeworm *T. saginata* [32]. They usually infest cattle via consumed food, e.g. grazing onpasture, polluted with human faeces which contains eggs of the tapeworm (faeces coming from sewage system or direct pollution). In 2009, Allepuz [3] discovered that the most likely way for the cattle to be infected is via water supply, while food and other routes are next possible ways the parasite can infect cattle. According to their research results, personnel and pasture appeared to be less common causes of infection. Cases of bovine cysticercosis have been registered in a number of different locations around the world, e.g. in Canada, Alberta [44] and New Zealand [51]. Humans got infected by bovine cysticercosis after ingesting raw or undercooked beef or products made of products. Subsequently, the larvae grow into adult tapeworms in the person's intestines. The disease is known as taeniosis when it affects people. Cysts do not display any resistance to heat and the transmission largely depends on dietary habits and culinary practices which involve consuming beef raw or lightly cooked [17].

Generally, the clinical signs and symptoms of cysticercosis depends on the number, size and location of the cysts, while they are also directly related to the host immune response to the parasite [6]. Light infections are more widespread than heavy ones among animals [17]. Commonly, the cattle do not show any signs of clinical illness [19,67]. Possible symptoms include mild fever, stiffness in gait, difficulties with feeding and inflammation of bowel; heavy infection may result in death due to myocarditis [76].

Taxonomic classification

T. saginata and its metacestode, *Cysticercus bovis* the unnamed beef tapeworm, is classified under the kingdom of Animalia, phylum of Platyhelminths, class of Cestode, order of Cyclophyllidea, family of Taeniidae, genus of Taenia and species of *T. saginata*.

Life cycle

Bovine cysticercosis appears in humans due to consumption of improperly cooked beef that is infested with viable cysts [3,20,37]. The presence of a single cyst is generally enough to launch an infection, but multiple infections also take place [19]. It is estimated that a single infected bovine carcass is able to infect 8-20 human individuals on average [21]. The adult tapeworm evolves in the human host's small intestine. These tapeworms become adults in two to three months and can reach significant sizes with a length of 3-12 meters [29]. If no treatment is carried out, the tapeworm is able to occupy human intestines for 20-25 years [36].

After reaching maturity, tapeworms release gravid proglottids containing 30,000-50,000 eggs on average [28]. They release between 3 and 7 proglottids every day [20]. These proglottids then leave the host's body travelling through the anus or within the feces. In the stools proglottids generally occupy the surface of the feces [8]. The eggs possess an oncosphere (a larva) that is infective starting from the moment when they are released by the host. The eggs are transferred to cattle through their grazing on a contaminated pasture. Contamination of the pasture may occur directly with human feces containing Taenia eggs, or indirectly via sewage sediment or flooding [29].

The digestive system of cattle is an ideal place for the eggs' hatching, and the oncospheres are released there after hatching. They penetrate through the walls of the intestines and start circulating across the body in lymphatic system and in the blood. Following such migration within the body the larvae gradually develops into cysts. This takes place in 8-10 weeks, and after that they already become infective to humans [28]. The cysts tend to occupy the smooth muscle tissue, such as heart, masseter muscles, tongue and diaphragm [29]. Within around nine months after infection the majority of cysts die and calcify [28] but some still remain viable in the muscle tissues. As a result of consuming raw beef containing viable cysts humans contract the infection and the cycle starts a new. Figure 1 illustrates the life cycle described above

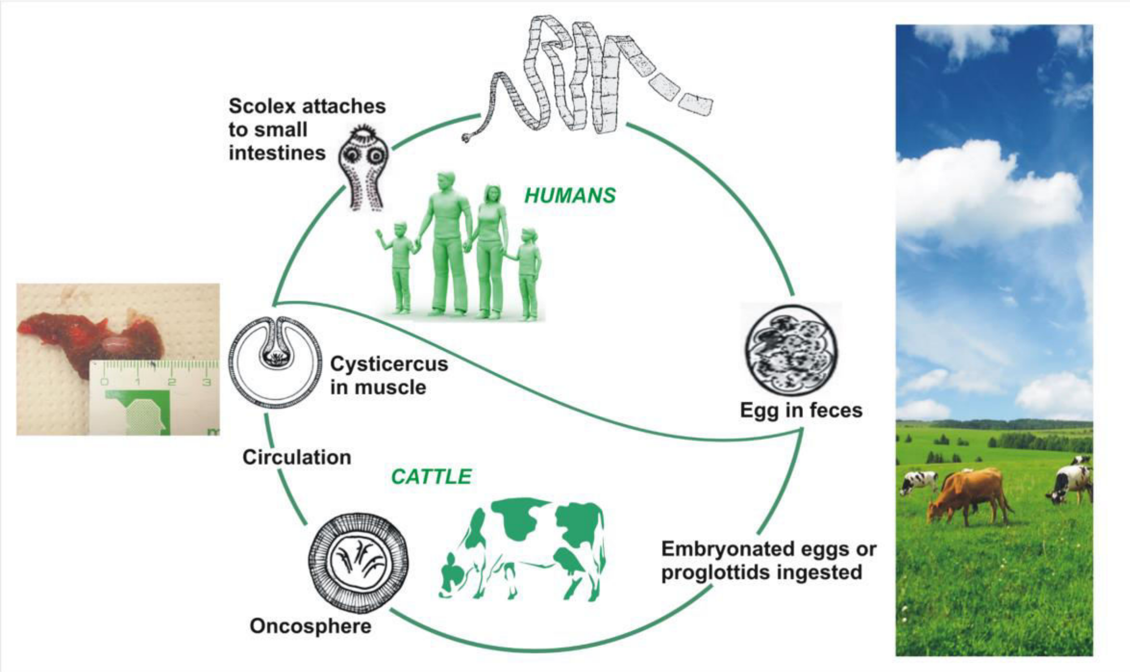

Figure 1. Life cycle of *T. saginata* and cysticercosis (Source: Kassahun S, 2014.)

Allepuz [3] discovered a statistically significant link between the status of infection and type of farm (dairy versus beef). According to their findings farms concentrated on production of dairy products have almost double likelihood to be affected compared to beef farms. They also found that infected farms had bigger farm size in terms of the number of animals in contrast with the uninfected ones. It was claimed that age of the cattle positively influences the measured sero-prevalence – older animals are significantly more likely to be infected [9,18,24,25]. Meanwhile, males seem to have lower risk in comparison with their male counterparts [9].

A research with mildly infected cattle showed that animals drinking from streams carrying effluent from sewage treatment plants constitute a serious risk factor of bovine cysticercosis [43]. Among other risk factors there is outdoor defecation near the grazing lands or cattle rearing facilities; improper fly and bird control facilities in the cattle farms; using water from sewage sludge or untreated human faeces in order to irrigate or fertilize crops and pasturelands; human carriers that take part of looking after the cattle; and misplaced faeces deposits on camping grounds and along highways and rail tracks [52]. Consumption of raw or undercooked beef and lack of satisfactory meat inspection were indicated as major risk factors for human infection from cattle by the same study.

Morphology

The mature tapeworm, *T. saginata*, is a large ribbon shaped, multi segmented, white flat worm, which is normally 4-15 m long and consists of many segments (proglottids) set up in a chain [76]. Its body consists of three different parts – head (*scolex*), neck and strobilla [33]. The head or *scolex* contains attachment organs, a short neck without segments as well as a chain of segments. The chain is referred to as strobilla while the segments are called proglottids. In contrast with other taeniids, the head does not have any *rostellum* or hooks. The proglottids are continually budded from the neck region and reach sexual maturity after they pass down the strobilla. Proglottids are hermaphrodite having one or two sets of reproductive organs. Gravid segments usually leave the host singly and often migrate spontaneously from the anus [5,68].

The shape of taeniid eggs present in the stool or released from ruptured segments are sub-spherical to spherical. They display great resistance, staying viable for six months in vegetables and pastures, five weeks in aquatic environment, 10 weeks in faeces or hay and 12 weeks in silage sludge. The diameter of taeniid eggs constitutes approximately 30-45 µm. They contain an *oncosphere* (hexacanth embryo) which has three pairs of hook. They an external membranous coating, oval in shape, the egg shell, lost from fecal eggs. In addition, they possess a thick, brown, radially striated embryophore or 'shell' consisting of hooks. [55]. The larval stage, or metacestode also known as "beef measles", can be observed in all striated muscles of the intermediate host. *Cysticercus bovis* is small, oval, pea-sized [55], translucent and has a single white *scolex* that is close to the *scolex* of the future adult tapeworm morphologically. They stay in a thin fibrous capsule, produced by the host [55].

Etiology

Bovine cysticercosis is a disease that impacts the muscles of cattle, caused by the metacestode stage of human intestinal cestode *T. saginata* [75].

2.2 Epidemiology

2.2.1 Geographic distribution: Status in the Czech Republic

Present information about the epidemiological situation of bovine cysticercosis on the territory of the Czech Republic is generally founded on findings of cysticerci in the carcasses of bovine animals while meat is inspected at the slaughterhouse [49].

Between 1989 and 1998 the percentage of bovine cysticercosis occurrence was 0.7-1.3%. After flooding in 1997, in an area seriously affected by it, cysticercosis were found in 17 out of 170 cattle in 1997 and in 18 of 54 in 1998 [46].

49 cases of human taeniosis caused *T. saginata* in the Czech Republic were recorded in the database of the National Institute of Public Health in Prague (EPIDAT) [27] during the period from 2006 to 2009 (Table 2). There was unsubstantiated evidence involving 24 individuals. EPIDAT [27] local disease database recorded 53 case reports in 2003-2013 in Moravian region of Czech Republic exclusively (Table 1).

Table 1: The number of reported cases of taeniosis in period 2003-2013 in Moravian Region

Place/year	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Bruntal	0	0	0	0	0	0	0	0	0	0	0
Frydek-Mistek	2	1	0	2	0	0	0	0	0	1	0
Karvina	0	1	0	0	1	3	0	0	0	0	0
Novy Jicin	0	1	0	0	0	0	0	0	0	1	0
Opava	1	1	1	0	0	0	0	0	1	1	25
Ostrava	2	2	0	1	2	0	0	0	1	2	0
Moravian region	5	6	1	3	3	3	0	0	2	5	25

(Source: EPIDAT)

Table 2. Reported cases of taeniosis in the Czech Republic period 2006-2009

Years	Cases	Prevalence (%)
2006	13	0.013
2007	26	0.026
2008	7	0.007
2009	3	0.003

(Source: EPIDAT)

2.2.2 Geographic distribution: Status in EU

T. saginata is known in the countries of the EU, but there is little exact information about the occurrence of this parasite [39,49].

