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Epidemiological survey of *Trichinella* infection in domestic, synanthropic and sylvatic animals from Argentina

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Abstract

The presence of *Trichinella* larvae was investigated in 247 samples taken from domestic, synanthropic and sylvatic animals, collected during 1996 to 2005 in 12 endemic provinces of *Trichinella* infection in Argentina. Muscle larvae of *Trichinella* from 65 infected animals were identified at the species level by single larva nested polymerase chain reaction (PCR) technique based on the variability within the expansion segment V (ESV) region of the ribosomal DNA. *Trichinella* infections were found in 97 of 164 pigs, 38 of 56 pork products, two domestic dogs, one domestic cat, 7 of 11 armadillos and 3 of 9 synanthropic rats. All *Trichinella* isolates were identified as *Trichinella spiralis* by nested PCR. These findings add new data on the epidemiology of trichinellosis and should be considered when implementing new strategies to control this zoonosis.

Introduction

Trichinellosis is a worldwide zoonosis caused by the consumption of raw or undercooked meat containing larvae of *Trichinella* sp. Eleven genotypes have been recognized in the genus *Trichinella*, eight at the species level (*T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. murrelli*, *T. nelsoni*, *T. papuae* and *T. zimbabwensis*) and three related to an uncertain taxonomic position (referred to as 'T6', 'T8', and 'T9') (Murrell *et al.*, 2000; Pozio *et al.*, 1999, 2002). However, morphological criteria alone are not enough to distinguish between the 11 genotypes of *Trichinella*. Thus, many biochemical and molecular methods have been developed for this purpose. Currently, the most useful methods for *Trichinella* differentiation are based on the polymerase chain reaction (PCR). Recently, a multiplex PCR test has been developed, based on the sequence length polymorphism within the expansion segment V (ESV) region and sequence variability within the internal transcribed spacer (ITS) regions of the ribosomal DNA repeat, for the unambiguous identification of all currently recognized species of *Trichinella* (Zarlenga *et al.*, 1999).

The genus *Trichinella* has a cosmopolitan distribution, infecting mammals, birds and crocodiles (Pozio, 2001). Although *Trichinella spiralis* can invade sylvatic habitats, it is transmitted and maintained in a domestic cycle. Meanwhile, all other genotypes are transmitted and maintained in a sylvatic cycle even though they can infect domestic animals (Pozio, 2001).

In Argentina, trichinellosis is an important public health problem, with notified cases in 18 of 23 provinces (Bolpe & Boffi, 1999). Several studies reported a great number of infected pigs in Argentina (Costantino *et al.*,1994; Venturiello *et al.*, 1998; Ortega-Pierres *et al.*, 2000), where 5217 human cases of trichinellosis, caused by the ingestion of pork meat or its derivative products, have been registered from 1990 to 1999 (Bolpe & Boffi, 2001). In addition, *Trichinella* larvae have been found in rats, cats, dogs, foxes and armadillos (Ossola *et al.*, 1969; Neghme & Schenone, 1970; Costantino *et al.*, 1994; Pozio, 2000), which might play an important role as reservoirs of *Trichinella* worms in nature, and serve as other sources of human infection. However, few *Trichinella* isolates from

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these animals have been identified at the species level and, to date, no report of sylvatic genotypes of *Trichinella* in the Neotropic region has been recorded.

The aim of the present study was to evaluate the presence of *Trichinella* infection in domestic, synanthropic and sylvatic animals from endemic regions of Argentina and to identify the aetiological agent.

Materials and methods

Parasites

From 1996 to 2005, 247 samples were collected from domestic, sylvatic and synanthropic animals from 12 provinces of Argentina. Domestic animals, including 164 pigs, that represent a source of infection for humans, two domestic dogs (*Canis familiaris*) and one domestic cat (*Felis catus*) suspected to be infected with *Trichinella* were analysed. Additionally, 56 pork derivative products (sausages and hams) suspected to cause trichinellosis in humans were examined. Pig muscle samples were taken from the diaphragm, intercostal muscles and tongue and dog and cat samples were taken from the diaphragm. The weight of each sample ranged from 10 g to 124 g. Sylvatic animal samples were collected from the intercostal muscles of 11 armadillos (*Chaetophractus villosus*), the forelegs of one hare (*Lepus europaeus*), the diaphragm of one mountain lion (*Felis concolor*) and the dorsal muscles of two foxes (*Dusicyon* sp.). Regarding synanthropic animals, samples were collected from the diaphragm of nine rats (*Rattus* sp.). The weight of samples from sylvatic and synanthropic animals ranged from 0.2 g to 30 g. The presence of infection and worm burden (larvae per gram, LPG) were evaluated by the artificial digestion method (0.7% HCl, 0.5% pepsin, 37°C for 3 h) according to standard procedures (Bell & McGregor, 1980).

PCR analysis

Muscle larvae of 65 *Trichinell*a isolates were identified at the species level by nested PCR of the ESV region of the ribosomal DNA according to Zarlenga *et al.* (1999) with some modifications. For DNA preparation, a single larva was placed in 5 μl of lysis buffer (10 mM Tris pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% tween-20, 0.45% NP-40, 0.01% gelatin) containing proteinase K (5 μg), overlaid with a drop of mineral oil, heated for 90 min at 65°C, then 15 min at 90°C, and frozen at −20°C. In the first and second round of nested PCR reactions the primers Ne (5′-TCTTGGTGGTAGTAGC + 5′-GCGATT-GAGTTGAACGC) and I (5′-GTTCCATGTGAACAG-CAGT + 5′- CGAAAACATACGACAACTGC) were used, respectively. Muscle larvae of *Trichinella* reference isolates of *T. spiralis* (ISS599), *T. nativa* (ISS532), *T. britovi* (ISS447), *T. pseudospiralis* (ISS13), *T. murrelli* (ISS103) and *Trichinella* T6 (ISS34) were used as controls.

