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Short communication

First isolation of *Trichinella britovi* from a wild boar (*Sus scrofa*) in Belgium

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Abstract

Since 1992, when the European Union Council Directive requires that wild boars (*Sus scrofa*) hunted in EU for commercial purpose should be examined for *Trichinella*, the infection has not been detected in wild boars from Belgium, despite serological evidence of the presence of anti-*Trichinella* antibodies in wildlife and previous reports of *Trichinella* larvae in this host species. In November 2004, *Trichinella* larvae were detected in a wild boar hunted near Mettet, Namur province (Southern Belgium). Larvae were identified as *Trichinella britovi* by polymerase chain reaction methods. This is the first report of the identification of *Trichinella* larvae from Belgium at the species level. The detection of *T. britovi* in wildlife in Belgium is consistent with findings of this parasite in other European countries and confirms the need to test game meat for *Trichinella* to prevent its transmission to humans.

Keywords: *Trichinella britovi*; Wild boar; Belgium; Wildlife; Trichinellosis; Epidemiology

1. Introduction

Four species of *Trichinella* (*Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi* and *Trichinella pseudospiralis*) are represented in the European

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Union (EU) (Pozio, 2001). In these countries, human infections are related to the consumption of meat from game, domestic pigs raised in organic farms in endemic areas or fed with offal from game, and horses imported from countries of Eastern Europe and America (Pozio, 1998; Pozio, 2001; Boireau et al., 2000). The European Union (Directive 92/45/EEC, 1992) obliges the examination of meat of wild boar, pig and horse for the presence of *Trichinella* spp.

In Belgium, *Trichinella* infection has not been detected in domestic pigs or horses and only one outbreak was documented in humans following the consumption of pork from a wild boar (Famerée et al., 1979). In wildlife Famerée et al. (1981) detected *Trichinella* larvae in 6.7% of wild boars (*Sus scrofa*), 2.2% of muskrats (*Ondatra zibethica*), 6.5% of brown rats (*Rattus norvegicus*) and 11.1% of black rats (*Rattus rattus*). However, at that time the parasites were not confirmed, and not identified at the species level. Since 1992, annually 8000 sport hunted wild boars are tested for *Trichinella* in Belgium, as imposed by Directive 92/45/EEC, and infection had until now not been detected. This paper presents the isolation of *Trichinella* larvae by artificial digestion and the subsequent confirmation and characterization of the isolate, from a wild boar in southern Belgium.

2. Materials and methods

Routine inspection by artificial digestion of pooled samples of 5 g of tongue and diaphragm muscle from 20 animals (EU Directive 92/45/EEC) was carried out in the laboratory of the Centre d'Economie Rurale of Marloie in Belgium. To trace back the infected animal, muscle samples of tongue, diaphragm and forearm from the 20 wild boars were digested separately. In order to identify the parasite at the species level, larvae were sent to the *Trichinella* Reference Laboratory of The Netherlands (RIVM, Bilthoven) and tested by a 5S rDNA based PCR followed by DNA sequencing (Rombout et al., 2001; Van der Giessen et al., 2005). For case registration, the larvae were also subjected to a multiplex PCR analysis (Pozio and La Rosa, 2003) in the International *Trichinella* Reference Centre in Italy (ISS, Rome).

3. Results

Five larvae were recovered after pooled sample digestion. Individual digestions revealed that the infected muscles originated from a wild boar shot near Mettet (50.19N, 4.40E). The average parasite load in mixed muscles from the tongue and diaphragm was 0.7 larva/g (LPG). No larvae were detected in 55 g of forearm muscles. The shape and movement of larvae were suggestive for *Trichinella*. The examination of larvae at higher magnification showed the presence of the stichosome and of a row of collateral dots, which are morphological characters of the *Trichinella* genus. In the reference laboratories, larvae were identified as belonging to *T. britovi*. Phylogenetic analysis of the 5S rDNA sequences (RIVM, The Netherlands) showed 99.5% similarity with *T. britovi* AY009943.1 from Genbank.

4. Discussion

Sylvatic carnivores (e.g. red fox, wolf and mustelids) represent the main hosts of *T. britovi*. The infection can be transmitted to wild boars and consequently it can easily reach the human being (Pozio, 1998). In the last decades, the wild boar populations of Europe have increased exponentially favoring *Trichinella* transmission and consequently increasing the biomass of this parasite (Hars et al., 2000). Even if the experimental infection of wild boars shows that swine is not the optimal host for *T. britovi* (Kapel, 2001), epidemiological data including the present work stress the role played by this animal species for spreading the infection in Europe. *Trichinella* infection can be maintained by a sylvatic cycle for decades as has been shown in Ireland, where *T. spiralis* was maintained among the fox population for >30 years, without any documented infection in domestic animals and humans (Rafter et al., 2005).

The only documented case of trichinellosis in Belgium was caused by the consumption of wild boar meat, originating from two home-fed animals in the northern part of the country, where wild boars are not present in natural conditions (Famerée et al., 1979).

In Belgium studies on wildlife species not intended for human consumption suggest low prevalence of *Trichinella* spp. In the season 2003–2004, 199 red

foxes (*Vulpes vulpes*), 32 badgers (*Meles meles*), 44 beech-martens (*Martes foina*) and 52 polecats (*Mustela putorius*) from Belgium were examined by artificial digestion of 25–33 g of tongue, diaphragm and hindleg muscles. *Trichinella* larvae were detected only in one fox (0.5%) from southern Belgium; however, larvae were not identified at the species level (unpublished results). From 1996 to 2000, no infection was detected in muscles of Belgian foxes, even if serum samples of 164 in 818 foxes (20%) were found positive for antibodies by ES-ELISA (Vercammen et al., 2002). Serological examination might be an alternative to assess the prevalence of *Trichinella* infection among wildlife and to follow trends in time in epidemiological studies but needs further evaluation (Gamble et al., 2004).

About one-fifth of the human cases that occurred in France, Germany, Italy and Spain, were caused by the consumption of pork from wild boar (Pozio, 1998; Geerts et al., 2002). Most of these infections were considered mild, causing fever, facial edema and/or myalgia. The present study shows a minor infection of 0.7 LPG in a wild boar, unlikely to cause any harm to the consumer, in case the predilection sites were eaten raw or undercooked (Gamble et al., 2004). Both *T. spiralis* and *T. britovi* have been associated with human infection. However, pathogenicity varies upon the species involved, indicating the need to identify the *Trichinella* species causing the infection (Kurdova et al., 2004).

As 3–5 g is required for reliable detection of larval load of 1 LPG, the routine examination of 5 g of predilection muscles of wild boars devoted to the market proves to be a good measure to protect the consumer (Forbes and Gajadhar, 1999). In addition, the habit to consume wild boar only ''well done'', i.e. at least 60 °C in the core for 1 min, is an extra preventive measure. An important measure to prevent spreading of the infection among wildlife is not to leave offal of animal carcasses in the field after skinning (Worley et al., 1994; Pérez-Martin et al., 2000; Pozio et al., 2001).

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