Detection of High Endemic and Zoonotic Risk Areas Regarding the Infestation with *Taenia Solium* Larvae in Pigs in Romania

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Abstract

In Romania, it is necessary to collect recent data about the prevalence of swine cysticercosis, to detect the areas of high endemicity and zoonotic risk and to prevent economic losses through proper implementation of control programs. A retrospective epidemiological study was performed during the period 2009-2013, by assessing report/surveillance systems and management of infected animals by analyzing the temporal and spatial distribution of swine cysticercosis in Romania.

The study was conducted by reporting the confirmed cases by the macroscopic examinations in slaughterhouses after slaughtering the animals, in accordance with the National Epidemic and Surveillance Strategic Program for swine cysticercosis.

In terms of spatial distribution, outbreaks were discovered in 7 counties (16.66%) of the 42 existing in Romania. During the studied period, a total of 10 disease outbreaks were identified. In terms of the temporal distribution, cases of the disease had been recorded in each year of the study period. In the north - west of Romania, were found five out of the seven recorded outbreaks. By analyzing the persistence/recurrence of the outbreaks, we can mention Alba County, located in the north-west of the country, where the infestation was maintained for 4 years (2009, 2010, 2012 and 2013). In Cluj County, the results were correlated with *T. solium* infestation in humans because of the pork meat consumption. The results demonstrate that in Romania some further studies are needed on the epidemiology of *Taenia solium* larvae infestation in swine, in rural communities, where control measures are not applied rigorously, in order to properly evaluate the scale of the phenomenon.

Keywords: *epidemiology, pig cysticercosis, Romania.*

INTRODUCTION

Muscle cysticercosis in pigs is caused by *Cysticercus cellulosae*, the larval form of *Taenia solium* (Stefan, 2000). In swine cysticercosis, the human are part of the life cycle of the parasite, being the final or intermediate host for it. The contamination of the two species (humans and pigs) is related to cohabitation, hygiene and

nutrition of both animals and humans. (Bourke and Petana, 1994).

T. solium has been ranked first on the global scale of foodborne parasites by FAO/WHO. (WHO, 2014). Regarding the human being, *Cysticercus celulosae* (*T. solium*) is located in the central nervous system and triggers neurocysticercosis, (NCC) (Garcia and Del Brutto, 2000), initiating

severe neurological disorders, the most common being seizures and chronic headache. NCC is estimated to be responsible for 30% of acquired epilepsy cases in endemic areas (Ndimubanzi *et al.*, 2010; Del Brutto *et al.*, 2005; Montano *et al.*, 2005) but may reach 50% according to Garcia (2005).

For more than one third of European Member States, the data related to the occurrence of porcine/cattle cysticercosis and taeniasis are missing and the studies regarding the incidence and transmission dynamics of these parasites are not systematically reported and therefore the magnitude of the phenomenon cannot be properly evaluated (Zammarchi *et al.*, 2013).

Despite of the available data regarding the prevalence of infection in pigs (0.6 to 60%) and humans (1.3 to 40%) their accuracy is questionable. (Ndimubanzi *et al.*, 2010; Willingham 2006; Preux *et al.*, 2005). Imperfect diagnostic tests, leading to underestimation of the real prevalence are responsible for this shortcoming.

T. solium was eradicated in the EU by inspection of pork, improving sanitation and modernization of breeding pigs (in shelters and indoor). However, as in the USA, a growing number of cases reported in EU is probably due to the increased migration (both immigration and the number of travelers to endemic areas). A review of Del Brutto published in 2012 on the NCC cases reported in Western Europe between 1970 and 2011 found that immigrants accounted for 53% of cases, European travelers 8% and 39% of the non-Europeans.

Data on the frequency of zoonotic transmission are limited. More than that, only a few studies, both in humans and animals are reported. All are based on post mortem examination in slaughterhouses and less on immunological methods.

Nowadays, many scientific approaches emphasize the need to study the epidemiological particularities of the invasion in specific socio-natural environments, in order to obtain more efficiency in surveillance, diagnosis, control and prevention (Assana *et al.*, 2012; Pondja *et al.*, 2015).

The purpose of our studies is the detection of high endemic and zoonotic risk areas regarding the infestation with *Taenia solium* larvae in pigs in Romania, and to improve the national prevention and control programs.

