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SYLVATIC TRICHINELLOSIS IN SOUTHWESTERN SPAIN

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ABSTRACT: The epidemiology of *Trichinella* spp. in their main sylvatic hosts, wild boar (*Sus scrofa ferus* and red fox (*Vulpes vulpes*), in Extremadura (southwestern Spain) was studied. We examined 88 *Trichinella* spp.-positive wild boar muscle-tissue samples from a total of 29,333 killed animals, referred to the Veterinary Parasitology Department (University of Extremadura, Spain) by the Extremadura Veterinary Service. Additionally, 227 red foxes killed during the hunting season and thus not subject to veterinary controls were examined for trichinellosis. *Trichinella* spp. larvae were found in six (3%) of the red foxes. All samples were examined using direct diagnostic techniques, including trichinoscopy and artificial digestion. The mean intensity of infection was 74.8 larvae/g (LPG) of muscle tissue in wild boars, compared to 30.6 LPG in foxes. *Trichinella spiralis* (*sensu stricto*) predominated over *T. britovi* in wild boars. Random amplified polymorphic DNA (RAPD) and alloenzyme typing showed that 74% of infected wild boars had only *T. spiralis,* 21% had only *T. britovi,* and 5% showed mixed infections. In contrast, 33% of infected foxes were infected only with *T. spiralis,* while 67% had *T. britovi,* suggesting a clear predominance of the latter in foxes. We suspect the existence of a paranthropic or sylvatic cycle in large areas of this region; given the ease of transfer between sylvatic and domestic or semi-domestic animals, this implies a high epidemiological risk.

Key words: Epidemiology, fox, survey, *Sus scrofa, Trichinella spiralis, Trichinella britovi, Vulpes vulpes,* wild boar.

INTRODUCTION

Following initial studies on trichinellosis epidemiology in a number of natural habitats, increasingly complex routes of transmission have been detected (Campbell, 1983). In addition to the traditional domestic or peridomestic cycles (limited to swine-swine or swine-rat), transmission of this genus has been reported in other animal species. Despite this situation, few studies address the role of either wild boar (*Sus scrofa ferus*) or red fox (*Vulpes vulpes*) as carriers and/or transmitters of *Trichinella* spp. or, by extension, their role in potential transmission to humans.

A thorough understanding of the role of these host species in the epidemiology of trichinellosis in the region under study is important. Trichinellosis is endemic in Extremadura (Spain) because of (1) the large swine population which are mainly Iberian pigs raised extensively on acorn pasture, and (2) due to the high density of wild boars and foxes in scrubland areas particularly suited to hunting. Thus, epidemiological data are of value in determining the most appropriate preventive measures for human trichinellosis to be implemented in the region.

MATERIAL AND METHODS

Eighty-eight *Trichinella* spp.-positive wild boar muscle-tissue samples were obtained by the Extremadura Veterinary Service (Regional Government Building, Cáceres, Spain) and submitted to the University of Extremadura Veterinary Parasitology Department (Cáceres, Spain) from 1985–97. The samples, obtained from wild boar killed by hunters and shown to be positive at compulsory veterinary inspection, are referred systematically for routine parasitological (e.g., infectivity) and pathogenic tests, as well as for typing. Following the recommendations of the International Commission on Trichinellosis (Secretary-General: Medical Academy of Poznan, Poznan, Poland), our working group is responsible for producing regular reports on the status of the genus in this area. These reports are sent to the *Trichinella* Reference Centre (TRC) of the International Commission on Trichinellosis, (Istituto Superiore di Sanitá di Roma, Rome, Italy).

A second part of this study was examination of 227 foxes killed during the hunting season and thus not subject to veterinary controls. Foxes were referred to the laboratory by collaborating veterinarians.

In order to ascertain the relationship between *Trichinella* spp. isolates and certain ecological considerations, all study samples were accompanied by a dataset describing the collection site, climatic and demographic aspects of the particular host population (Vicente Calle et al., 1993), in order to be able to incorporate useful epidemiological information into the ultimate typing of the species involved.

A 1 g sample of each wild boar muscle was examined with a stereoscopic microscope (Nikon SMZ-10, Nippon Kogaku K. K., Chiyoda-ku, Tokyo, Japan), and between 5 and 50 g, depending on sample weight, were used for artificial pepsin digestion (Serrano et al., 1992). Samples were generally taken from the pillars of diaphragm, a site for which *Trichinella* spp. larvae show particular affinity. Identical-sized samples were obtained from limb flexor and extensor muscles of foxes, again a preferred site for the genus (Kapel et al., 1994).

Upon confirmation that samples were positive, mean larva counts/g were done for each sample. In cases of scarce infection, larvae were counted individually following artificial digestion of muscle tissue; where intensity was higher, the method of Pérez-Martín (1994) was used. Briefly the methods is outlined as follows. The volume of larva-containing liquid obtained after artificial digestion, was distributed homogeneously in a magnetic stirrer and a McMaster (Weber Scientific International Ltd., Teddington, Middlesex, UK) chamber filled. The number of larvae in the chamber was multiplied by a correction factor (6.66) for chamber volume, and by digestion volume in ml., to give the number of larvae in the digested muscle tissue. This was repeated six times and the arithmetical mean of these counts was used.

Material for typing was referred to the Istituto Superiore di Sanità (Rome, Italy; International Reference Centre for *Trichinella* and Trichinellosis) (Pozio et al., 1989a), where isolates were typed by random amplified polymorphic DNA (RAPD) and/or alloenzyme analysis (Pozio et al., 1989b).

