

ZOONOTIC ASPECTS AND MOLECULAR APPROACH IN TRICHINELLOSIS IN GORJ COUNTY, ROMANIA

ASPECTE ZOONOTICE ȘI ABORDĂRI MOLECULARE ÎN TRICHINELOZĂ ÎN JUDEȚUL GORJ, ROMÂNIA

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ABSTRACT | REZUMAT

Trichinellosis is a cosmopolitan foodborne disease caused by nematode species of the genus *Trichinella* recognized as important zoonotic pathogens in Eastern Europe, transmitted by the ingestion of raw or undercooked infected meat from pigs, horses and game (wild boars and bears).

The aim of the study was to provide data on the prevalence of *Trichinella* spp. in pigs from Gorj county, and to identify etiological agents presents in domestic pigs and wild boars. Samples represented by striated intercostal, diaphragm and sublingual muscle were examined by trichineloscopy and larvae digestion method. Identification and molecular characterization of larvae at the species level was conducted by multiplex PCR technique according to the methodology described by Pozio and La Rosa (2003). Infections were detected in one out of 24000 (0.0041%) slaughtered and examined domestic pigs and four out of 216 (1.8518%) hunted wild boars. The *Trichinella* larvae isolates were identified as *T. spiralis*.

Keywords: PCR, *Trichinella*, pigs, wild boar

Trichineleza este o boală cosmopolită transmisibilă prin alimente, produsă de specii de nematode din genul *Trichinella*, recunoscute ca importanți patogeni zoonotici în Estul Europei, transmise prin ingestia de carne infestată crudă sau insuficient gătită de la porci, cai sau vânat (mistreți și urși).

Scopul studiului a fost de a furniza date cu privire la prevalența *Trichinella* spp. la porci din județul Gorj, precum și pentru a identifica agenți etiologici la porcii domestici și mistreți. Probele reprezentate de musculatură striată de la nivel intercostal, pilier diafragmatic și sublingual, au fost examinate prin trichineloscopie și metoda digestiei artificiale. Identificarea speciei și caracterizarea moleculară a larvelor, la nivelul speciei, a fost realizată prin tehnica PCR multiplex după protocolul descris de Pozio și La Rosa (2003). Infestația a fost identificată la unul din 24000 porci domestici sacrificați și examinați (0.0041%) și patru din 216 (1.8518 %) mistreți vânați. Larvele izolate de *Trichinella* au fost identificate ca *T. spiralis*.

Cuvinte cheie: PCR, *Trichinella*, porci, mistreți

Trichinella species that causes trichinellosis, a serious human disease transmitted by consume of meat, are recognized as important pathogens in the Eastern Europe (2, 7).

In Romania, *Trichinella* species are transmitted within and between two cycles: sylvatic and domestic one (1). When man fails in the proper management of domestic animals and wildlife, *Trichinella* spp. infections, and particular *Trichinella spiralis* infection, are transmitted to the sylvatic at domestic environment, sometimes by the synanthropic animals.

In addition, certain *Trichinella* species can be

transferred in reversible way from domestic animals to the wildlife (16).

In Europe, red foxes (*Vulpes vulpes*) are considered the most important *Trichinella* reservoirs of sylvatic cycle and also predictive sentinels for parasite circulation among wildlife (13, 14).

Information on the molecular characterization of *Trichinella* species isolated are limited in Romania and not many were identified to species level (3, 9, 12).

In accordance with European Directive no. 2003/99 / EC (European Commission, 2003), zoonotic agents, such as those from *Trichinella* genus, should be monitored in wildlife in European Union countries.

The aim of the study was to provide data on the prevalence of *Trichinella* spp. in pigs from Gorj county, and to identify etiological agents presents in domestic pigs and wild boars.

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MATERIALS AND METHODS

Animals surveyed were from Gorj county, from several localities (Dănești, Bucureasa, Urichești, Mușetești, Crasna, Runcu, Turceni, Bălești, Lelești) and additional information was gathered from DSVSA Gorj.