MATERIALS AND METHODS

Data on the occurrence and evolution of swine cysticercosis in Romania are missing, so it is necessary to carry out systematic and accurate research on the incidence of *T. solium* larvae infestation. Current programs of prevention and control of swine cysticercosis diagnosis in Romania are mainly based on the slaughterhouse necropsy exam (ord. No. 29/20014; ord. 25/2008). Teniosis is not a notifiable disease, and therefore cases are not reported nationwide, the frequency of the disease is evaluated based on laboratory tests or epidemiological investigations.

In order to assess monitoring, reporting and the management systems of infested animal with *T. solium* larvae, a retrospective epidemiological study over a period of 5 years, (2009-2013) was performed. The method consisted of analyzing the spatial and temporal distribution in 42 counties in Romania. Officially reported data were processed by Districtual sanitary veterinary and food safety authority (DSVSA). Confirmation tests on postmortem collected samples were initially performed in veterinary authorized slaughterhouses following macroscopic examination in accordance with the National Epidemic-surveillance Strategic Program for swine cysticercosis. In Cluj County, the data were correlated with *T. solium* infestations in humans following pork meat consumption. Information's were compiled for several years, regarding the number of samples from animals diagnosed with post-mortem muscle cysticercosis, by various mathematical methods, implemented in tables and figures. Prevalence of an infection in a herd is appreciated as reduced when the value is less than 3%, moderate when the value is below 10%, and very intense over this limit.

RESULTS AND DISCUSSIONS

The result of epidemiological investigations, the number of positive samples and the percentage incidence of infestation is shown below (Table 1). In terms of temporal distribution, we found that infected animals were identified during all studied years (Table 1).

Thus, we see that during this five years study, a total number of 2.220 samples were sent to the laboratories for diagnostic confirmation, of which only 10 samples confirmed the infestation.

Tab. 1. Evolution of the outbreaks number and samples during the studied period

Year	No. of samples	No. of positive
2009	162	3
2010	741	3
2011	896	1
2012	302	1
2013	119	2
TOTAL	2220	10

Tab. 2. The evolution of the number of slaughtered animals diagnosed with swine muscular cysticercosis in the studied period

No.crt.	Specification	Year				
		2009	2010	2011	2012	2013
1	Number of slaughtered animals					
	Alba	31596	87060	106051	111775	122566
	Bistrita-Nasaud	6451	19494	26299	51519	57971
	Cluj	9792	6184	574	3326	8036
	Dolj	54987	44388	50906	58094	61327
	Ilfov	18596	10206	12765	12629	14535
	Maramures	238645	224359	237260	248152	269522
	Satu Mare	70837	88134	94671	94790	89204
	Total	430904	479825	528526	580285	623161
2	Animals diagnosed with cysticercosis					
	Alba	1	1	0	1	1
	Bistrita-Nasaud	1	0	0	0	0
	Cluj	0	1	0	0	0
	Dolj	1	0	0	0	0
	Ilfov	0	0	1	0	0
	Maramures	0	1	0	0	0
	Satu Mare	0	0	0	0	1
	Total positive %	3 0.0006	3 0.0006	1 0.0001	1 0.0001	2 0.0001

During the study period, between 2009 and 2013, there was a number of 2642701 swine (tab. 2) that were slaughtered in authorized slaughterhouses in the counties where the disease was declared (Alba, Dolj, Bistrita-Nasaud, Cluj, Maramures, Satu Mare and Ilfov). After analyzing the obtained data (tab.2), an annual variation in the number of slaughtering was found, with an increase in pig slaughtering.

Compared to the number of slaughtered animals, it was found that the percentage of swine muscular cysticercosis in Alba was 0.0001% (2012 and 2013), 0.001% (2010) and 0.003% (2009); in Bistrita-Nasaud was 0.015% (2009); in Cluj

was 0.016% (2010); in Dolj was 0.001% (2009); in Ilfov was 0.007% (2011); in Maramures was 0.0004% (2010) and in Satu-Mare was 0.001%.

During the studied period (2009-2013), outbreaks were identified in 7 counties out of 42, respectively Alba, Dolj, Bistrita-Nasaud, Cluj, Maramures, Satu Mare. That represent 16.66% of the country's area while, in the other counties (35), the infestation was absent (figure 1).

