

**TRICHINELLA STATUS IN THE WILD FAUNA OF CATALONIA (NE SPAIN):
FIRST REPORT OF *T. SPIRALIS* IN A WILD BOAR FROM MONTSENY
(BARCELONA PROVINCE)**

Torrents, Alfredo. Veterinary

Fàbregas i Comadran, Xavier. Veterinary

Bolás, Francisco. Ph D, Professor, Parasitology Department, Complutense University of Madrid

Nogal, Juan José. Lecturer, Parasitology Department, Complutense University of Madrid

SUMMARY

The confirmation and identification of a *T.spiralis* isolates from a wild boar coming from the Montseny Massif (Barcelona, Catalonia, Northeastern Spain) is reported. The detection of *Trichinella* larvae was confirmed using a digestion reference method (larvae load range 10.4-22 lpg). We revise the available information of *T.spiralis* isolates found in the wild fauna of Montseny and Catalonia. This is the first reported identification of *T.spiralis* in a wild boar at the Montseny Natural Park.



INTRODUCTION

In Barcelona province (Spain) more than 8 million pigs are slaughtered every year for human consumption, most of them produced under controlled housing. The presence of *Trichinella spiralis* parasitizing the population of wild boar, which is constantly expanding, may represent a risk for the food safety.

Trichinella inspection on gamemeat has been practiced for years. Trichinoscopy had frequently been used until the artificial digestion tests have been recommended for the detection of low larvae load infection by international standards (OIE Terrestrial Manual, 2012).

Although, pigmeat production in Catalonia could be considered *Trichinella*-free, several veterinarians find each year positive-trichina wild boars hunted in mountainous areas (personal commun.).

Very few studies have been published regarding this parasite in the susceptible wild fauna of Catalonia, (wild boar, wild carnivores and rodents), which can act as reservoir. The aim of this study is to present a case report in which a positive finding is contrasted with two different digestion variations methods for try to improve the counting load of parasitization. One variation counts the larvae in 2-gram sample, reducing the volum digestion fluid, while the other use the Mac Master egg-feces counting technique applied in parasitology. Further research may be useful to elucidate the *Trichinella* status in the wild fauna in the province of Barcelona, and farther in Catalonia.

MATERIAL AND METHODS

In Winter 2011, specimens of an adult wild sow (*Sus scrofa ferus*) from the massif of Montseny was tested for *Trichinella*. About 12.5 grams of pillars of diaphragm were pooled up to 100 gram sample with further negative meat for *Trichinella* detection using the method of the magnetic stirred artificial digestion. This positive specimen was further investigated to estimate the larvae load: 5 pieces of 2 grams of diaphragm pillar fresh specimen were each submitted to modified digestion (minced with a pair of scissors, digested in 300 ml of 44° warm tap water with 2.4 ml of HCl (25%), and 1.5 g of pepsin).

Further specimen was used for identification.

Counting estimation was also performed using the Mac Master technique after pepsidic digestion, as described by Pérez-Martín et al (2000). Meat sample was minced with a pair of scissors and domestic blender, and digested in 0.5 % pepsin and HCl 0.7 % + NaCl 0.9 %, in a ratio 10 gram/100 ml of digestive fluid. Agitation at 37 ° C and 200 rpm was maintained during 90 minutes. Then, the digestion was filter through cotton tissue in a conical tube. After 10-15 min of sedimentation, the sediment was recovered with a pipette Pasteur in a glass beaker. For the counting in the Mc Master camara, the

sediments was resuspended in magnetic agitation and aliquots were sampled for counting.

Identification of species was performed using PCR-ISSR as described in Fonseca-Salamanca et al (2006).

RESULTS AND DISCUSSION

| | |
|---------------------------|--|
| Reference method | 10.4 larvae/gram |
| Modified reference method | 16 larvae/gram 22 larvae/gram 14 larvae/gram 19,5 larvae/gram 15 larvae/gram |
| Average (n=5) | 17,3 larvae per gram |
| Standard desviation | 3,35 |
| Mac Master counting | 22 larvae/gram |

A load of about 10.4 lpg (130 larvae in 12.5 g) were detected in the diaphragm sample of the wild sow submitted to examination after standard digestion. Trichinoscopy only detected 2 larvae in about 1 gram divided in 24 small pieces compressed between glass plates.

Afterwards, an average (n=5) load of 17.3 lpg was estimated using the above mentioned modification of the reference method. Standard desviation was calculated in 3.35 lpg. The recovery of the reference digestion test performance over the modified one was approximately 60 %. Furhter, using the McMaster technique, 22 lpg were recorded. The isolated larvae were identified as *T.spiralis*.

This larvae load is above the detection limit of the reference technique and it could be considered a high infective dose according to the minimal infectious intake -60 to 750 infective larvae- by Teunis et al (2012).