Samples represented striated muscle, from intercostal, diaphragm pillars and sublingual were examined by the trichineloscopy at CSVSA Small Square, Targu Jiu, Gorj county.

Positive samples were subjected to examination by technic described in the following. Five grams of muscle samples from each animal were examined for *Trichinella* using the magnetic stirrer method, according to Commission Regulation (EC) No. 2075/2005 (6). After digestion, the resulted fluid was poured through a 180- μ m sieve. The larvae were counted under a trichinoscope with horizontal table at 50–100 $\times$ magnification, washed several times in cold water, preserved in 90% ethyl alcohol until species identification (9).

Five larvae from each isolate were tested for identification and molecular characterization at the species level by multiplex PCR technique according to the methodology described by Pozio and La Rosa (2003) (15, 18).

RESULTS AND DISCUSSIONS

Of the total number of registered pigs slaughtered, 24000, in the Gorj county has identified one case of trichinosis from pork. On the other hand, after examining samples from wild boars was observed that the degree of infestation was higher. From 216 wild boars hunted and subjected trichineloscopy study were identified 4 positive for *Trichinella* spp. infection.

Overall, one out of 24000 (0.0041%) slaughtered and examined domestic pigs and four out of 216 (1.8518%) hunted wild boars were found to be infected with *Trichinella* spp. larvae

In order to determine the species involved and their belonging to domestic or sylvatic cycle was performed the molecular characterization of isolates. In our study this protocol to Multiplex PCR revealed the presence of clear bands at 173 bp specific of *T. spiralis* according to Pozio și La Rossa 2003 (15).

The fact that at PCR exam, the identified species was *T. spiralis*, highlights a very important epidemiological aspect, namely that domestic cycle is one that maintains infection both in domestic pigs and wild boars.

Even if the first *Trichinella* infection in a pig in Romania has been recorded 101 years ago, and currently represent a black mark (15) for Romanian pig industry (8) comprehensive research were made only in the last 26 years.

Blaga et al. (2009b) during the period 1997 - 2004 has done an investigation in order to analyse the extent of *Trichinella* infection in slaughtered pigs, using routine *Trichinella* test data's, when they revealed a prevalence of *Trichinella* infection of 0.09% (9.648/10.813.350;) in home slaughtered pigs and 0.07 (18.280/23.726.965) in abattoir slaughtered pigs (3).

Borza et al. (2012) report, from January 1998 to November 2011, in pigs from Timiș County *Trichinella* infection at 681/5.586.431 (0.01%) of domestic pigs from farms and 407/609.325 (0.07%) of pigs from private households (5).

Nicorescu et al. (2015) made an extensive investigation at nationwide level, included all territory of Romania, and revealed that overall prevalence was 0.20%, which means that 227 out of 113,383 domestic pigs raised in non-controlled conditions, were infected with *Trichinella* spp. (12).

The molecular data on *Trichinella* species has been provided by Blaga et al. (2009c) (4) and Nicorescu et al. (2015) (12) using multiplex PCR, and *T. spiralis* and *T. britovi* were the only species detected throughout the territory of Romania in domestic pigs.

In the opinion of same researchers, in 2004 Romania was the country with the highest incidence of trichinellosis in the world (2, 9, 10, 11) and the majority of the outbreaks have been linked to the consumption of pork from home-slaughtered free-ranging and backyard pigs that escaped any veterinary *Trichinella* testing (9, 14).

In Romania, *T. spiralis* was the most prevalent species identified in domestic pigs compared to *T. britovi* and mixed *T. spiralis* and *T. britovi* infections (4, 9, 12, 18).

Ten Member States reported positive findings in pigs and wild boar. Accordingly 204/ 191, 332, 813 (0.0001%) pigs were reported to be positive, most were found in Romania. In hunted wild boar 0.12% tested positive and originated mostly from eastern EU Member States (17).

CONCLUSIONS

Prevalence of *Trichinella* infection in Gorj county was 0.0041% in domestic pigs and 1.8518% in hunted wild boars.

By multiplex PCR the *Trichinella* larvae isolates were identified as *T. spiralis*.

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