

Seroprevalence of porcine cysticercosis and influence of some associated risk factors in Northwestern Romania

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Abstract

Cysticercosis is a zoonotic disease that may have a significant impact on the public health. The aim of the current study was to assess the seroprevalence of porcine cysticercosis in Northwestern Romania and the influence of several risk factors such as age, sex and season. The study included 1,288 pigs originating from 7 different counties and was conducted between May 2014 and May 2015. All pigs were reared in the traditional backyard pig system. Of these, 226 were young and 1,062 were adult pigs, 188 were males and 1,100 females. Serum samples were collected. The apDia Cysticercosis Antigen ELISA (apDia, Belgium) commercial kit was used to identify the viable cysticerci of *Taenia sp.* Out of the 1,288 serum samples, 83 were seropositive (seroprevalence: 6.4%). The risk factors' analysis showed that age (3.1% juveniles vs 7.1% adults [$P < 0.03$]) and season (9.3% fall vs 11.8% winter [$P < 0.00001$]; 3.4% spring vs 1.2% summer [$P < 0.00001$]) had a significant influence on the seropositivity, while sex had no significant influence (3.7% males vs 6.9% females, $P = 0.1$). Considering the genus specificity of ELISA commercial kit, the serology should be correlated with the *post mortem* examination in order to confirm the disease and have a *Cysticercus cellulosae* specific prevalence.

Pigs, ELISA, prevalence, age, sex, season

Cysticercosis is a tissue infection caused by the larval stage of an adult tapeworm. In pigs, two different diseases have been described, the visceral cysticercosis caused by the metacestode stage of *Taenia hydatigena* (previously known as *Cysticercus tenuicollis*, definitive hosts: canids) and the muscular cysticercosis produced by the metacestode stage of *Taenia solium* (formerly known as *Cysticercus cellulosae*, definitive hosts: humans) (Tsang and Wilson 1995). Accidentally, humans become an aberrant intermediate host of *C. cellulosae* and develop subsequent neurocysticercosis because of the faecal-oral transmission through person-to-person contact, autoinfection or consumption of contaminated food. This neglected, emerging or re-emerging disease is mostly diagnosed in developing countries (Cantey et al. 2014). Meat processing industry and pork meat consumption are economically affected by these metacestodes (Pawlowski et al. 2005).

Gaps in knowledge and control of these diseases are still present despite the emerging or re-emerging character of the muscle infection in different regions of the world. Although the *T. solium*/metacestode stage of *T. solium* complex in humans and pigs is important, neurocysticercosis is rarely reported in Romania (Dulamea 2015). Moreover, the backyard system of rearing pigs, known as a major risk factor in the epidemiology of porcine cysticercosis, is still prevalent in Romania.

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The purpose of this study was to evaluate the seroprevalence of cysticercosis in pigs bred in the traditional pig farming system in Northwest Romania and to assess the influence of some risk factors such as the season, age and sex.

Materials and Methods

Study area and time period

The study was conducted in pigs bred in the traditional outdoor system in seven counties of Northwestern Romania, between May 2014 and May 2015. The animals had access to pasture. Four villages were randomly selected in each county for sampling: Bihor (BH), Bistrița-Năsăud (BN), Cluj (CJ), Maramureș (MM), Mureș (MS), Sălaj (SJ) and Satu-Mare (SM) (Fig. 1).

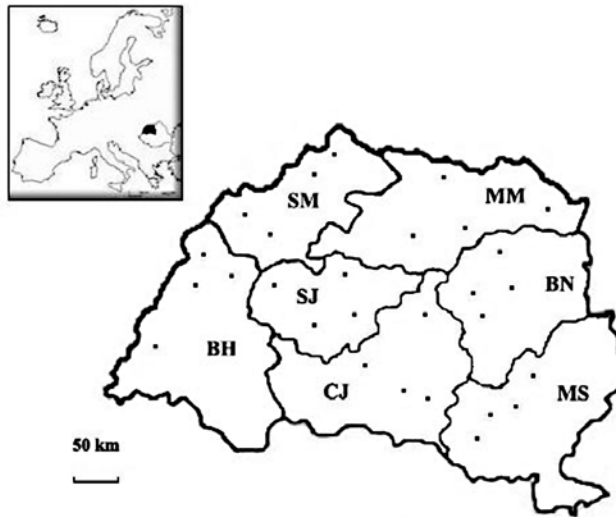


Fig. 1. The geographical origin of the collected samples from Northwestern Romania (• collection sites)

Collecting and processing of the samples

Blood samples were collected from the jugular vein. The serum was separated from the clot to avoid haemolysis and preserved by freezing at -20 °C until use.

