



Manzano-Lorenzo<sup>1</sup>, R.; Nogal-Ruiz, J.J.<sup>1</sup>; Fonseca-Salamanca, F.<sup>1</sup>; García-Sánchez, R. N.<sup>1</sup>; Arroyo Díaz, J.M.<sup>2</sup>; Jiménez, S.<sup>3</sup>; Fábregas, X.<sup>4</sup>; Colomer, A.<sup>4</sup>; Bolás-Fernández, F.<sup>1</sup>; Martínez-Fernández, A.R.<sup>1</sup>

<sup>1</sup>Depto. de Parasitología, Facultad de Farmacia, UCM, 28040 Madrid, España. Telf. 913941818, e-mail: francisb@farm.ucm.es.

<sup>2</sup>Equipo Veterinario de la Delegación Provincial de Sanidad de Toledo (Castilla-La Mancha). Telf. 925232041, e-mail: emtoledo@jccm.es.

<sup>3</sup>Consejería de Salud y Servicios Sociales, Logroño (La Rioja).

<sup>4</sup>Veterinarios de Lleida y Girona, e-mail: xfabregues@hotmail.com

## 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.

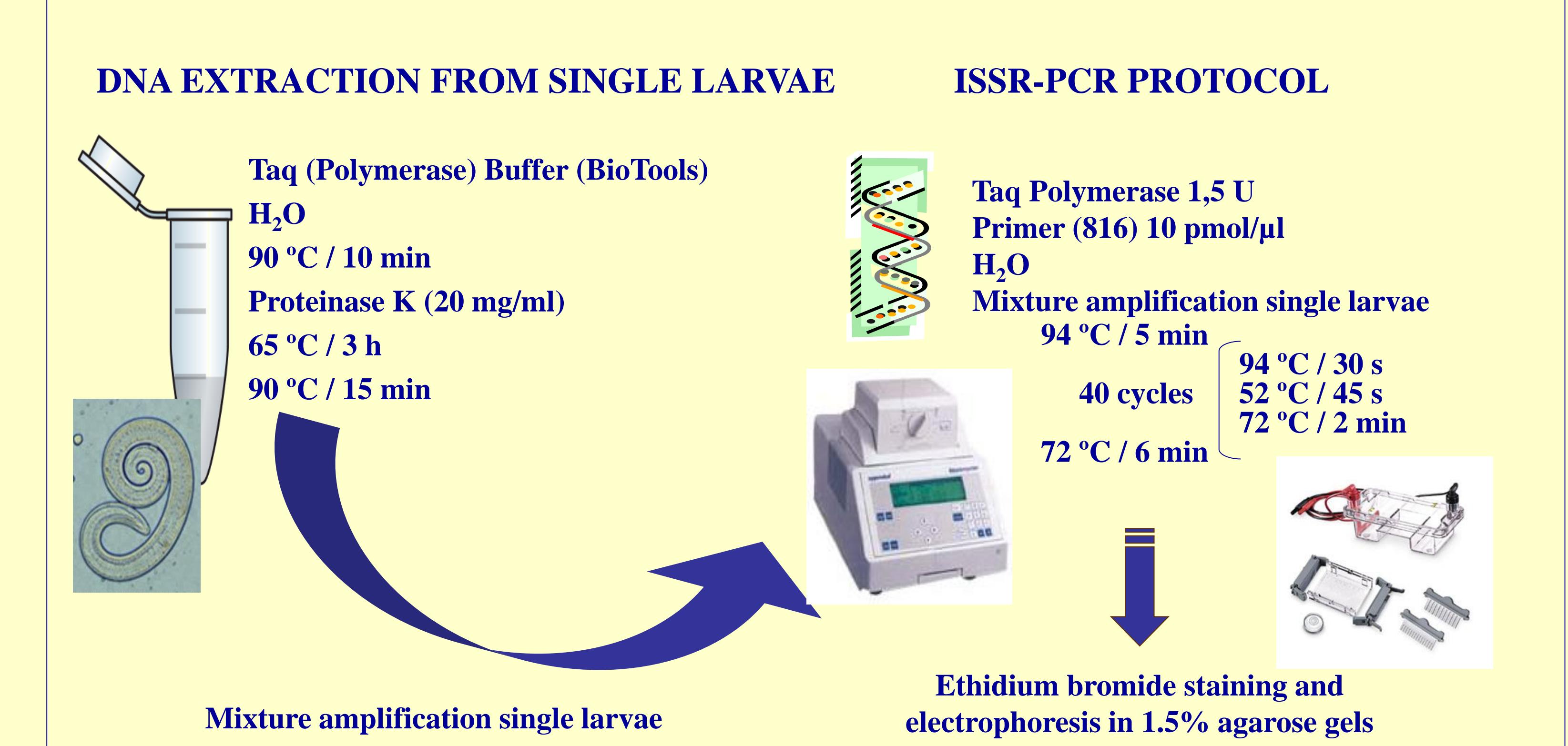
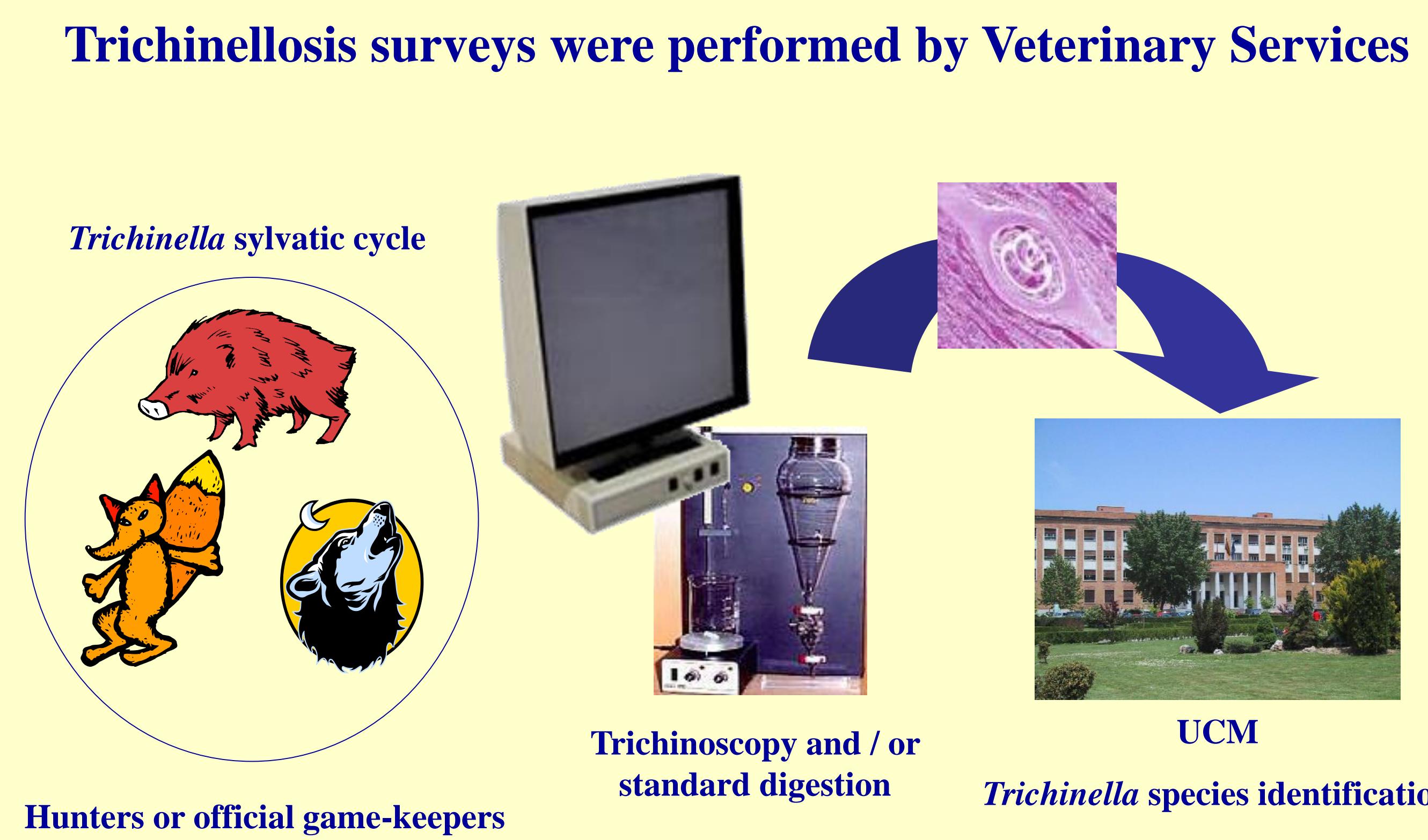
La Rioja is a natural geographic area in the NE of Spain. This region is formed by a succession of mountains, valleys and rivers. During trichinellosis survey (2001-2003), the Veterinary Services of the Govern of La Rioja Autonomous Region processed meat samples of 1278 wild boars and 70 foxes by trichinoscopy 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



