Trichinellosis survey in wild fauna from various regions of Spain

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INTRODUCTION

In Spain, three trichinellosis surveys were carried out in the wild fauna of Cataluña, La Rioja and Castilla-La Mancha regions in the context of a surveillance program on wildlife diseases. *Trichinella spiralis* and *T. britovi* live in apparent sympatry in this fauna of the Iberian Peninsula.

La Rioja is a natural geographic area in the NE of Spain. This region is formed by a succession of mountains, valleys and rivers. During trichinellosis survey (2001-2003), the Veterinary Services of the Govern of La Rioja Autonomous Region processed meat samples of 1278 wild boars and 70 foxes by trichinoscopy and/or standard digestion. Trichinellosis prevalence in wild boars in this study was 0.70%. Only 3 foxes among the 70 examined (4.2%) were parasited.

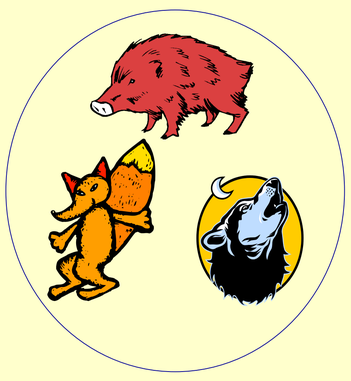
In Castilla-La Mancha, a broad open region in Central Spain at 600 meters above sea level, a total of 2216 wild boars were examined by trichinoscopy in the local abattoir (Toledo) during the trichinellosis survey (2007-08 campaign). Here the prevalence of trichinellosis was next to 0.72%.

In Cataluña, an autonomous region placed in the NE of Spain, a total of 1069 wild boars and 156 foxes were captured during the hunting season and the prevalence of trichinellosis, as determined by standard digestion, was 0,93% and 0,64%, respectively during the trichinellosis survey (2006-08 campaign).

MATERIALS AND METHODS

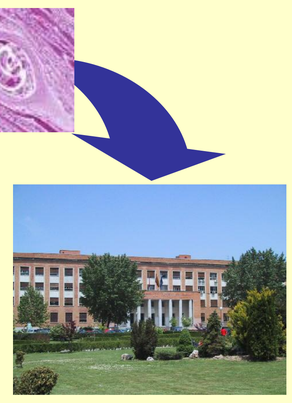
Trichinellosis surveys were performed by Veterinary Services

Trichinella sylvatic cycle



Hunters or official game-keepers

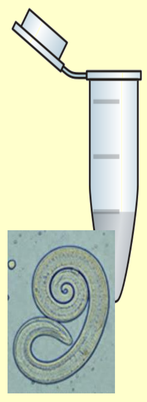
Trichinoscopy and / or standard digestion



UCM

Trichinella species identification

DNA EXTRACTION FROM SINGLE LARVAE



Taq (Polymerase) Buffer (BioTools)
H_2O
90 ºC / 10 min
Proteinase K (20 mg/ml)
65 ºC / 3 h
90 ºC / 15 min

Mixture amplification single larvae

ISSR-PCR PROTOCOL

Taq Polymerase 1,5 U
Primer (816) 10 pmol/µl
H_2O
Mixture amplification single larvae
94 ºC / 5 min
40 cycles
- 94 ºC / 30 s
- 52 ºC / 45 s
- 72 ºC / 2 min

72 ºC / 6 min

Ethidium bromide staining and electrophoresis in 1.5% agarose gels

RESULTS

TRICHINELLOSIS SURVEY (POSITIVE SAMPLES)

LA RIOJA (2001-2003)

	Host	Location	Species
La Rioja 1	wild boar	Panzares	*T. spiralis*
La Rioja 2	wild boar	Lagunilla	*T. britovi*
La Rioja 3	fox	La Pineda	*T. britovi*
La Rioja 4	fox	n/d	*T. spiralis*
La Rioja 5	dog	Enciso	*T. britovi*
La Rioja 7	dog	Laguna de Cameros	*T. britovi*
La Rioja 8	wild boar	Muro en Cameros	*T. spiralis*
La Rioja 9	wild boar	Laguna de Cameros	*T. spiralis*
La Rioja 10	fox	la Pineda	*T. britovi*
La Rioja 11	wild boar	n/d	*T. spiralis*
La Rioja 12	wild boar	n/d	*T. spiralis*
La Rioja 13	wild boar (sausage)	Enciso	*T. britovi*
La Rioja 14	wild boar	Villoslada	*T. spiralis*
La Rioja 15	wild boar	n/d	*T. britovi*
La Rioja 16	wild boar	Vadillo	*T. britovi*
La Rioja 17	wild boar (sausage)	Brieva de Cameros	*T. spiralis*

CASTILLA-LA MANCHA(2007-2008)