The apDiaCysticercosis Antigen (Ag) ELISA (apDia, Belgium), a genospecific test, was used to detect viable cysticerci of *Taenia* sp. from the serum samples. The kit has monoclonal antibodies (IgG isotype) against excretory-secretory products (ESP) of viable *T. saginata* cysticerci (Brandt et al. 1992; Dorny et al. 2004). Each sample, including positive and negative control, was double tested according to the manufacturer's instructions.

The test is considered valid if the individual absorbance value of the optical density (OD) is > 1.000 for the positive control and < 0.100 for the negative control. According to the kit specifications, serum samples with Ag Index ≤ 0.8 were considered negative, ≥ 1.3 positive, and an Ag Index varying between 0.8 and 1.3 was considered doubtful.

Statistical analysis

The prevalence and frequency distribution of the overall infection were tested by Epi Info software using Chi-square test. The analysis was performed considering independent variables such as age (≤ 12 months old, > 12 months old), sex (males and females), and season (fall, winter, spring, and summer). *P* was considered significant if < 0.05.

Ethics

Serum samples were collected within The Surveillance, Prevention, Control and Eradication of Animal Diseases National Program; therefore, the approval from the Ethics Commission of the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania was not required.

Results

Collected samples

A total number of 1,288 samples were collected from all counties during the 4 seasons. The age of animals ranged between 6 and 44 months. They were divided into 2 age categories: youth 6–12 months old and adults > 12 months old. Of all samples, 226 were collected from young animals and 1,062 from adults, and 188 from males and 1,100 from females.

Seroprevalence of porcine cysticercosis

The ELISA test was validated by an OD of 2.305 for the positive control and 0.66 for the negative control. The values of positive samples ranged between 1.756 and 3.123, and of negative samples between 0.063 and 0.126. No doubtful results were recorded.

Out of the 1,288 serum samples tested, 83 were seropositive; the overall prevalence was 6.4%. The *Cysticercus* sp. infection was diagnosed in all territorial units. The lowest prevalence was recorded in the Bistrița-Năsăud county (3.3%, 95% CI: 1.2–7.0) and the highest in Cluj County (11.4%, 95% CI: 7.2–16.9) (Table 1).

Table 1. Seroprevalence of *T. solium* cysticercosis in pigs by Ag-ELISA examination in Northwestern Romania.

Factor		Frequency	Prevalence (%)	95% CI	P-value
County	BH (n=184)	8	4.3	1.9-8.4	0.03**
	BN (n=184)	6	3.3	1.2-7.0	
	CJ (n=184)	21	11.4	7.2-16.9	
	MM (n=184)	12	6.5	3.4-11.1	
	MS (n=184)	10	5.4	2.6-9.8	
	SJ (n=184)	10	5.4	2.6-9.8	
	SM (n=184)	16	8.7	5.1-13.7	
Season	Fall (n=322)	30	9.3	6.5-13.2	0.00001***
	Winter (n=322)	38	11.8	8.6-16.0	
	Spring (n=322)	11	3.4	1.8-6.2	
	Summer (n=322)	4	1.2	0.4-3.4	
Age class	Grower (6-12 months) (n=226)	7	3.1	1.3-6.3	0.03**
	Adult (≥12 months) (n=1,062)	76	7.2	5.7-8.9	
Sex	Females (n=1,100)	76	6.9	5.5-8.6	0.1*
	Males (n=188)	7	3.7	1.5-7.5	

n - number of specimens; CI - Confidence Interval; *Non-significant; **Significant ($P < 0.05$); ***Significant ($P < 0.01$)

Risk factors analysis in the study area

Seasonal seroprevalence of *Cysticercus* sp. infection was higher in fall (30/322 [9.3%, 95% CI: 6.5–13.2]) and winter (38/322 [11.8%, 95% CI: 8.6–16.0]) compared to spring (11/322, [3.4%, 95% CI: 1.8–6.2]) and summer (4/322, [1.24%, 95% CI: 0.4–3.4]) (Table 1). The differences between fall vs winter, and spring vs summer, respectively, were significant ($P < 0.00001$).

Based on the age, the prevalence of porcine cysticercosis was 7/226 (3.1% [95% CI: 1.3–6.3]) in the 6–12 months age category and 76/106 (7.2% [95% CI: 5.7–8.9]) in the > 12 months age category (Table 1). The difference between the age categories was significant ($P < 0.03$).

Based on sex, the seroprevalence was 7/188 (3.72%, 95% CI: 1.5–7.5) for males and 76/1100 (6.9% [95% CI: 5.5–8.6]) for females. The statistical analysis showed no significant difference (Table 1).