23 countries, mostly situated in Western and Central Europe, had available reports on occurrence of *T. saginata*, and for some of them there was only information gathered before 1990. Frequency of occurrence determined by meat inspection was mostly low (less than 6.2% in 95 % of the records). This data was different for different countries and their parts. Higher range of prevalence was found by serology and detailed examination of meat (0.41–14%), according to Minerva Laranjo-González et al., [49].

Their study calculated occurrence rates of bovine cysticercosis in various European countries. Most of the data was derived from usual inspection while only a few studies used other methods of diagnostics, including serological tests and detailed meat inspection [49].

Data from 50 different sources were gathered by Minerva Laranjo-Gonzales et al., [49] on prevalence of bovine cysticercosis in Europe on the basis of meat inspection. There was a small number of published reports and/or personal communications per year as only three reports or less were published in most of the years. According to the reports, bovine cysticercosis has been present in Europe for decades and still can be observed today (Figure 2). Most of the data was related to the period after 1990, as only reports which were published after that year were chosen to be included. Still, using the reports which were included, we managed to extract and determine data on bovine cysticercosis in the period between 1918 and 2013 while for certain other countries, namely, Hungary, Greece, Slovenia, Serbia and the Netherlands, it was possible to identify reports on prevalence before 1990 (Figure 3). There was a generally low prevalence rate recorded by ordinary meat inspection throughout Europe as the prevalence was found to be below 6.2% in 95% of the records and below 4.3% in 90% of the records [49].

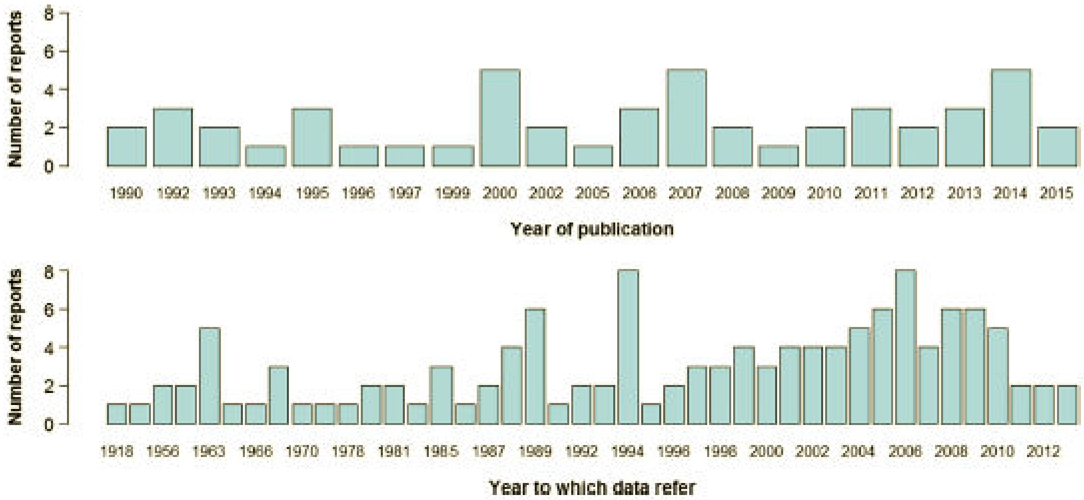

Figure 2. Number of sources of publication reporting prevalence and per year of data collection

(Source: Minerva et al., 2016)

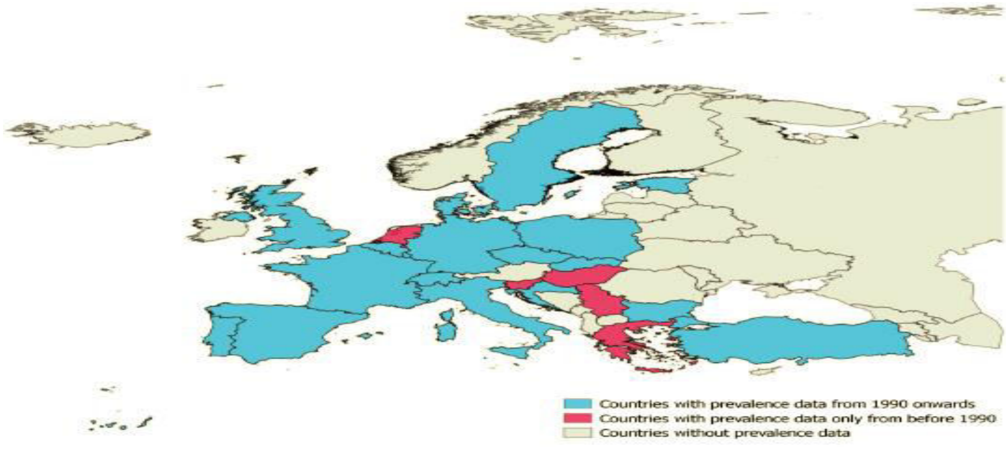

Figure 3. Map of Europe representing availability of prevalence data per country.

(Source: Minerva et al., 2016)

Sweden and Belgium reported data referring to *cysticercus* in slaughtered cattle for the period 2011-2013. 808,075 cattle were inspected at the slaughterhouse in Belgium and 994 (0.12%) carcases were found to be positive for *T. saginata* cysts in 2013, of which 16 were severely contaminated. In 2012 and 2011, the percentages of positive results were higher at 0.15% and 0.16%, respectively [23].

417,384 bovine carcases were inspected in Sweden for C*ysticercus* cysts (*T. saginata*) in 2013 and one of them was reported positive, which does not differ from the findings which were reported in 2012 and 2011 [23].

2.3 Pathogenesis and diagnosis

Disease in human

Mostly, human taeniosis does not display any specific clinical signs or it show itself as a mild non-specific gastrointestinal illness, showcasing the symptoms of abdominal pain, digestive disturbance, nausea, diarrhoea, and anorexia [41,77].

Disease in cattle

Cysticercosis is generally asymptomatic in cattle even when the infection is rather heavy [41]. The fact that cysticerci can be found in the in the cattle's muscle tissues is not looked upon as a symptom under natural conditions. However, when calves were massively infected by *T. saginata* eggs, a serious case of myocarditis and heart failure was observed, which is associated with the presence and development of cysticerci in the heart which may result in fatal outcome in 14-16 days [77].

Diagnosis in human

Due to the absence of specific clinical signs of *T. saginata* infection, humans are diagnosed on the basis of laboratory findings. Since it is generally shows no symptoms, the infection seldom diagnosed based on clinical picture. When the patient complains about crawling sensation in the perianal area, this disease is under suspicion [58]. The physician can diagnose *T. saginata* after finding eggs or proglottids. Eggs and proglottids begin occurring in the sample of stool three months after the start of the infection. Within the initial three months the methods of identifying antibodies can be deployed in order to find antibodies in a blood sample. The eggs of all *Taenia* species are almost identical so identification can only be performed at genus level. Stool PCR and loop-mediated isothermal amplification (LAMP) can differentiate *T. saginata* from

T. solium. However, these methods are not generally available, while their benefit for case detection in field conditions still needs to be proven [48,53]. Gravid proglottids can be examined in order to identify the particular species for educational purposes. Additionally, endoscopic examination can be done for diagnosis [58].

The following morphological features should be emphasized in *T. saginata* diagnosis. Identification of specific species is done by examining gravid proglottids. Microscopic examination reveals gravid segments of *T. saginata* which show more than 13 uterine branches [58].

Diagnosis in cattle

Meat inspection concentrates on the internal and external masseter muscles while the tongue and the heart are incised and checked [41]. During inspection, each predilection site check was performed in compliance with the guideline by MoA as follows; deep linear incisions were made for masseter muscle parallel to the mandible; examination of the tongue was made from base to top, the hearts were incised from base to apex to open the pericardium and the cardiac muscle was also incised for detailed examination. Deep, adjacent and parallel incisions were performed above the point of elbow in the shoulder muscles. Kidney and liver were examined accordingly as well. If a carcass is found to be severely infected, then the carcass, meat, offal and blood are all condemned. Severe infection is described in different ways, but generally the infection is considered to be heave when cysts are detected at two of the predilection sites plus two sites in the legs [55].

The focus of immunological diagnosis is detection of serum level of specific antibodies (IgG, IgM) by enzyme-linked immunosorbent assay (ELISA), according to Parija [58]. The immune reaction to taeniid parasites is reported to be antibody-mediated. A positive report of antibody ELISA indicates the exposure of the animals to the infection, but they may not necessarily have a current infection [34].

2.4 Prevention, control and treatment

There are potential risks of bovine cysticercosis infection of food animals that arise from human activities such as animal husbandry, sanitation and waste disposal (Figure 4). Practical solutions to mitigate the risks of transmission of taeniid eggs from infected humans to cattle are described below [79].

In cattle Sanitary measures are crucial for upgrade of cattle farming techniques, for example, livestock farms with regulated hygienic conditions where the cattle does not have access to grazing lands polluted by human faeces [45].