	Host	Location	Species
CLM-1	wild boar	Villamantilla (Madrid)	*T. spiralis*
CLM-2	wild boar	Casas de Miravete (Cáceres)	*T. spiralis*
CLM-3	wild boar	Monterubio de Serena (Badajoz)	*T. spiralis*
CLM-4	wild boar	Monterubio de Serena (Badajoz)	*T. spiralis*
CLM-5	wild boar	Brazatortas (Ciudad Real)	*T. spiralis*
CLM-6	wild boar	Fuencaliente (Ciudad Real)	*T. spiralis*
CLM-7	wild boar	Casas de Miravete (Cáceres)	*T. spiralis*
CLM-8	wild boar	Hontanar (Toledo)	*T. spiralis*
CLM-9	wild boar	Mazarambroz (Toledo)	*T. spiralis*
CLM-10	wild boar	Cañamero (Cáceres)	*T. spiralis*

CATALUÑA (2006-2008)

	Host	Location	Species
CA-1	wild boar	Areny (Huesca)	*T. britovi*
CA-2	wild boar	Anoia (Barcelona)	*T. britovi*
CA-3	fox	Guils del Cantó (Lleida)	*T. britovi*
CA-4	wild boar	Bonansa (Huesca)	*T. britovi*
CA-5	wild boar	Sant Hilari Sacalm (Girona)	*T. spiralis*
CA-6	wild boar	Sant Hilari Sacalm (Girona)	*T. spiralis*
CA-7	wild boar	St. Esteve Llémena (Girona)	*T. spiralis*
CA-8	wild boar	St. Esteve Llémena (Girona)	*T. spiralis*
CA-9	wild boar	Tremp (Lleida)	*T. britovi*
CA-10	fox	Tremp (Lleida)	*T. britovi*
CA-11	wild boar	Guilleries (Girona)	*T. britovi*

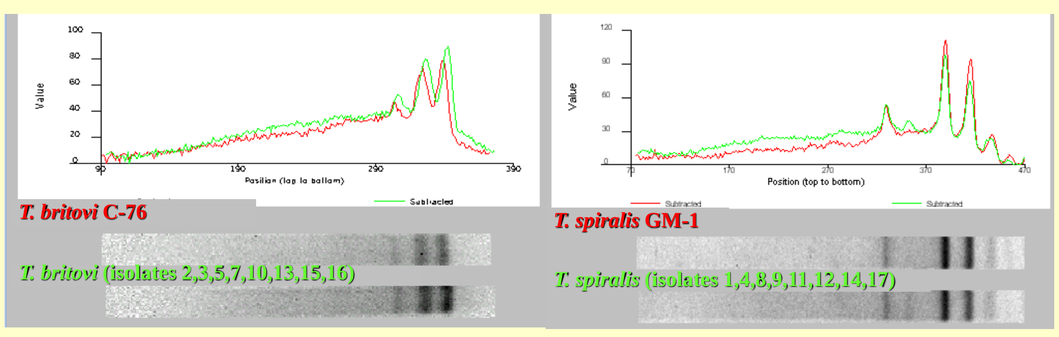


Fig.1. Comparison of electrophoretic profiles using QUANTISCAN. *Trichinella* isolates from La Rioja *versus T. spiralis* (MFEL/SP/62/ISS48 GM-1) and/or *T. britovi* (MCAN/SP/76/ISS11 C-76) reference strains

In all cases, the positive samples were sent to our laboratory for their isolation and specific identification by inter-simple sequence repeat-PCR (ISSR-PCR). These analyses of ISSR-PCR markers provide a quick, reliable and highly informative DNA single larva fingerprinting. According to our results in these studies, we found a dramatic predominance of *T. spiralis* in wild boars from La Rioja (83.3%) and Castilla-La Mancha (100%) whereas in Cataluña both *T. spiralis* and *T. britovi* where equally represented (50%). *T. britovi* was the only species detected in foxes. The results show that all isolates identified as *T. spiralis* were indistinguishable from *T. spiralis* (ISS48) using ISSR-PCR with the primer 816, whereas four variations were clearly distinguished among those belonging the *T. britovi*. Among all of them the ISS2 and ISS11 isolates were found to be the most frequent (Fig.1).

DISCUSSION AND CONCLUSIONS

The uniformity found within *T. spiralis* isolates suggest its perhaps recent introduction whereas the *T. britovi* isolates suggest that this species represents one of the original endemic *Trichinella* in this West-End of Eurasia. Orographical diversity of these regions would preserve its population variation. The high prevalence of *T. spiralis* is a good example of the persistence in sylvatic conditions of a species from the domestic cycle. Our observations confirm the sympatric coexistance of the two species and the risk to human health represented by the consumption of non-inspected wild boar meat. In addition, we confirmed that ISSR-PCR is a robust technique for the molecular identification of Trichinella species and genotypes.

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