Discussion

The *T. solium* taeniasis/cysticercosis complex is a prevalent disease worldwide, with a hyperendemic distribution in many developing countries in Africa and Asia, being rarely diagnosed in North America, Europe, and Australia (Rajshekhar et al. 2003; Bouteille 2014). In 2010, Dorny et al. reported porcine cysticercosis in five European countries: Austria, Estonia, Lithuania, Poland, and Romania. However, up to date, limited data are available regarding the visceral or subserosal cysticercosis prevalence in pigs. Different studies showed an 8.30% prevalence in Uttar Pradesh (India) (Pathak and Gaur 1982), 3.22% in Jos Plateau (Nigeria) (Fabiya 1979) and 50.8% in New Zealand (Ineson 1953).

Given the potential impact of this disease on public health, our study is the first regional screening designed to assess seroprevalence of porcine cysticercosis in Romania. We identified an overall seroprevalence of 6.4%. The apDia Cysticercosis Ag ELISA kit (apDia Belgium) we used has a specificity of 99.6% for pig serum samples (Cysticercosis AG Elisa Technical Guide 2014). Despite its high sensitivity, the assay is genus-specific and does not allow the differentiation between *T. solium* and *T. hydatigena* larvae infections in pigs. Therefore, we may also have positive crossed reactions between these two larvae. Even so, the seroprevalence identified in this study was lower than the serosal cysticercosis prevalence (12%) reported in wild boar in Romania (Cucoranu 2010).

Emergence or re-emergence of *Taenia* sp. larvae infection in pigs is influenced by several risk factors, mainly by poor sanitation, the pig rearing system and their slaughter within the household under unsuitable conditions (Enander et al. 2010; Gabriël et al. 2015). In our study, the main risk factor is considered poor sanitation in the selected locations because of low health education; in some cases, animals even have access to human excrements. This may support the high prevalence found in the pigs in our study. However, the reported morbidity of taeniasis in Romania is low in humans (ranges: 0.4% and 0.75%), but a high prevalence of *Taenia* sp. infections was reported in dogs (1.78% in southern Romania, Costin et al. 2011). The latter may contribute to a significant extent to environmental pollution and contamination of pigs, but it would be interesting also to screen the dogs in the selected areas of our study.

Traditional backyard pig rearing with outdoor access is widely spread in Transylvania where the pigs are raised in coops made of solid material, generally concrete. Sanitation measures are easily applicable and consist of removing the faeces and washing the floor daily. Given this, the chance of contact between pigs and the parasites' eggs eliminated by humans or dogs is low. However, the practice of feeding pigs with green mass harvested from the pasture creates an opportunity for this contact. Pollution of these pastures with *Taenia* sp. eggs is possible as some persons defecate outside, in the environment, or by subsequent contamination from stray and shepherd dogs. In addition, the pigs from mountain hamlets are freely reared on these contaminated pastures, which increases the contamination risk.

Another common risk factor in Romania is the traditional home slaughter of pigs especially during the winter holidays, performed by the owners. Local legislative regulations only request meat examination by accredited veterinarians for trichinellosis, but the pillar muscles of the diaphragm are not the preferred sites of *T. solium*. The possibly infected viscera are usually given uncooked to dogs. This way, the biological cycle of cestodes is maintained in the area.

The influence of age as a risk factor in the prevalence of infection in pigs is supported by the animals' exposure throughout their lifetime to accidental consumption of tapeworm eggs. It has been shown that the prevalence slowly increases from 46.8% in piglets of 2–4 months to 80.9% in adults over 1 year in Peru (Garcia et al. 2003). We have found

a similar significant increase with age: the prevalence in adults (7.15%) was 2-fold higher than in young pigs (3.1%).

Regarding the sex, different studies suggest that this factor has no influence on cysticercosis prevalence in pigs (Ngwing et al. 2012; Yulianto et al. 2014). Our results were similar to previous findings. The difference between the seroprevalence in males and females (3.7% versus 6.9%) was not significant.

Seasonal influence of cysticercosis prevalence in pigs may be explained by 1) higher frequency of human faeces consumption by pigs (Copado et al. 2004) and increased frequency of the farmers' defecation in the field in the dry season (Yulianto et al. 2014); 2) pigs housing in the warm, sunny and dry season, when they are left free to graze around households or garbage sites (Kagira et al. 2010). In our study, the higher seroprevalence was recorded during autumn fall (9.3%) and winter (11.8%). As the biology of larval forms requires 2 to 3 months to complete the development stages (Stewart and Hoyt 2006), it means that seropositive animals were contaminated during the spring and summer, when pigs graze on pasture.

This study has shown that porcine cysticercosis is present in Northwestern Romania and that age and season are significant risk factors in traditional backyard rearing system of pigs.

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