Veterinary inspections during slaughter in abattoirs should be reinforced and more meat

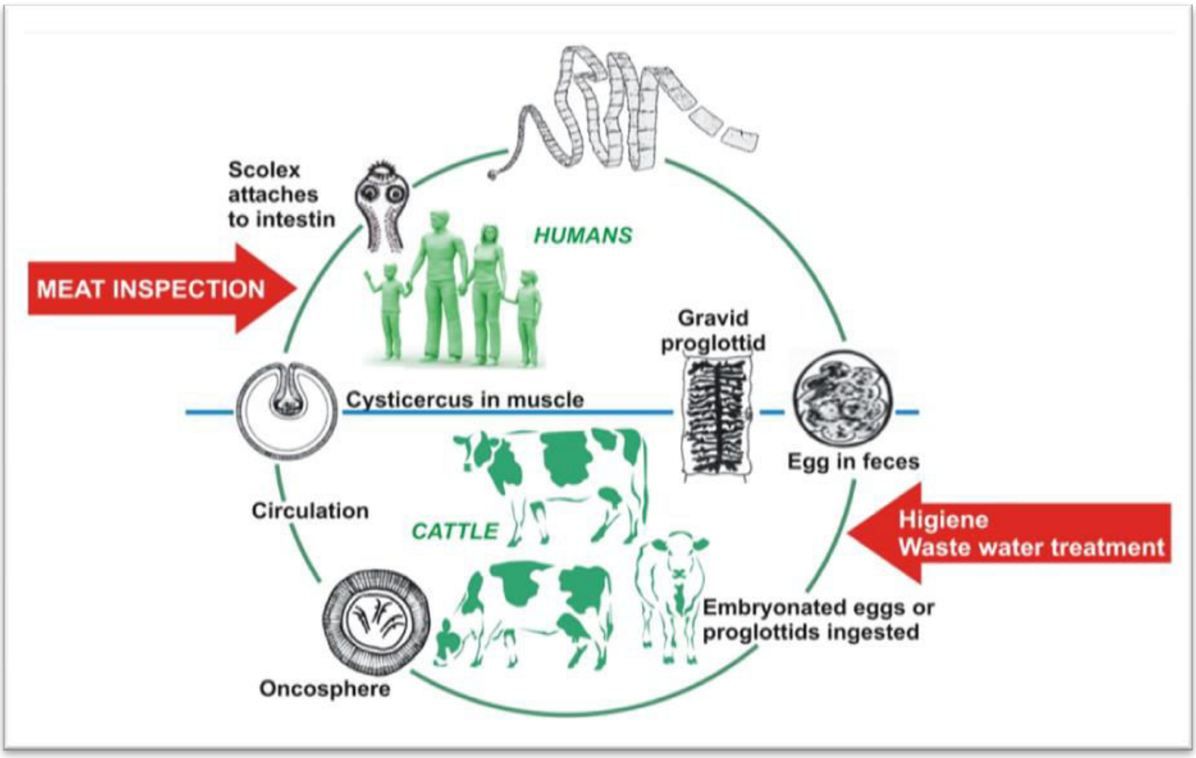

Figure 4. Control of *T. saginata* and cysticercosis (Source: Tetyana B, 2015.)

inspection both in municipal slaughter houses and slaughter establishments at markets should be undertaken [5].

Cattle producers are able to cut the risk of beef measles by means of providing enough toilets and facilities for handwashing, while also ensuring that all visitors and personnel use them in order to avoid contamination of feed bunks, feed storage areas, ditches or other places with human waste. Portable mobile toilets must be cleaned properly to prevent contamination of lagoon water used to wash areas where cattle are kept [12].

In humans

As was stated by Blancou [5], in order to ensure control of infection among humans, it is necessary to diagnose the carriers and treat them with taenicides (e.g. niclosamide or praziquantel) in order to eliminate the parasite which is considered to be the source of contamination of the cattle and environment. Enhancement of personal hygiene and establishment of proper sanitary facilities for family use is necessary. Good level of environmental hygiene should be established and suitable drainage facilities of waste water should be installed. Additionally, it is essential to provide public health education for the population, emphasizing the risks related to consumption of raw or partially cooked beef. The population should be educated to use latrines as well.

Treatment

Taeniosis is treatable with praziquantel (5–10 mg/kg, single-administration) or niclosamide, for adults and children over 6 years: 2 g, single-administration after a light meal followed 2 hours later by a laxative; children aged 2–6 years: 1 g; children under 2 years: 500 mg [65].

2.5. Echinococcosis

Echinococcosis in humans is caused by larval stages of taeniid cestodes of the genus Echinococcus. Identification of six species has been made, while four remain a public health concern: *Echinococcus granulosus* (the cause of cystic echinococcosis), *Echinococcus multilocularis* (the cause of alveolar echinococcosis), *Echinococcus vogeli* and *Echinococcus oligarthrus* (the cause of polycystic echinococcosis). The following two new species have been recognized recently: *Echinococcus shiquicus* in small mammals from the Tibetan plateau and *Echinococcus felidis* in African lions, but their potential for animal-to-animal transmission is yet unknown. According to a number of studies, these diseases represent an increasing public health concern and they can be considered to be emerging or re-emerging diseases [61].

Alveolar echinococcosis is a human disease which is caused by the metacestode stage of *Echinococcus multilocularis (E. multilocularis).* It is a considerable public health concern in Europe due to the severity of the disease, which results in fatal outcome in untreated patients [60].

Taxonomic classification

E. multilocularis is classified under the kingdom of Animalia, phylum of Platyhelminths, class of Cestode, order of Cyclophyllidea, family of Taeniidae, genus of Echinococcus and species of *E. granulosus*.

Life cycle

Small intestine of the host serves as a habitat for adult worms. Their reproduction takes place by means of releasing eggs outside into the environment in the stool of the host animal. The eggs are ready and well adapted for the challenges in the external environment and to survive there for a year if they stay in cool moist conditions, but are susceptible to desiccation. Such feature of the freshly released eggs as their stickiness assists them to adhere to the fur of definitive hosts thus setting the conditions for their spread. The eggs are transmitted to the intermediate host incidentally while grazing, foraging or drinking. Hatching of the eggs takes place in the small intestine. The resultant larvae penetrate the gut wall, and reach different organs via circulatory system. The cysts, referred to as hydatid cysts or metacestodes, are formed there. Larvae-containing cysts either comprise bladders filled with fluid, containing larval pre-tapeworms (protoscoleces), and cause cystic echinococcosis due to *E. granulosus,* or alternatively, for *E. multilocularis*a multivesiculated lesion or mass, which contains protoscoleces, that grows rapidly through exogenous budding and causes alveolar echinococcosis in rodents and other small mammals [41], on the Figure 5.

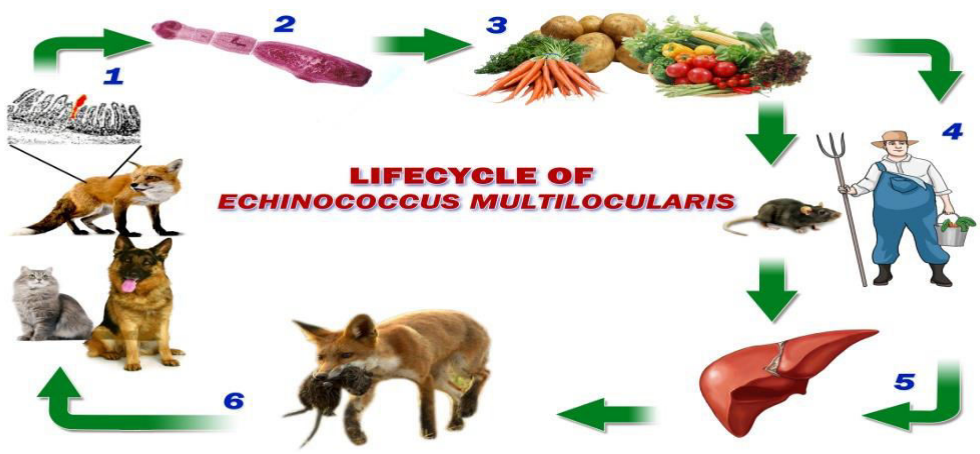

Figure 5. Life cycle of *E. multilocularis* (Source: Tetyana B, 2015.)

Morphology

Adult worms have quite little size, only 4 to 6 mm. They contain only 3 to 4 segments, and the last segment is the largest and gravid, i.e. filled with eggs. There are four suckers on the head (*scolex*), which also has numerous hooks which are needed in order to attach to the gut's wall. Otherwise, similarly to other tapeworms. *E. multilocularis* does not possess either a digestive tube**,** or circulatory or respiratory systems**.** It does not have any necessity in them as each proglottid absorbs what it needs directly through its tegument. Proglottids are hermaphroditic as each individual proglottid has reproductive organs of both sexes. They also have excretory cells which are known as flame cells (*protonephridia*). Each proglottid's reproductive organs have a genital pore – a common opening. All these organs remain in rudimentary stage in young proglottids. Their development takes place progressively, increasing the size of the proglottid while it moves towards the tail. Adult gravid proglottids are full of eggs and detach from the strobila (the chain of segments) in order to be carried into the environment with the host's faeces [41,68].

Etiology

Such types of echinococcosis as a alveolar echinococcosis, alveolar hydatid disease, multilocular echinococcosis or multivesicular hydatidosis are caused by *E. multilocularis*. It has been classified into Eurasian, North American and Chinese 'strains,' which are less distinct than those of *E. granulosus*. One group of organisms has been proposed as a distinct species, *Echinococcus shiquicus (E. shiquicus)* has been isolated only from small mammals and Tibetan foxes (*Vulpes ferrilata*) from the

Tibetan Plateau region of China. Whether it should receive its own species designation is still uncertain [13].

2.6 Epidemiology

2.6.1 Geographic distribution: status in the Czech Republic

In the Czech Republic, the cases of *E. multilocularis* occurrence in definitive (red fox, dog, cat, raccoon dog) and intermediate hosts was observed and reported [69]. Pijacek [62] reported the prevalence of *E. multilocularis* in foxes in the Czech Republic in the period between 2008 and 2009. The reported prevalence constituted 31% and 27% respectively. Following the first report on discovery of the parasites in foxes in 1995 [59], physicians began to make requests for laboratory tests for alveolar echinococcosis in persons with liver lesions, suspicious clinical signs, or both. In the period 1998–2014, examinations of 1,892 patients revealed 20 cases of alveolar echinococcosis (12 females, 8 male patients); the first two of these cases were reported in 2007 [38]. In all cases, alveolar echinococcosis characteristic imaging by using ultrasonography, computed tomography, magnetic resonance imaging, or a combination of these methods were used as the basis for the diagnosis; in 95% of the cases (19 cases), confirmation of the results was provided by *E. multilocularis* – specific serology [11] Figure 6.