Only one outbreak of *Trichinella spiralis* originated in wild boar has been declared in Catalan Barcelona province (Martin Granado et al, 2007) from 1994 to 2006 according to the Weekly Epidemiological Bulletin. Between 2001 and 2010, only four cases of human trichinellosis were declared in the 2006' Catalan Epidemiological Bulletin, 3 at Pallars Jussà (Northwestern Catalonia) and 1 at Vallès Occidental (central Catalonia, neighbored to Montseny).

Published data of *Trichinella* detection and identification in Catalonia (Northeastern of Spain) are scarce. Although, Thichinella presence is well documented and knowledged in Spain, and thus it is expected in Barcelona province, very few publications are found

regarding the wildlife infection in Catalan Barcelona province. And this is one of the reasons we have had to refer to veterinarian personal communication.

In wild boar, a veterinarian (personal communication) reported a 0.17% (n=576) prevalence in 2005 hunting campaign at Alt Empordà (Girona province, northeastern of Catalonia), where a positive, *T.britovi*, wild boar was caught at Susqueda area, Guilleries, contiguous to the Montseny Natural Park. During 2006-08 hunting seasons Manzano-Lorenzo et al reported the detection of two pairs of *T.spiralis* strains neighbouring to the same area (Sant Hilari Sacalm, 2/15; Sant Esteve de Llémena, 2/19), as well as *T.spiralis* at Susqueda and Sant Hilari Sacalm; meanwhile, *T.britovi* at Guilleries, and *Trichinella spp* within this area (Osor, La Selva, Sant Hilari Sacalm), (personal communication). At the same mountainous area 500 meters above sea level (Susqueda, Sant Hilari Sacalm, Arbúcies, Anglès, Sant Feliu de Pallerols), trichina-borne wild boars have been detected during 2011-12 (personal communication) hunting campaign, as well as during 2012-13 hunting season at Vilanova de Sau, Folgueroles, Sant Feliu de Buixalleu, Rupit and Fogars de Montclús (within Natural Park of Montseny, local press, 2012), and at Sant Feliu de Llobregat and in the Montseny Massif.

Table

Human outbreaks (Catalonia) and *Trichinella*-borne wild boar cases (Girona and Barcelona provinces), 2005-2013.

Concerning other wild fauna, few studies are published on *Trichinella* in Catalonia, although some of them were sampled at the Montseny Massif. Feliu et al (1985) detected larvae in *Rattus norvegicus* sampled from Catalonia, by muscle trichinelloscopy. Miquel et al (1994) found an adult *Trichinella sp* in one badger *Meles meles* in Tarragona province (South of Catalonia). Various surveys were taken on intestinal parasitisation of wild carnivores from the Montseny Massif and Catalonia (Feliu, Miquel, Torres et al, 1992, 1994, 1996). In Mustelids, Segovia et al (2007) detected *Trichinella*-infected diaphragms of the pine marten *Martes martes* in Spain (Cantabria, Mallorca, Pirineos) with a prevalence 4.44% (n=90). Finally, Manzano-Lorenzo et al (2008) reported *T.britovi* in red foxes (*Vulpes vulpes*) from the Northwest of Catalonia.

The wild boar is considered a good indicator of the circulation of *T.spiralis* in Europe (Pozio et al, 2010). Its presence at the Montseny Natural Park may indicate its persistence in this wildlife environment, and might act as a reservoir for the domestic cycle which is absent in Catalonia.

Map

Historical records of *Trichinella* in wild boars found in Catalonia (Girona and Barcelona provinces), species, years and localities.

CONCLUSION

The wild boar population may expand for feed and reproduction through a series of contiguous mountainous areas, where wild boar dwells, (Diputació de Barcelona, 2010), and towards some areas naturally and geographically connected in the center-northeastern of Catalonia, comprising the Montseny Natural Park, the Guillerics, Susqueda, Collsacabra and Garrotxa mountainous areas and even the Montnegre i Corredor Litoral Natural Park, Sant Llorenç de Munt-Serra de l'Obac Natural Park or the Collserola metropolitan Natural Park.

Red foxes could also harbor *T.spiralis* in regions where wild boar is present (Poizio, 2010). There are not enough data in Catalonia to discard the presence of *T.spiralis* in the red fox or other sylvatic carnivore reservoir (Mustelidae, raccoon dog). Whether a wild carnivore, rodent or the wild boar behaviour (cannibalism, scavengerism, snout-digging,) closes the *T.spiralis* cycle continues to be a mystery.

Aknowledgments

This work was in part supported by MED-VET-NET project (FOOD-CT-2004 506122, Trichimed WP27).