RESULTS

Trichinella spp. larvae were found in 227 foxes (3%). The prevalence in wild boar, as detected by the Veterinary Inspection Service, was 0.3% (88 *Trichinella* spp.-positive animals from a total of 29,333 hunter-killed animals).

Mean intensity of infection was greater in wild boar (74.8 larvae/g-LPG-) than in foxes (30.6 LPG). Overall, 35% of positive animals had levels of <10 LPG.

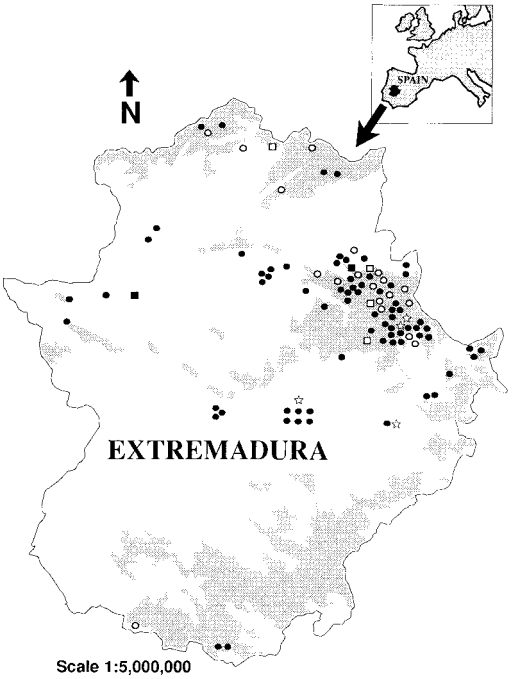

FIGURE 1. Distribution of sylvatic trichinellosis in Extremadura (Spain). Subscripts are ■ *T. spiralis* in red fox, ● *T. spiralis* in wild boar, □ *T. britovi* in red fox, ○ *T. britovi* in wild boar, ☆ *T. spiralis* and *T. spiralis* in wild boar, and ▨ Altitude > 500 m above sea level.

Concerning species prevalence, there is a predominance of *Trichinella spiralis* (74%) in wild boar compared to *T. britovi* (21%). The reverse is true of foxes, where *T. britovi* (67%) was far more frequently isolated than *T. spiralis* (33%). Particularly noteworthy was the finding of mixed *Trichinella* spp. infection in four wild boars (5%).

With respect to geographical distribution (Fig. 1), overall findings indicate that *T. spiralis* occurs over a wider area than *T. britovi.* Most animals infected by either species were killed near mountainous areas or in natural parks.

DISCUSSION

The prevalence of *Trichinella* spp. in foxes (3%) indicates the huge importance of this host in the parasite's sylvatic cycle (Kapel et al., 1994; Pozio et al., 1997). The prevalence recorded in wild boar over the

study period closely matches that of 0.3% reported by Calero et al. (1989) from 1985–88. However, it is higher than that reported in neighboring countries (Pozio et al., 1996). Given that *Trichinella* spp. is frequently found in domestic pigs in districts where it also is most prevalent in sylvatic mammals, it may be that a large proportion of infections of domestic pigs originate in the sylvatic cycle, and vice versa.

The fact that 35% of positive animals displayed <10 LPG, in spite of the poor sensitivity of the legally-established diagnostic method, suggests a distribution trend, in the wild, towards lower intensity, which is in agreement with other authors (Famerée et al., 1982). This situation can include an important health risk, due to the constant possibility of eating infected wild boar meat.

The differing prevalence of the two *Trichinella* spp. species may be due to the degree of host-parasite adaptation. It seems to be a marked association between *T. britovi* and carnivores, which is confirmed in the finding of *T. britovi* in 67% of infected foxes. The importance of mixed infection also has been cited elsewere (Pozio et al., 1997).

The wild boar is an accidental host in some countries where trichinellosis in carnivores is frequent (Pozio et al., 1996). However, in Extremadura, both parasite species are widespread in this host, in spite of the domestic cycle of *T. spiralis* and the moderate infectivity for domestic pigs and wild boars of *T. britovi* (Serrano et al., 1992).

In general, most infected animals were found at >500 m sea level (msl), and many at >700 msl (Fig. 1) in the "Los Ibores" and "Las Villuercas" areas. Most of this land is used for forestry and hunting, where agricultural activity is very scarce. Human population density also is low (around 5 inhabitants/km^2), with the villages being very small, relatively isolated, and placed between two Natural parks of Monfragüe and Cijara. For these reasons there are a very few habitat alterations and wild animals are very abundant, allowing a more frequent overlap between the so-called domestic form (*T. spiralis*) and sylvatic form (*T. britovi*) of trichinellosis.

Human activity seems to be of major importance in the epidemiology of sylvatic trichinellosis in Spain. There are frequent hunts during the season, and the negligence of the hunters does much to favor the transmission of this parasite. Both the viscera of dead animals and the bodies of unlocated wounded wild boar are generally left *in situ*. This is the case with all foxes, because of the low market price of pelts. Thus, foxes are a source of the parasite to hunting dogs, other wild animals, and occassionally pigs. Thus, some districts such as Extremadura, today have a significant amount of sylvatic trichinellosis which can have human and veterinary implications. Thus, some measure of pubic health education may be advisable in these areas.

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