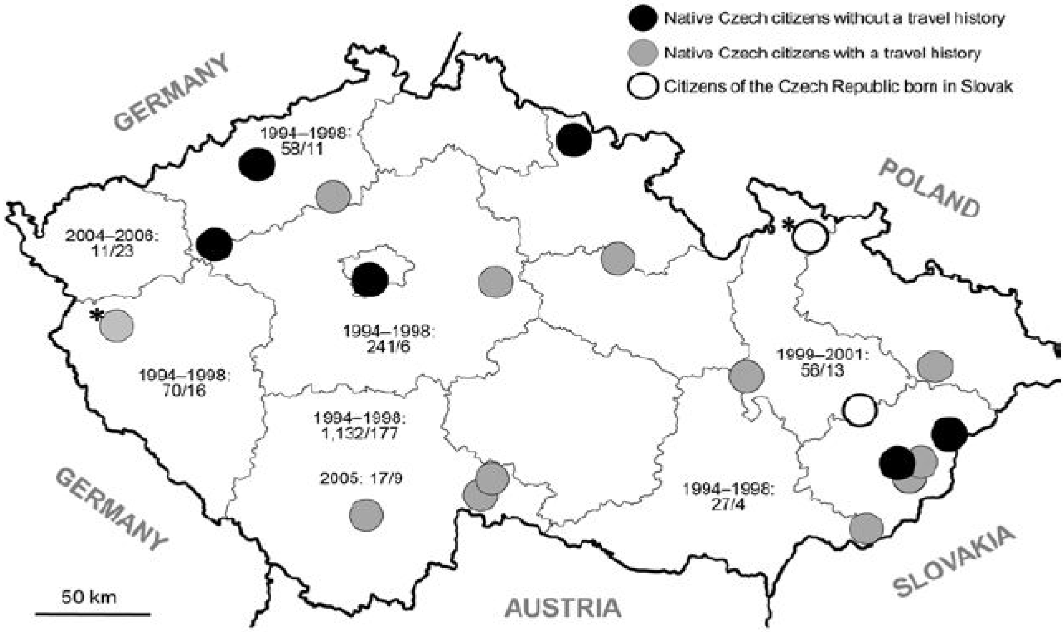

Figure 6. Distribution of human alveolar echinococcosis in the Czech Republic during 2007–2014.
(Source: CDC.).

The map of the distribution of human alveolar echinococcosis above is based on the sites of residence of 20 case patients, including their travel history. Alveolar echinococcosis cases which were already published are indicated by asterisks (*). Six patients claimed that they did not travel outside the country; 2 patients' country of birth was Slovakia and they lived in the Czech Republic for 5 and 14 years prior to the initial alveolar echinococcosis diagnosis; the rest of the patients traveled out of the Czech Republic to different countries, including those where alveolar echinococcosis is endemic. Non periodic examinations of red foxes showed that *E. multilocularis* was present in the country. Date ranges indicate the examination period; numbers separated by virgules indicate the number of foxes examined and those that were reported positive, respectively [11].

2.6.2 Geographic distribution: Status in the EU

As a result of the lifting of border restrictions in Europe, pet owners were mostly able to move across borders freely with their pets. Nevertheless, countries that remain historically non-endemic (such as the UK, Finland, Malta, Ireland and Norway) have specific regulations that demand dogs and cats to be treated for tapeworms before entry (EU Directive 998/2003). The treatment requirements were only coordinated among different countries starting from January 1, 2012 (EU Directive 1152/2011). For many pet owners and veterinarians these different regulations appear to be problematic, and veterinarians' recommendations to their clients is often improper or, sometimes, inaccurate [16]. Due to insufficient border controls and the paucity of easily available veterinary consultation, it does not come as a surprise that many pet owners do not meet the requirements of the treatment regulations. For example, Norwegian authorities demand two-time treatment of dogs and cats (once before entry and once after entry), but there is no control ensuring compliance with this regulation [54]. Infrequent and insufficient border checks, human error and oversight may result in incidental introduction of *E. multilocularis* into new territories. Thus, it is not surprising that dogs are more under suspicion for the introduction of *E. multilocularis* into Sweden and Denmark, rather than wild animals [57].

Illegal import of pets is also a significant problem, especially in Europe. It is well known that tens of thousands of pets are illegally trafficked in Europe every year. They are transported with fake or forged documents or lack them altogether. Therefore, these illegally trafficked pets are the likeliest suspects in transporting *E. multilocularis* and other pathogens, into new regions [63].

There is an increasing number of cases with *E. multilocularis* in Europe, particularly in regions where the density of foxes is quite high [14,15,26,57,70].

The confirmed cases in Europe are provided by country reports from Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, the United Kingdom (Figure 7).

Figure 7. Reported confirmed echinococcosis cases: number of cases, EU, 2014 (Source: ECDC)

2.7 Pathogenesis and diagnosis

Disease in humans

A specific feature of alveolar echinococcosis is 5–15 years of asymptomatic incubation period and slow evolution of a primary tumor-like lesion which usually can be found in the liver. Among the symptoms there is weight loss, abdominal pain, general malaise and symptoms of hepatic failure [7].

Larval metastases may spread to organs which are located next to the liver (e.g., the spleen), or they may spread to distant locations (e.g., the lungs, or the brain) after dissemination other parasite via the blood and lymphatic system. If not treated, alveolar echinococcosis can progress and result in death [7].

Disease in animals

While staying in the definitive host's small intestine tapeworms cause few ill effects. In the intermediate host, fibrosis in normal tissue is gradually displaced or induced by the cysts, and cause manifestation of the disease. The clinical signs in humans are related to the area in the body where the cyst evolves, as well as to the size and numbers of cysts or metacestode mass. *E. multilocularis* (as well as other species) seldom infect livestock and when exposure takes place the cysts are unlikely to be viable [68].

Diagnosis in human

Ultrasonography imaging is used in order to diagnose both cystic and alveolar echinococcosis. Computed tomography (CT) and/or magnetic resonance imaging (MRI) scans are normally used in addition to this technique as a back-up or a means of proving and validating the diagnosis [7].

Radiography can incidentally reveal the cysts. Different serological tests detect the specific antibodies which can support diagnosis. Biopsies and ultrasound-guided punctures can be helpful for differential diagnosis of cysts from tumors and abscesses [7].

Diagnosis in animal

In view of the life cycle, the best means to diagnose echinococcosis in definitive hosts is the demonstration of the adult worm in the intestine at post mortem or in the mucus after a diagnostic test (arecoline purgation), or finding the proglottids (tapeworm segments) in feces. In wild carnivores necropsy is commonly undertaken, for example foxes in *E. multilocularis* endemic areas. There are also tests for specific antigens in feces (coproantigens) which are highly genus specific for Echinococcus and exhibit greater sensitivity than arecoline purgation.Post mortem examination of small mammals can be used to detect *E. multilocularis* cysts but prevalences are usually low, and fox prevalence data is therefore more useful [41].

2.8 Prevention, control and treatment

In animals

There are much more difficulties in controlling *E. multilocularis* because of the existing wildlife cycle between foxes and rodents, but through the use of paraziquantel baits for foxes and dosing of owned dogs where spill-over into the dog population occurs, it has been made possible to reduce transmission [76]. Dogs also constitute a part of the transmission cycle. Dogs, which are unattended by owners, can get into contact with wild animals this way increasing their risk of infection [42].

In humans

It is difficult to prevent and control alveolar echinococcosis because the transmission cycle involves wild animals as both definitive and intermediate hosts. Regular deworming of domestic carnivores which can come into contact with wild rodents should help to cut the risk of infection in humans [41].

Studies conducted in Japan and European countries used anthelminthic baits for wild and stray definitive hosts which caused considerable decrease in prevalence of alveolar echinococcosis. Culling of foxes and stray dogs seems to be highly ineffective. Sustainability and cost–benefit efficiency of such campaigns are debatable [41].

Treatment

It is frequently very expensive to treat cystic and alveolar echinococcosis amd it is also complicated. Sometimes, trteatment requires the use of extensive surgery and/or prolonged drug therapy. Overall, there are four possible ways of treating cystic echinococcosis: treatment of the hydatid cysts percutaneously with the PAIR (puncture, aspiration, injection, re-aspiration), technique surgery, anti-infective drug treatment, "watch and wait" [68].

Treatment selection should be based on ultrasound images of the cyst, following a stage-specific approach, and also based on the medical infrastructure and human resources available [68].

In order for treatment of alveolar echinococcosis to be effective, it is crucial to diagnose the illness on early stages while radical (tumor-like) surgery and anti-infective prophylaxis with albendazole after it are still essential. If the lesion is limited to a particular area, radical surgery can be effective and curative. Unfortunately, it is usually the case that many patinests are diagnosed of alveolar echinococcosis at a later stage. Consequently, if palliative surgery is carried out without complete and effective anti-infective treatment, relapses of the disease will often occur [68].

3. AIMS OF THE THESIS

Diseases transmitted from vertebrates to humans are referred to as zoonoses. The aim of this work was focused on evidence and zoonotic aspects of parasitic developmental stages *Cysticercus bovis* and *echinococcus* in foxes. A post-mortem inspection of cattle and a laboratory examination of the developmental parasitic stages of both zoonoses were carried out. The prevalence of cysticercosis/taeniosis in the Czech Republic and human alveolar echinococcosis in the Czech Republic and individual EU countries, were also monitored.

Hypotheses

Our hypotheses were:

$\mathbf{H_1}$: detection of cysticerci in livestock was higher in the Czech Republic (increase of cysticercosis/taeniosis) in comparison with EU countries during the period 2010-2016.

$\mathbf{H_2}$**:** detection of echinococci in foxes was higher in the Czech Republic (increase of human alveolar echinococcosis/echinococcosis) in comparison with EU countries during the period 2010-2014.

4. METHODOLOGY

The master thesis was focused on cysicercosis/taeniosis and human alveolar echinococcosis/echinococcosis, which are zoonotic diseases spread between animals and people. Data were collected at the slaughterhouse and from databases in the Czech Republic and EU, using *post mortem* inspection and laboratory procedures. Afterwards, those data were statistically analyzed.

4.1 *Post mortem* inspection protocol at slaughterhouse

I had the opportunity to complete a veterinary inspection of 240 animals, under the supervision of a State Veterinary Service at the slaughterhouse, with owner's permission, in October 2016 and 2017 and there were slaughtered around 120 animals per day. The research analyzed the results of *post mortem* inspections of cattle, slaughtered at local abattoir in 2016 (Table 3), when the total number of 33,944 cattle was slaughtered; 33,756 of which was adult cattle and 188 young calves.