Results

Of 247 samples from various animal sources and pork derivative products, 148 were found to be positive for *Trichinella* larvae using artificial digestion (table 1). Ninety seven of 164 pigs and 38 of 56 pork products were positive. Two domestic dogs and one domestic cat were also infected, whereas in the wild, 7 of 11 examined armadillos were infected. All other sylvatic animals studied were negative. In relation to synantropic animals, 3 of 9 rats were found to be positive.

Table 1. *Trichinella* infection in samples examined from a variety of domestic, synanthropic and sylvatic animals from 12 endemic provinces of Argentina from 1996 to 2005.

	No. of samples		
Province and sample	Examined	Infected	Analysed by PCR
Buenos Aires			
Pig	53	30	13
Pork products	41	29	7
Armadillo	11	7	2
Hare	1	0	–
Fox	1	0	–
Rat	1	1	1
Catamarca			
Pig	14	3	2
Fox	1	0	–
Chubut			
Pig	23	20	16
Córdoba			
Pork products	3	3	2
La Pampa			
Pig	2	2	1
Neuquén			
Pig	14	13	6
Pork products	2	2	0
Cat	1	1	1
Dog	2	2	1
Rat	7	1	1
Río Negro			
Pig	19	13	8
Rat	1	1	1
San Luis			
Pork products	1	1	1
Santa Cruz			
Pig	7	5	0
Mountain lion	1	0	–
Santa Fe			
Pig	9	7	1
Pork products	2	2	0
Santiago del Estero			
Pig	21	3	0
Pork products	7	1	1
Tierra del Fuego			
Pig	2	1	0
Total		148	65

PCR analysis carried out on 65 *Trichinella* isolates (table 1), from 47 pigs, 11 pork products, one dog, one cat, two armadillos and three rats, generated a fragment of 173 bp, in accordance with the *Trichinella spiralis* reference isolate (fig. 1).

Discussion

Most pork samples came from pigs raised under poor hygienic conditions and slaughtered at homes without any sanitary inspection. Moreover, all pigs and pork products analysed came from regions where numerous human infections are reported each year. (Bolpe & Boffi,

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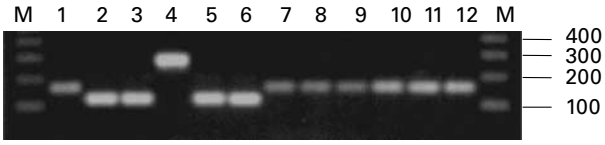

Fig. 1. Nested polymerase chain reaction analysis of the expansion segment V region of the ribosomal DNA from domestic, synanthropic and sylvatic isolates of *Trichinella*. Lane M, 100 bp molecular ruler (Bio-Rad); lane 1, *Trichinella spiralis*, reference isolate ISS599; lane 2, *Trichinella nativa*, reference isolate ISS532; lane 3, *Trichinella britovi*, reference isolate ISS447; lane 4, *Trichinella pseudospiralis*, reference isolate ISS13; lane 5, *Trichinella murrelli*, reference isolate ISS103; lane 6, *Trichinella* T6, reference isolate ISS34; lane 7, pig isolate, Buenos Aires; lane 8, pork product isolate, Buenos Aires; lane 9, dog isolate, Neuquén; lane 10, cat isolate, Neuquén; lane 11, armadillo isolate, Buenos Aires; lane 12, rat isolate, Neuquén. *Trichinella* isolates from pig, pork product, dog, cat, armadillo and rat, generated a fragment of 173 bp, in accordance with *T. spiralis* reference isolate ISS599. Furthermore, it is important to note that the PCR product of 173 pb, derived from amplification of the ESV region, differentiates *T. spiralis* from the ten other genotypes of *Trichinella* (Zarlenga *et al.*, 1999; Pozio *et al.*, 1999; Pozio *et al.*, 2002).

2001; Ortega-Pierres *et al.*, 2000). The molecular identification shows that all *Trichinella* isolates analysed belonged to *T. spiralis* (table 1). This species of *Trichinella* is highly pathogenic and is responsible for most human infections (Murrell & Pozio, 2000). Although veterinary control of pigs for public consumption is compulsory in Argentina, it is worth noting that most human outbreaks of trichinellosis have been caused by food coming from pigs that had not been examined. Thus, it is necessary to carry out more intense control of *Trichinella* infections in swine and to provide consumers with more information to improve prevention of human trichinellosis in these regions.

In sylvatic and synanthropic animals, 2 of 7 species were infected (table 1). In this context, it is important to note that in Buenos Aires province, 63.6% of infection was found out of 11 armadillos examined and two isolates were identified as *T. spiralis* (table 1). The high prevalence in armadillos suggests that these animals can serve as reservoirs of *Trichinella* infections. Presumably, scavenging on carcasses by armadillos can play an important role in the spread of *Trichinella*. Larvae of *Trichinella* might follow the infection route from armadillo via synanthropic animals to production animals and finally to ingestion by man. Alternatively, armadillos are only victims of improper pig slaughtering. Armadillo meat is widely consumed in this region. The lack of reports of human infections caused by the consumption of this animal could be related to the prolonged cooking time of armadillo meat.

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