REFERENCES

Diputació de Barcelona (2010) Programa de seguiment de les poblacions de senglars a Catalunya 2009/2010 <http://www.diba.cat/parcsn/parcs/fitxers/pdf/p06d097.pdf>

Feliu C, Mas-Coma S, Gallego J (1985) (in Catalan) Coneixements actuals sobre l'helmintofauna paràsita dels Múrids (Rodentia) a Catalunya Butll. Inst. Cat. Nat., 50: 255-261 <http://publicacions.iec.cat/repository/pdf/00000126%5C00000063.pdf>

Feliu C, Miquel J, Casanova JC, Torres J, Segovia JM, Fons R, Ruiz-Olmo J (1996). Helminthfaunas of wild carnivores in the Montseny Massif: An atypical ecosystem in the northeast of the Iberian Peninsula. *Vie et Milieu*, 46(3-4):327-332.

Fonseca-Salamanca J, Nogal-Ruiz J, Benito C, Camacho MV and Martínez-Fernández (2006) Molecular characterization of *Trichinella* genotypes by inter-simple sequence repeat polymerase chain reaction (SIR-PCR) *J Parasitol*, 92 (3), pp 606-610.

Local Press Lavanguardia (19/11/12) (In Spanish) <http://www.lavanguardia.com/local/girona/20121119/54354612404/valles-oriental-la-selva-garrotxa-jabali-carne-infectada-triquina-alerta-sanitaria.html>

Manzano-Lorenzo R., Nogal-Ruiz J.J., Fonseca-Salamanca F., García-Sancho R.N., Arroyo-Díaz J.M., Jiménez S., Fàbregas X., Colomer A., Bolás-Fernández F., Martínez-Fernández A.M.. 2008. Trichinellosis survey in wild fauna from various regions of Spain. EPI 27. Abstract Book. 4th Annual Scientific Meeting. MED-VET-NET. Saint-Malo, France.

Martin Granado, A, Martínez Sánchez, EV, Varela Martínez, MC, Sánchez Serrano, LP, Ordóñez Banegas, P, Torres Frías, A, Díaz García, O, Hernández Pezzi, G. (2007) (In Spanish)Vigilancia epidemiológica de brotes de triquinosis en España. Temporadas 1994/1995 a 2005/2006 Boletín Epidemiológico Semanal semanas 7-8, 2007 vol.15 nº4/37-48.

Miquel J, Torres J, Feliu C, Casanova JC, Ruiz-Olmo J (1992). On the helminthfauna of carnivores in Montseny Massif (Catalonia, Spain). I. Parasites of Viverridae and Mustelidae. *Vie et Milieu* 42 (3-4): 321-325.

Miquel J, Torres J, Feliu C, Casanova JC, Ruiz-Olmo J, Segovia JM (1994). Helminthfauna of Canidae and Felidae in the Montseny Massif (Catalonia, Spain). *Doñana, Acta Vertebrata*, 21 (2): 131-142.

Miquel J, Torres J,, Casanova JC, Feliu C (1994). Helminths paràsits de carnívors silvestres a Catalunya. Treballs del MDG-CCN 3. Museu de Granollers, Granollers.

OIE Terrestrial Manual 2012, Chapter 2.1.16. Trichinellosis.

Pérez-Martín JE, Serrano FJ, Reina D, Mora JA, Navarrete I (2000) Sylvatic trichinellosis in southwestern Spain *J Wildlife Dis.* , 531-534.

Pérez-Martín JE, Robledo-Berrocal J, González-Ruibal L, Gamito-Santos JA, Calero-Bernal R, Pariente-Palomino FJ, Serrano-Aguilera FJ, (2007) Evolución de la prevalencia de trichinellosis en Extremadura: epidemiología de los ciclos doméstico y silvestre. Congreso Ibérico de Parasitología, Madrid, 2007.

Pozio et al. (2010). Development of harmonised schemes for the monitoring and reporting of *Trichinella* in animals and foodstuffs in the European Union Scientific report submitted to EFSA.

Segovia, J. M., Torres, J., Miguel, J., Sospedra, E., Guerrero, R., Feliu C. (2007). Analysis of helminth communities of the pine marten, *Martes martes*, in Spain: Mainland and insular data. *Acta Parasitol.*, 52: 156-164.

Teunis, PFM, Koningstein, M, Takumi K, and Van Der Giessen JWB (2012) Human beings are highly susceptible to low doses of *Trichinella spp* *Epidemiology and Infection* Feb 2012 140: pp 210-218.

Torres J, Miquel J, Casanova JC, Motje M, Feliu C (1996) Health involvements relating to the helminths parasitizing wild mustelids in Catalonia [Spain] *Medicina Veterinaria* Jul.Ago 1996.

Torres J, Miquel J, , Motje M (2001) Helminth parasites of the eurasian badger (*Meles meles* L.) in Spain: a biogeographic approach. *Parasitol Res.* Apr;87(4):259-63.