Table 3. Slaughtered cattle at the local abattoir in 2016

Slaughter house(2016)	Total number of slaughtered cattle adult/young
January	3185
February	2581/20
March	2789/16
April	2820/25
May	2657/15
June	2533/13
July	2558/12
August	2993/7
September	2816/27
October	2810/37
November	3187/17
December	2827/17
Total (2016)	33,756/188

Post mortem inspection was done in accordance to the Regulation (EC) No 854/2004 of the European Parliament and of the Council (2004).

The suspicious pieces of muscle were assessed by slicing head muscles and heart (Figure 10-11). Then veterinary inspection was continued, when liver, lung, intestines, fore stomachs, mammary gland and skeletal muscles were carefully inspected by knives and other tools.

Mammary glands and mammary lymph nodes were completely cut away from the body. The lymph nodes were examined and incised. The bronchial lymph nodes were examined by palpating and incising. The larynx, trachea, bronchi and small bronchioles were opened and looked for worms. Heart was removed from lungs at the level of large blood vessels. Liver was removed and examined visually. The muscles of various parts of the body, especially lumbar and thigh muscles, were examined and incised.

During the meat inspection, carried out at slaughterhouse, cow-facial muscles, heart, tongue and muscles of diaphragm were inspected to confirmation for further diagnosis, mainly cysticercosis. Skeletal and heart muscles were examined by cutting *musculus masseter*, *musculus pterygoideus* and *septum cordis*, tongue and *oesophagus* palpation, as well as by inspecting other visible muscles or, if necessary, cutting into them.

4.2 Detection of viability of *Cysticercus bovis* and *E. multilocularis* in laboratory conditions

The tissue samples of were placed into the fridge at the slaughterhouse, then transported to State Veterinary Instiute (SVI). The laboratory procedures were carried out according to Good Laboratory Practice (GLP), mandatory for SVI in the EU countries. The tissue samples were investigated and results compared with positive controls (cysts in muscle samples from the earlier bovine infections). The cysts were put on the Petri dishes to the thermostat (+ 37^{o}C) and after 48 hours the samples from Petri dishes were taken. If the head of the parasite (*scolex*) was visible, test was positive and bovine cysticercosis was confirmed; if not, the result of the laboratory test was negative respectively (Figure 8). All preparation of *E. multilocularis* eggs was carried out at SVI in Prague laboratory using inactivated eggs which had been frozen for more than 3 days at below -80°C, and kept in ethanol. Eggs were transferred from the storage container to a plastic Petri dish containing physiological saline then transferred to the thermostat (+37^{o}C) and after 48 hours the samples from Petri dishes were taken. If *scolex* was visible, test was positive and *E. multilocularis* was confirmed; if not, the result of the test was negative. Laboratory procedures carried out in SVI in Prague was under supervision of Ing. Ivan Pavlásek, DrSc.(Figure 9).

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Figure 8. Test to viability of *Cysticercus bovis* in muscles

(Source: Sikasunge et al., 2008)

Figure 9. Laboratory procedures at SVI in Prague under the supervision by Ing. Ivan Pavlásek, DrSc (photo by Lukesova 2017)

4.3 Collection of data from databases

Cysticerci data related to period 2010-2015 were collected from the State Veterinary Institutes. However, in the period of 2016 was obtained from local abattoir in Highland and taeniosis data, from years 2010-2016, from the Czech Republic (EPIDAT) and EU (ECDC) databases. Data, related to fox echinoccosis in the Czech Republic and EU, was used from published studies. In human alveolar echinococcosis, the data was downloaded from the Czech Republic (EPIDAT) and EU (ECDC) databases, related to period 2010-2014.

The data were obtained from the official database of the European Centrum for Disease Control and Prevention (ECDC), then I processed reported cases of alveolar echinococcosis (the number per 100,000 population in the EU), by means of statistical analysis of the data.

The individual states of the European Union (EU) were divided into four geographic regions: Central Europe (C) which involves the Czech Republic; Western Europe (W), Southern Europe (S) and Northern Europe (N) which are shown in Table 4.

4.4 Statistical data processing

The obtained data were transferred to Microsoft Office 2007. The program Statistica.cz, version 12 (StatSoft, USA) was used for statistical evaluation of the results. Data were expressed as means ± SD (standard deviation). One-way analysis of variance (in case of the cases of echinococcosis in European regions for all years*)* and two-way analysis of variance (ANOVA) and by following select POST-HOC test (Fisher´s LSD test) were utilized. 95% of confidence interval was selected. The figures were created by the statistical program Statistica.cz (version12) as well.

Table 4. Reported confirmed human alveolar echinococcosis cases

Region	Countries	2010	2011	2012	2013	2014
C	Austria	21	7	3	11	14
	Czech Republic	5	0	0	2	20
	Hungary	9	11	6	5	2
	Poland	36	19	28	39	48
	Slovakia	9	2	3	20	8
W	Belgium	1	1	6	15	15
	France	33	45	49	34	32
	Germany	117	146	118	127	112
	Ireland	1	0	0	1	0
	Luxembourg	1	1	0	0	0
	Netherlands	35	45	50	33	30
	United Kingdom	7	9	7	14	25
S	Bulgaria	291	307	320	278	302
	Croatia	*	*	0	0	20
	Cyprus	0	2	0	0	0
	Greece	11	17	21	10	13
	Malta	0	0	0	0	0
	Portugal	3	1	2	3	4
	Romania	55	53	96	55	36
	Slovenia	8	8	6	6	5
	Spain	82	53	96	94	77
N	Estonia	0	0	3	3	1
	Finland	1	1	3	4	0
	Iceland	*	*	*	0	0
	Latvia	14	10	8	7	13
	Lithuania	23	24	23	23	22
	Norway	1	3	2	2	0
	Sweden	30	19	16	16	21

Legend: C – Central Europe; W – West Europe; S – South Europe; N – North Europe,
* = no data reported

5 RESULTS

During period 2010-2016 total number of 224,185 heads of cattle were slaughtered at the abattoir in region the Highland. There were generally identified 24 positive cases of *Cysticercus bovis* and there was a prevalence of cysticercosis (0.01%), which was shown in Table 5.

The highest number of positive cases was demonstrated in eight cases in 2010 and later, mainly in monitored period 2010-2014. Cysticerci were found mostly in *septum* of heart and less frequently in masseteric muscles (Figure 10-11).

Negative cases were observed by State Veterinary Service at the slaughterhouse in period 2015-2016, which means 0% prevalence.

Table 5. The number of slaughtered cattle and the presence of zoonotic parasites *Cysticercus bovis* during period 2010-2016

Years	Total number of slaughtered cattle	Number of positive animals	Prevalence (%)
2010	**34,709**	**8**	**0.02**
2011	**33,337**	**5**	**0.01**
2012	**29,786**	**3**	**0.01**
2013	**29,447**	**7**	**0.02**
2014	**31,869**	**1**	**0.003**
2015	**31,093**	**0**	**0**
2016	**33,944**	**0**	**0**
Total	**224,185**	**24**	**0.01**

Figure 10. Incising of *musculus masseter* at abattoir (Photo by Lukesova, 2016)

Figure 11. Inspecting of bovine heart at abattoir (Photo by Lukesova, 2016)

Detection of viability was carried out at SVI, during period 2010-2016, by using the test of *Cysticercus bovis* viability. The veterinary inspection of samples was not observed positive cases of *Cysticercus bovis,* either in active (infectious) or inactive (calcified) form of the parasite, during the whole year 2016.

Positive cases of cysticerci in previous years (2010-2014), although they were found but were not viable because were saturated with calcium salts (so called calcification).

According to State Veterinary Authority of the Czech Republic a total number of 244,604 cattle in 2016 was slaughtered, while during the same period were only 33,944 cattle, with no positive cases at abattoir in region Highland (Table 6).

Firstly, it was made a comparison on the regional level (slaughterhouse at Highland), according to obtained data in 2016, month by month. Afterwards these results were compared to the Czech national data, which showed the total number of slaughtered animals with detected prevalence of bovine cysticercosis after *post mortem* inspection at abattoir in the Czech Republic.

For the same reporting period of cysticercosis were confirmed positive cases of *T. saginata* tapeworms in the stool of people, with suspected human taeniosis. According software EPIDAT, from the National Institute of Public Health in Prague (SZÚ Praha), were reported 78 positive cases of taeniosis [47]. Positive cases, however, differed in each year (Table 7), the highest number of 30 reported cases were recorded in 2013. In the following years there was a gradual decline in reporting positive human cases with this diagnose. The prevalence of taeniosis, caused by *T. saginata*, was not recorded in the European ECDC database and no reported data were available for comparison between the Czech Republic and the EU countries.

Table 6. Positive samples of cysticerci in slaughtered cattle at the local abattoir in 2016

Slaughter house(2016)	Total number of slaughtered cattle adult/young	Positive samples (cysticerci)
January	3185	-
February	2581/20	-
March	2789/16	-
April	2820/25	-
May	2657/15	-
June	2533/13	-
July	2558/12	-
August	2993/7	-
September	2816/27	-
October	2810/37	-
November	3187/17	-
December	2827/17	-
Total (2016)	33,756/188	-

Table 7. Reported cases of taeniosis in the Czech Republic from 2010-2016

Years	Number of human cases	Prevalence (%)* number per 100 000 population
2010	4	0.004
2011	9	0.009
2012	6	0.006
2013*	30	0.03
2014	18	0.018
2015	6	0.006
2016	5	0.005

*registered outbreak of taeniosis in Opava (Martinkova, 2016)

(Source: The National Institute of Public Health (EPIDAT, 2017))

In the next part of our work were examined the data obtained from the reports on the occurrence of human alveolar echinococcosis in the EU countries (including the Czech Republic) in the period of 2010-2014.

Figure 12 showed that the highest occurrence of human cases of alveolar echinococcosis was demonstrated throughout the period in South Europe (S). The highest positive cases occurred in the years 2010 (61.22 ±94.26), 2011(60.22 ±98.60) and 2012 (60.11 ±105.35); although later there was a slight decline in reported cases during the years of 2013 (49.55 ±91.64) and 2014 (50.77 ±97.36).