Analyze of the spatial distribution of identified outbreaks during the mentioned period revealed that outbreaks were grouped in the northwest of the country.

Fig. 1. Outbreaks distribution between 2009 and 2013

Analyzing the maintaining and recurrence of infection in outbreaks it is established that outbreaks were present during all those 4 years just in one single county, respectively Alba. In six counties outbreaks were identified in one year: Bistrita-Nasaud (2009); Dolj (2009); Cluj (2010); Maramures (2010); Ilfov (2011); Satu Mare (2013) and lack of infestation was revealed for 35 counties.

Prevention and control of *T. solium* infestation in pigs are based on complete information related to the epidemiological characteristics, obtained by applying the measures for the surveillance and monitoring. Efficient action can be implemented to prevent hazards and infestation transmission by collecting, synthesizing and analyzing these data.

The reduced sensitivity, less than 15%, of antemortem diagnosis of swine cysticercosis, explains the failure of measures designed to limit or eradicate the disease in Romania. Explanation consists in the fact that vesicles of *C. cellulosae* are difficult to differentiate from large cysts of *Sarcocystis* (Stefan N., 2000).

Eichenberger *et al.* (2013) states that meat inspection against the cysticercosis requires several sections in muscle tissue. This method reports an increase in sensitivity of the post mortem examination if more incisions are made.

About 75% of swine herds, in Romania, are kept in individual holdings (backyard production) and 25% on commercial farms. It should be noted that nationwide, the number of veterinary authorized slaughterhouses is too small compared with the existing swine herds. The majority of animals slaughtered in authorized slaughterhouses are coming from commercial farms. This led to the slaughtering of pigs in household system without being subjected to veterinary control for cysticercosis. Considering these aspects, we can say that the actual prevalence of swine cysticercosis in Romania is underestimated.

In Cluj country, in 2009-2013 the data obtained in the veterinary field (1 case of cysticercosis declared in 2010) cannot be correlated with the data obtained from human sector, because the individuals submitted for diagnosis were recorded as negative, but people with high risk of exposure (from rural areas) does not follow the investigations for teniasis.

On the contrary in Botosani country, there were no cases of swine cysticercosis (probably because the slaughter take place in the backyards, in the absence of veterinary control), but in November 2014, a male person aged 62 years, was diagnosed with neurocysticercosis.

To avoid these discrepancies and to perform a correlation between data obtained in the

veterinary sector with the data obtained in human field, decision makers should consider the inclusion of taeniasis among the official reportable diseases, in order to develop a monitoring and reporting program.

Although the risk factors in the case of swine cysticercosis were identified (such as leaking sewage sludge from wastewater treatment, flooded pastures, water consumption effluent flows by tourists), meat inspection remains, for now, the only instrument for control.

New molecular and serological diagnostic techniques have been developed for the detection of tapeworm and larvae in humans and animals, which are promising candidates to replace routine diagnostic procedures in most laboratories and slaughterhouses, but they are very expensive and therefore could not be applied to all slaughtered pigs. Moreover, since the lack of cross-sensitivity, new approaches (genomics, proteomics, transcriptomics and nanotechnologies), should be used for diagnosis.

The data obtained after performing epidemiological study in Romania, are similar to data on the worldwide evolution of *T. solium* infestation. Because the lack of accurate diagnostic tools and knowledges, swine and human cysticercosis remains neglected, with an underestimated real prevalence (Rajshekhkar *et al.*, 2003; Zoli *et al.*, 2003; Praet *et al.*, 2009).

This original study represents one of the first investigations of this pathology involving elements of animal health and public health in Romania.

CONCLUSIONS

This nation wide retrospective study on the epidemiology and diagnosis of *T. solium* infestation between 2009 and 2013 shows that the highest endemic area is represented by the northwestern Romania, where outbreaks have been reported in five counties: Alba, Cluj, Bistrita-Nasaud, Maramures and Satu Mare. Following the results, we believe that, at national level, the intensity of infestation with *Taenia solium* larvae is poor (10 disease outbreaks during a period of 5 years), but the true prevalence of cases is underestimated, because the number of authorized slaughterhouses is too small compared with the existing swine herds, which is why in the rural area veterinary measures are not rigorously applied. By estimating the global burden of disease, we urgently needed

to inform public authorities and advocate for more funding both for research and control/prevention.

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