## RESULTS

### TRICHINELLOSIS SURVEY (POSITIVE SAMPLES)

#### LA RIOJA (2001-2003)

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

#### CASTILLA-LA MANCHA(2007-2008)

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

#### CATALUÑA (2006-2008)

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

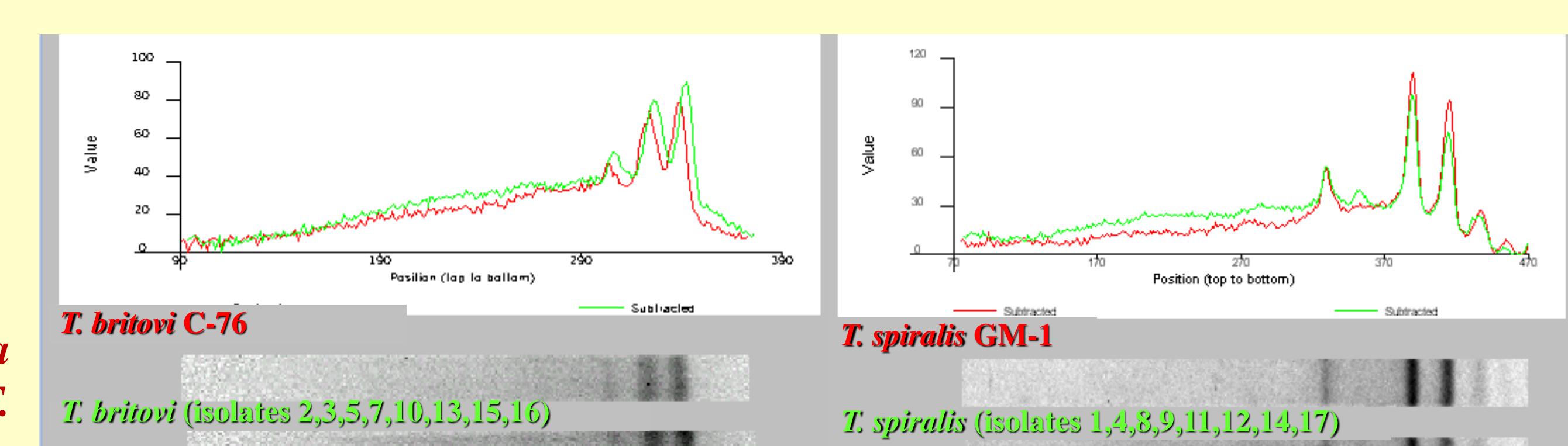


Fig.1. Comparison of electrophoretic profiles using QUANTISCAN. *Trichinella* isolates from La Rioja versus *T. spiralis* (MFEL/SP/62/ISS48 GM-1) and/or *T. britovi* (MCAN/SP/76/ISS11 C-76) reference strains

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* were 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 to *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 coexistence 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.