Next to South Europe we can see West Europe (W). As we can see from figure 12 cases of alveolar echinococcosis are also higher than Central Europe (C) and North Europe (N). Most highest occurrence of human cases of alveolar echinococcosis was reported period 2011 (35.28 ±52.81), lowest demonstrated period 2010 (27.85 ±42.07). Next remained three years we can see slight declining with results by years 2012 (32.85 ±43.49), 2013 (32.00 ±44.02) and 2014 (30.57 ±38.24)

The lowest occurrence of human alveolar echinococcosis cases was found in Central Europe (C) between 2011 (7.80 ±7.59) and 2012 (8.00 ±11.37). Later, in 2013 (7.85 ±8.47) and 2014 (8.14 ±10.25) were reported almost identically the lowest occurance of human cases of alveolar echinococcosis in North Europe (N). Years 2010 (16.00 ±12.68), 2013 (15.40 ±14.87) and 2014 (15.60 ±18.62) were quite higher occurrence of human alveolar echinococcosis than 2011 and 2012 in Central Europe (C). In North Europe (N) years 2010 (24.28 ±35.82), 2011 (22.57 ±35.78) and 2012 (22.28 ±35.57) were unpleasant comparing to years 2013-2014.

While no statistically significant difference in reported cases of human alveolar echinococcosis between the reference years, depending on the manifestation in different regions of EU.

Figure 13 resulted statistically evaluated data related to the occurrence of human alveolar echinococcosis in all European regions (C, W, S, N) and the whole monitored period from 2010 to 2014.

A statistical evaluation of the data was showed the highest occurrence of human cases of alveolar echinococcosis, especially in Southern Europe (56.38 ±93.16). The second position in the EU countries hold Western Europe (31,71 ±41,77). A smaller number of cases was registered in Northern Europe (17.03 ±27.63) and ranked third. Last position in the EU hold Central Europe (12.56 ±12.95).

Statistical significance between regions in Central Europe (C) and South Europe (S) was demonstrated, as well as between regions in North Europe (N) and South Europe (S). Other differences between regions were not significant.

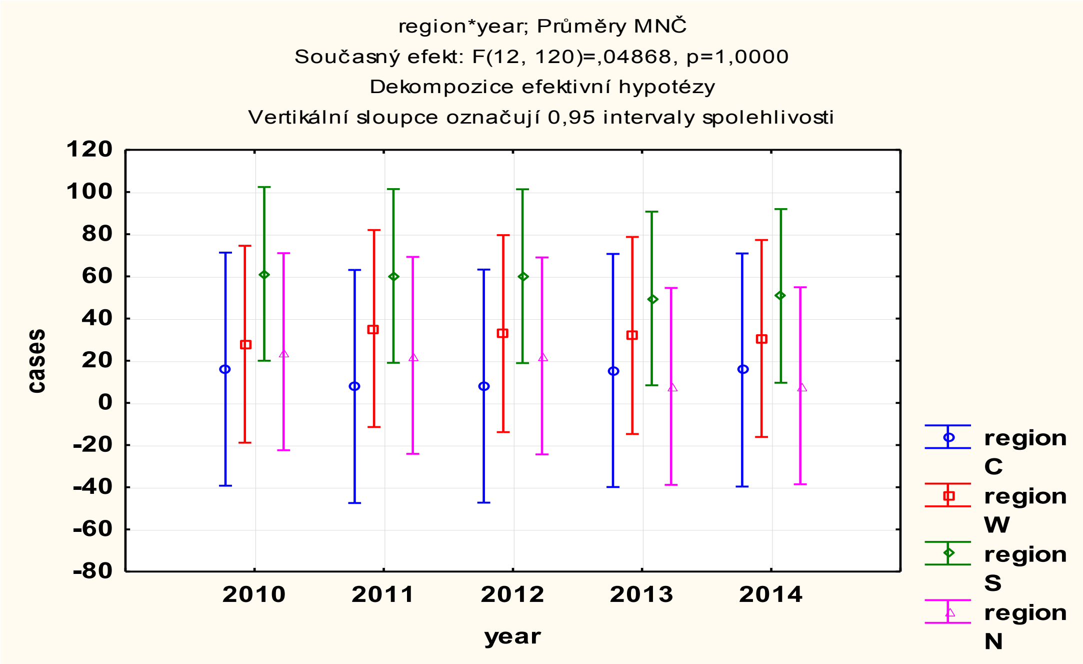

Figure 12. The average cases of human alveolar echinococcosis in particular years depending on European regions

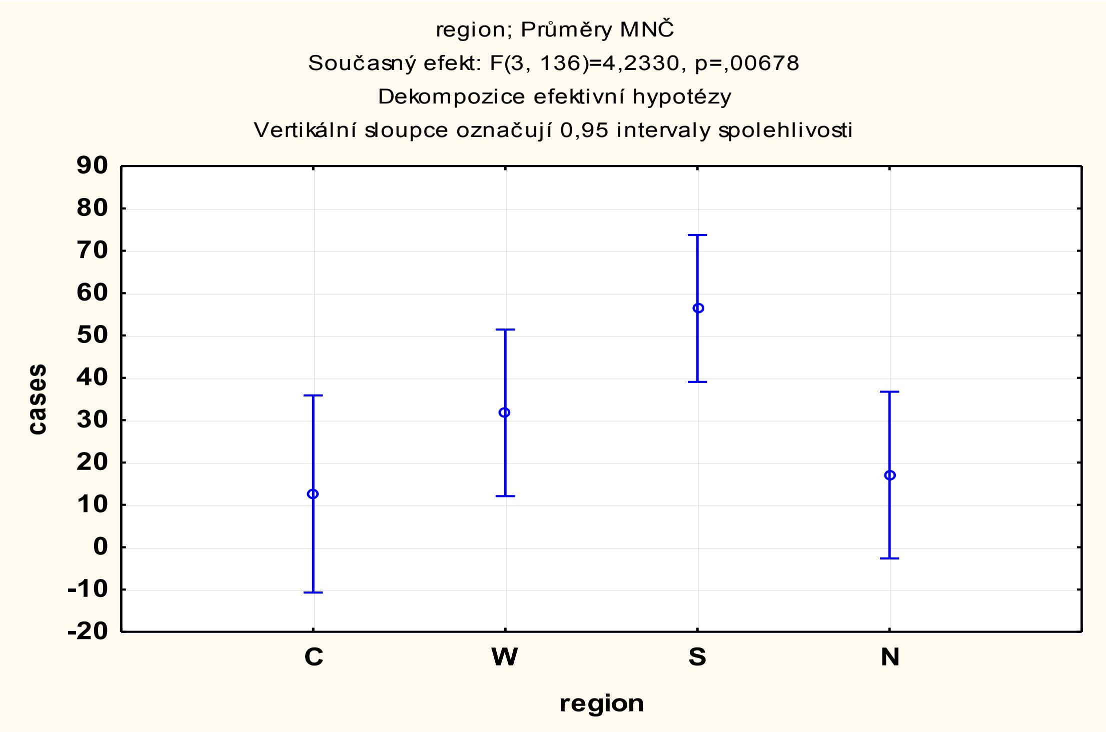

Figure 13. The average cases of human alveolar echinococcosis in European regions for the entire period (2010-2014)

The Figure 14 shows the number of reported cases of human echinococcosis that may be caused by human developmental stages (metacestods) by different types of parasites such as *E. granulosus* or *E. multilocularis*.

The results showed that the highest occurrence of *E. granulosus* was confirmed by laboratory tests in South Europe (74.00 ±133.48), West Europe (41.25 ±25.51), Central Europe (8.71 ±5.59) and the latest in North Europe (3.60 ±1.81). Confirmed cases related to *E. multilocularis*, has not been recorded in South Europe, but the highest occurrence was recorded in West Europe (31.75 ±4.78) and Central Europe (6.50 ±4.13), too. From the ECDC database was also obtained data when the European laboratory could not distinguish between the parasite genes, such as these kinds of parasites (*E. granulosus* or *E. multilocularis*). This reports were included in the table as "unknown/not reported", the results showed that the highest occurrence was reported from South Europe (27.18 ±31.37), then West Europe (20.57 ±11.25), North Europe (8.22 ±6.67) and at the end from Central Europe (7.86 ±8.69).

Statistical analysis showed that reports related to *E. granulosus* has been statistical differences ($p<0.05$) between Central Europe (C) and South Europe (S), and between North Europe (N) and South Europe (S), while parasitic zoonotic species *E. multilocularis* showed no significant differences between regions. As was shown in Figure 14 (reports related to "unknown/not reported"), no significant differences between regions were recorded.

In all European countries were reported the higher incidence of human diseases caused by *E. granulosus* parasite, during both years 2013 (36.09 ±82.18) and 2014 (33.76 ±82.35) in Figure 15, while reported cases as "unknown / not reported" in years 2013 (15.89 ±23.57) – 2014 (18.00 ±16.63) and *E. multilocularis* years 2013 (14.85 ±14.11) – 2014 (11.71 ±12.28) were reported less frequently in the EU.

Statistical analyses showed no significant differences between parasitic species *E. granulosus* and *E. multilocularis* or "unknown/not reported" during period 2013 and 2014.

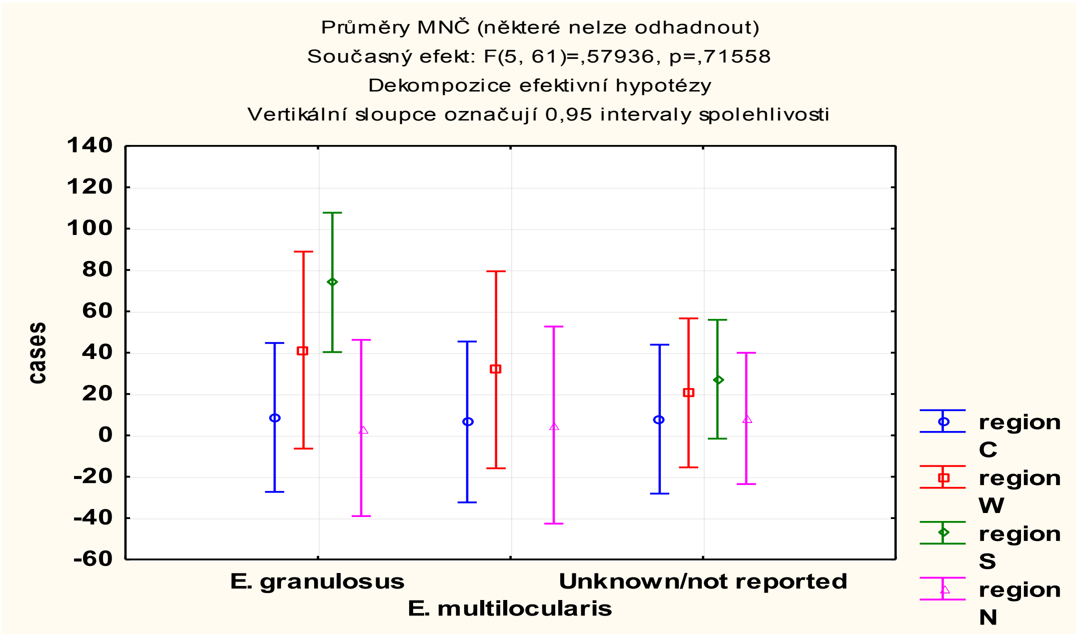

Figure 14. The average cases of echinococcosis depending on the species in European regions

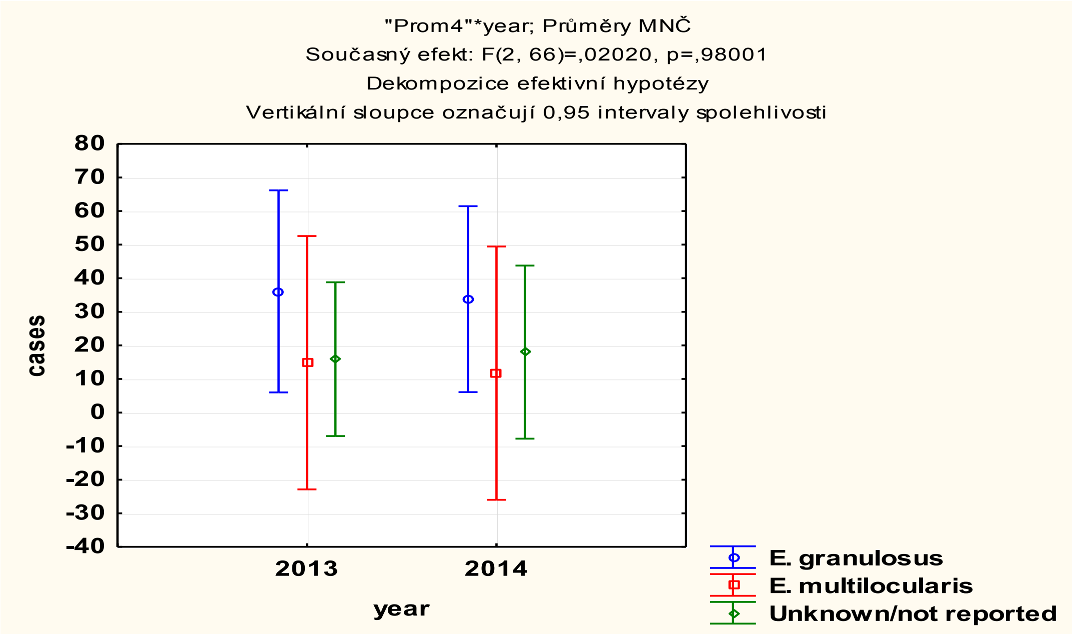

Figure 15. The average cases of echinococcosis depending on the species in years 2013 and 2014 in all Europe

6. DISCUSSION

The results of our research shown the low prevalence of cysticercosis given the high number of the examined carcasses and organs, and as compared to the previous researches in local level [46], the percentage of detected to cysticerci cattle was significantly lower. However, over floods can impact can impact to change correlation of prevalence of cysticecosis which took place in 1997 and 2002 in the Czech Republic.

Our result with prevalence of cysticercosis was 0.01% within some European countries, as Belgium and Sweden, according to EFSA and ECDC [23]. They reported higher prevalence (0.12%) infected cattle in Belgium in 2013, which means rejection of hypothesis related to cysticercosis. However, same organizations reported very low prevalence of cysticercosis in Sweden, with just one positive case in 2011 and 2012. Representation of bovine cysticercosis was not in databases of EU like OIE. The rest of EU countries did not provide any reports related to bovine cysticercosis after 1990s. We assume that it is connected with business purposes of those countries.

A few researchers interested the age of inspected cattle. According to Minerva Laranjo-González et al., [49] only in a few cases prevalence was given for different groups of age. During our *post mortem* inspection *C. bovis,* mainly detected from adult cattle. Zdolec et al., [81] reported during 2005-2010 the prevalence confirmed in calves (0.014%) was lower than in steers (0.093%) and much lower than in cows (0.69%). In the United Kingdom, period 2008-2011 the prevalence detected in calves and adults was 0.008% and 0.032%, respectively [35]. Author Dorny et al., [20] during personal communication with Minerva Laranjajo- Gonzales et al., [49] described epidemiological situation observed in Belgium where positive cattle are normally adult cattle and calves are generally negative at meat inspection. Also, Zdolec et al., [81] find correlations of *C. bovis* in cattle age by older animals are exposed to risk for a longer period than younger.

Browsing research articles we found few studies, which reported results based on more sensitive inspection methods such as serology or detailed meat inspection. Observation carried out in Belgium [20] and eastern Spain have detected, through antigen ELISA three to 55 times higher prevalence then obtained prevalence by through meat inspection [3]. Also, research carried out in Germany under effective determination prevalence through antibody test and *post mortem* inspection showed effectiveness of antibody test than through meat inspection [1]. Other reports based detailed meat inspection in Spain, Switzerland and Belgium showed 2 to 50 times higher prevalence than reported prevalence through by routine meat inspection [24,31].

However, situation with taeniosis in the Czech Republic was not good like situation with cysticercosis. There was one registered outbreak in 2013 in the Czech Republic [47]. During the period 1982-2012, a total number of 4127 cases was registered taeniosis cases. Noticeable trend of cases with taeniosis turned out to be 739 cases (incidence of 7,2/100,000) in 1982 to 3-9 cases (incidence of 0.03-0.09/100,000) in 2008-2012 [56]. Outbreak of taeniosis in the Opava which enters to Moravian region was registered by Regional Health Authority between 2013 and 2014 with 25 cases [47]. According to The National Institute of Public Health database EPIDAT cases of human taeniosis from 2013 is extremely going up comparing same database of same organization from 2008 to 2012 cases of taeniosis is declining, which means acceptation of hypothesis related to taeniosis. In EU estimating of taeniosis prevalence is impossible. The rest of countries EU not provide reports to ECDC cases of taeniosis. Only few studies reported results related with teaniosis. Cabaret et al., [8] estimate prevalence of taeniosis in EU from 0.01% to 10%.

Taeniosis is not notifiable disease. According to selling taenicidal drugs usually estimates taeniosis incidence [19]. Eggs of *T. saginata* and *T. solium* are quite similar and problem with distinguish of them still actual [50]. Diagnosis of taeniosis by stool microscopy is not effective, therefore this method is not recommended [2]. Even antigen test such as ELISA based coproantigen detection cannot distinguish two species of Taenia. However, molecular diagnostic method such Stool PCR and loop-mediated isothermal amplification (LAMP) can distinguish between *T. saginata* and *T. solium* but these analysis is not routinely available. Usefulness of this method at the field conditions also has yet to be established [48,53]. It means estimating real prevalence of taeniosis related to species makes difficulties.

Finally, in Europe there is no sufficient information for the introduction of such systems, especially in the eastern countries. Data quality and reporting of cases of cysticercosis of *T. saginata* in Europe should be improved. Research identifying risk factors should be conducted in different countries and for different production systems. This information should provide a better understanding of the epidemiological situation and identify factors that determine the level of risk and therefore the implementation of risk-based approaches.

Echinococcosis is a rare disease in the EU. Since information on species was available only for 27% of registered confirmed cases of echinococcosis, it is impossible to draw conclusions about the trends of alveolar echinococcosis and cystic echinococcosis. The general trend of echinococcosis provides limited information for public health actions, as these two diseases require different prevention and control strategies. Thus, speciation in the diagnosed cases is significant.

Human alveolar echinococcosis, as we mentioned in results that showed low occurrence in the Czech Republic, and as compared to previous studies carried out on the local level [38] confirmed cases of human alveolar echinococcosis increases 1-1.5 times. The same authors reported that first confirmed diagnosed cases were registered in 2007, while database of The National Institute of Public Health EPIDAT reports first diagnosed confirmed cases related to human alveolar echinococcosis in 2016 [27].

Cases of echinococcosis are registered in most EU countries, although not very often, with most cases reported from certain several countries. The greatest risk of population risk was observed in Bulgaria, where the level of registered cases with human alveolar echinococcosis in 2012 was 23 times higher than the overall incidence rate in the EU, which is deemed as the rejection of hypothesis related to human alveolar echinococcosis. Over the past five years, the incidence rate of the EU has remained virtually unchanged. As for age, the higher morbidity rates in the elderly can be explained by a long incubation period of several years. The observed increase in alveolar echinococcosis is a cause for concern, since with the absence of treatment, the disease can be fatal. In a study conducted in Germany by the case-control method, 65% of cases can be attributed to farming. Cases were also more likely than control of dog ownership, and in particular, leaving the dog in the garden unattended or having dogs that killed the game [42]. French studies reported similar results, where people living in rural areas in endemic areas were at greatest risk, and then horticulture in non-agricultural settings in endemic areas [64]. Observation of *E. multilocularis* in foxes is important for assessing the prevalence of this parasite in Europe, in particular, since there is evidence that the distribution of *E. multilocularis* in Europe is increasing, where there are more endemic regions, high density of foxes, also in urban areas, as well as a higher density per fox [14,15,26,57,70].

Investigation of Pijacek [62] related to fox echinococcosis in the Czech Republic reported 300 positive cases from tested 1348 foxes (22%) in 2010 and 344 positive cases from 1053 foxes (33%) in 2011. After 2011 no investigation was carried out related to estimating of fox echinococcosis prevalence in the Czech Republic. We assume that it is connected with finance. However, databases of the EU also were empty to estimate real prevalence of fox echinococcosis in EU areas. Only few studies reported about prevalence of fox echinococcosis but this studies cover only one region or country. In the investigations of Bagrade et al., [4], the prevalence of fox echinococcosis in foxes were estimated in the period of 2010-2011, 23.7% and 15.9%, respectively. Detailed studies in Hungary show a higher proportion of infected foxes in the northern regions of the country and impact of environment to the infection correlation [10,74].

Tolnai et al., [74] found higher levels of fox infection in areas with constant water bodies, as well as herbaceous and arable land. Except red fox, the raccoon dog, as well as other wild canid, such as the golden jackal (*Canis aureus*) and the wolf (*Canis lupus*), can bet as definitive hosts, but there is real proof that they can support the life cycle of the parasite [22].

To sum up, most of available data related to fox echinococcosis in the EU and in the Czech Republic are scarce and its quality is low. Lack of data and no investigation after 2011 in the Czech Republic will not give any chance to estimate current situation of fox echinococcosis in Czech areas. Similar problem will arise when estimating current prevalence in the EU areas.

7. CONCLUSION

The aim of this work was summarize up to date existing knowledge about the problem related to cysticercosis/taeniosis and human alveolar echinococcosis/fox echinococcosis.

The topic of zoonoses is very important in whole world and many publications and scientific articles are constantly updated, especially from the WHO, OIE, FAO, CDC, ECDC databases, as well as from national databases from the State Veterinary Administration of the Czech Republic in Prague and State Veterinary Institutes (SVA and SVI), in cooperation with The National Institute of Public Health in Prague (SZÚ Praha) and their EPIDAT database.

Origin of parasitic zoonoses are widespread throughout the world and have been found in the Czech Republic. It is therefore important to follow the basic preventive measures and regular monitoring of the animal health status, thanks to the cooperation of the SVA and the SVI and the Ministry of Agriculture of the Czech Republic. This concerns in particular the veterinary inspection of slaughtered bovine animals for the presence of *Cysticercus bovis* and the use of tests of their viability and the preparedness of contingency plans for the flood periods, where a significant risk can be the flooding dry toilets in recreational sites or the collapse of a sewage treatment plants, resulting in an increase in the number of reported cases in grazing cattle at the slaughterhouses. The National Institute of public Health in Prague, in cooperation with their authority (Ministry of Health of the Czech Republic), monitor the presence of stages of tapeworm in infected patients who consumed raw or heat-treated beef and suggests appropriate antiparasitic treatment of taeniosis.

As regards of echinococcosis in the Czech Republic, the danger arises above all in the limited possibilities of regular hunting of foxes, which takes place only in the winter months, as well as in the sophisticated diagnostic procedures. Infecting of humans occurs the most often in the summer months, with the consequence of human alveolar echinococcosis, when a number of tourist activities, such as harvesting of blueberries, are found in the forest.

From a global perspective, various factors affecting the sources and routes of transmission of infectious agents and the monitoring of zoonoses are examined on the basis of reported epidemiological studies. The result of this work was confirmed by the fact that, cysticercosis/taeniosis and human alveolar echinococcosis/fox echinococcosis are mainly distributed in the less developed countries of the European Union. Due to the significant efforts of veterinary services in the EU countries, the epizootic and epidemiological situation has improved over the past few years due to the lower number of reports of positive cases.

The incidences were mainly monitored in rural areas around villages, often in the family of epidemies. The number of cases was closely related to hygiene and the food cooking way, which connected prevalence of diseases as well. The good epidemiological situation of both diseases is linked to the regulation rules of the European Union that all member states of the European Union are obliged to comply to.

On the other hand, we should also pay great attention to the veterinary hygiene training of gamekeepers and authorized persons who will take care of the animals. In addition, we need to cover more about zoonoses with the help of the media, about what effect zoonoses lead to and what kind of preventive methods are available in order not to become infected.

Finally, in countries where the incidences of cysticercosis and echinococcosis are sporadic, it is necessary to carefully monitor possible sources of infection imported from another country.

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List of the Appendices

APPENDICE I.

REGULATION (EC) No 854/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004

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.

APPENDICE II

Europe COMMISSION REGULATION (EC) No 2054/2004

of 29 November 2004

amending Regulation (EC) No 998/2003 of the European Parliament and of the Council as regards the lists of countries and territories

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Regulation (EC) No 998/2003 of the European Parliament and of the Council of 26 May 2003 on the animal health requirements applicable to the non-commercial movement of pet animals and amending Council Directive 92/65/EEC (1), and in particular Articles 10 and 21 thereof,

Whereas:

(1) Regulation (EC) No 998/2003 lays down the animal health requirements applicable to the non-commercial movement of pet animals and the rules applying to checks on such movement. Part C of Annex II to that Regulation contains a list of third countries where the risk of rabies entering the Community as a result of movements from their territories of pet animals has been found to be no higher than the risk associated with such movements between Member States.

(2) Under Regulation (EC) No 998/2003 a list of third countries was to be drawn up before 3 July 2004. To be included on that list, a third country should demonstrate its rabies status and that it complies with certain conditions relating to notification, monitoring, veterinary services, prevention and control of rabies and regulation of vaccines.

(3) In order to avoid any unnecessary disturbance in the movements of pet animals, and to allow time for the third countries to provide where necessary additional guarantees, it is appropriate to establish a provisional list of third countries. That list should be based on the data available through the International Office of Epizootic Diseases (OIE - World Organisation for Animal Health), the results of inspections carried out by the Commission's Food and Veterinary Office in the third countries concerned and information gathered by Member States.

(4) The list should also be based on the data provided by the World Health Organisation (WHO), the WHO Collaborating Centre for Rabies Surveillance and Research in Wusterhausen and the Rabies Bulletin.

(5) The provisional list of third countries should include countries which are free of rabies and countries in respect of which the risk of rabies entering the Community as a result of movements from their territories has been found to be no higher than the risk associated with movements between Member States.

(6) Following requests of the competent authorities of the Russian Federation to be included in the list in Part C of Annex II to Regulation (EC) No 998/2003, it appears appropriate to modify the provisional list established in accordance with Article 10.

(7) In the interest of clarity of Community legislation, it is appropriate to replace Annex II to Regulation (EC) No 998/2003 in its entirety.

(8) Regulation (EC) No 998/2003 should therefore be amended accordingly.

(9) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

Article 1 Annex II to Regulation (EC) No 998/2003 is replaced by the text in the

Annex to this Regulation.

Article 2 This Regulation shall enter into force on 3 December 2004.

APPENDICE III

I am pleased to inform that the received presentation

„THE GEOGRAPHICAL DISTRIBUTION OF PARASITIC ZOONOSES: THE ROLE OF WILDLIFE IN THE TRANSMISSION"

By

Lukešová D., Hailu F.T., Khatanbaatar I., Pebriansyah A., Azimov S.

has been accepted to be presented as a POSTER presentation at **THE IMPACT OF GLOBAL CHANGE ON THE ENVIRONMENT, HUMAN AND ANIMAL HEALTH** ***that will be held in Košice, Slovakia, in May 2nd-6th, 2017***

April, 05th 2017

Martin Tomko

Chairman of International Organizing Committee

THE GEOGRAPHICAL DISTRIBUTION OF PARASITIC ZOONOSES: ROLE OF WILDLIFE IN THE TRANSMISSION

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ABSTRACT

The main objective of this topic has been focused on the risk factors for various helminths with zoonotic potential from contrasting types of urbanized areas. Ecological changes significantly contributed to these trends: the high wild animal population and the high density of freely roaming predators (Canidae, Felidae) maintain a permanent infection pressure of these and other parasites. The direct method (necropsy of animals) and indirect method (serological investigation) has been used due to close cooperation with the State veterinary institutes in the Czech Republic, where we have had a strong support to solve this problems (e.g. study of interactions of vectors, reservoir hosts, the transmission of pathogens to humans and/or domestic animals, prevalence of diseases, biosecurity plans, etc).

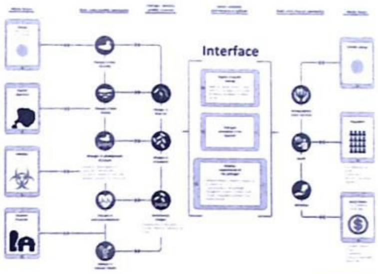

Figure 1 Cascades of Abiotic Factors, and the Components of Host–Pathogen Biotic System (for a Directly Transmitted Pathogen) That Are Affected by These Factors, Are Represented on Either Side of a Hypothetical Wildlife–Human Interface

INTRODUCTION

The changes in rural landscapes due to urbanization has a huge impact on the transmission of zoonotic parasites. Since many wildlife species are unable to adapt to these alterations in their environment (decline of biodiversity in areas of urban development), in contrast, some wild animals are attracted to peri-urban and urban habitats (e.g. availability of an abundant food supply).

In many areas composition of wildlife communities differs between rural and urban areas. Some of these highly adaptable species are also hosts for a number of parasites of public health and veterinary importance (e.g. toxoplasmosis, cryptosporidiosis, toxocarosis, echinococcosis etc.) More than 80% of human diseases are of zoonotic origin. It will be important to understand the dynamics between wildlife and domestic animal species and human population.

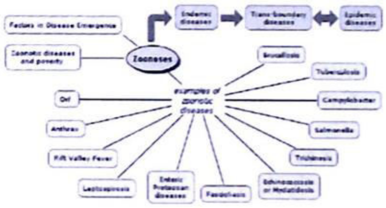

Figure 2 Zoonotic Diseases

CONCLUSION

The supposed results can be used by: veterinary authorities (planning and implementing of effective prevention strategies and a close collaboration between veterinary and public health professionals in a 'One Health' concept); veterinary and pharmaceutical industries (monitoring the effectiveness of suitable therapeutic animal products); public health professionals (knowledge of prevalence of antibodies in animal population and health risks for humans) in different regions of the Czech Republic and EU countries.

REFERENCES

Part of the results has been published:

Jankovská I, Brožová A, Matějů Z, Langrová I, Lukešová D, Sloup V. 2016. Parasites with a possible zoonotic potential in the small intestines of red foxes (Vulpes vulpes) from Northwest Bohemia (CR). Helminthologia, 53 (3): 290-293.

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