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Description of an outbreak of human trichinellosis in an area of Argentina historically regarded as *Trichinella*-free: The importance of surveillance studies

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ABSTRACT

Trichinellosis is an important food-borne zoonosis which is not treated as a major public health concern in Argentina. After more than 20 years without reports of infection in an area regarded as *Trichinella*-free, research studies reported that infection occurred in humans, pigs and game animals, including the recent outbreak of human trichinellosis revealed here.

The outbreak, affecting 64 individuals, occurred in July 2010 in the province of Entre Ríos. Epidemiological studies, clinical observations, laboratory analyses and immunoserological specific assays (indirect immunofluorescence, IIF, and ELISA) were performed. Food samples were analyzed by artificial digestion, and *Trichinella* larvae isolates were identified to the species level by multiplex PCR.

The main source of infection, commercially available food, had a parasite load of 1.1 muscle larvae per gram. Larvae were identified as *Trichinella spiralis.* Patients presented predominantly with oedema, fever and myalgia; and laboratory findings and/or immunoserological tests were positive for trichinellosis. Individuals received outpatient treatment. No deaths or secondary sequelae were recorded.

Results suggest that the presence of *T. spiralis* infection should be suspected in all endemic areas, especially where animal husbandry and official food safety controls are not properly conducted. The lack of the cases reported ought not to be taken as a proof of parasite absence. We highlight the importance of the urgent need to implement interdisciplinary and inter-institutional programs aimed to control infection transmission, to guarantee food safety and to conduct epidemiological surveillance studies.

1. Introduction

Trichinellosis, a zoonotic disease caused by the ingestion of raw or undercooked meat containing larvae of *Trichinella* nematodes, occurs globally (Pozio, 2000; Bruschi and Murrell, 2002; Murrell and Pozio, 2011) and has commonly been reported in Argentina (Bolpe and Boffi, 2001; Bruschi, 2012). The major sources of trichinellosis in humans are pork and pork-products.

Despite the fact that in Argentina pigs are not a major source of food, it is cash income for small farmers. Human behaviour as well as the lack of compliance to ensure food safety remains the most important factor responsible for the persistence of trichinellosis: husbandry practices vary within villages, pigs may be penned or tethered; it is also common for pigs to roam freely in the village with access to

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garbage dumps. The scarce number of trained professionals, laboratories accurately equipped to analyze meat, and the lack of information provided to small producers and/or the difficulties they face to reach these laboratories worsen the problem of food-borne parasitic diseases.

Data from north Argentina is limited. Only a few isolated human cases were reported over the last 10 years (Ribicich et al., 2005). In contrast, major trichinellosis outbreaks have been reported in other provinces of the country such as Buenos Aires, Santa Fe, Córdoba, San Luis and Mendoza. Between 1990 and 2005, 5221 cases of human trichinellosis were reported with an incidence of 1.48/100,000 persons-years caused by the consumption of pork meat and/or meat products. Argentina represents the country with the highest number of cases in the region of the Americas (Murrell and Pozio, 2011).

To date, the official criterion applied to consider an area as *Trichinella*-free is the absence of reports of human or porcine trichinellosis outbreaks. Probably, some of those areas where no cases are reported are not in fact free of *Trichinella* but no appropriate epidemiological surveillance has been carried out to detect its presence, since some of the cases are mistaken for other pathologies or they are not reported. Reliable epidemiological information in Argentina on animal and human infection has been unevenly collected and only a few studies have shown an incidence in the areas studied (Bolpe et al., 2001; Ambrosioni et al., 2006; Krivokapich et al., 2006; Scialfa et al., 2010; Cohen et al., 2010; Montali and Pouzo, 2011), resulting thus in fragmentary information.

Preliminary studies conducted in the province of Entre Ríos, a province historically free of *Trichinella* according to the official recording system (http://www.msal.gov.ar/index.php/home/funciones/institucional/boletines-epidemiologicos), revealed the presence of the infection in humans, pigs and wild animals through immunoserological studies and/or parasitological studies. The seroprevalence of trichinellosis (positive sera by at least two of the three methods used) was 8.3% in humans (Costantino et al., 2009; Barlasina et al., 2009), 24.5% in pigs (Costantino et al., 2009) and 11.4% in wild boars (*Sus scrofa*) (Cohen et al., 2010); the parasite could be detected in the carcasses of wild boars and pigs (Costantino et al., 2009; Cohen et al., 2010). Despite conferring appreciable zoonotic risks, these results were ignored by provincial and national authorities, as a consequence, preventive measures to avoid a potential outbreak were not developed.

Although the research mentioned above was performed in the same area by national universities, an outbreak of human trichinellosis began in early July 2010 and lasted until middle August, affecting 64 individuals with suspicion of trichinellosis infection. The aim of this study was to describe the first human trichinellosis outbreak in an area regarded as *Trichinella*-free.

2. Materials and methods

2.1. Study area

An outbreak of trichinellosis occurred in Pueblo General Belgrano, Department of Gualeguaychú, province of Entre Rios, Argentina (latitude −32°30′, longitude −58°33′) (Fig. 1) in July–August 2010, affecting the city of Gualeguaychú as well. This village is located at 15 km from the capital of the Department (Gualeguaychú) which, at the time of the outbreak, had a population of 1500 persons. In this village there is an urban area and a rural area with many farms of small farm producers who are engaged in pig-raising, in some cases as an activity for subsistence. Porcine husbandry practices prevailing in the region are typical of the rural areas of Argentina where small farm products are intended for consumption among family members and/or, as is the case of this outbreak, the owner of this farm is a butcher who also processes pork-meat which products are sold. The local authorities of this area lack a laboratory for food safety control of pork-meat for domestic consumption. The nearest laboratory is located at about 250 km from this farm. Due to the official history of the area and the lack of information on trichinellosis in this place, controls are not performed. The pigs of this farm were raised within a perimeter fence.

Fig. 1. Location of the area studied in Argentina.

Tourism in Pueblo General Belgrano is the main industry.

2.2. Patients and epidemiological features of the outbreak

Sixty-four patients with suspicion of infection were recorded during the outbreak. The classification of the

individuals was made according to the algorithm for diagnosing the probability of being infected with acute *Trichinella* in humans drawn up by Dupouy-Camet and Bruschi (2007)—Case definition: (1) A probable case was defined as all the inhabitants of Pueblo General Belgrano and Gualeguaychú or those persons who had visited the areas during the outbreak and had eaten pork meat and/or meat products (salami, sausages) as well as those presenting typical infection signs and/or symptoms (fever, myalgia, facial oedema, diarrhea, elevated muscle enzymes and/or eosinophilia). (2) A confirmed case was defined as a patient presenting at least one positive immunoserological test result for *Trichinella* antibodies (ELISA, IIF and/or Western blot, WB) and signs and/or symptoms of the acute phase of infection. (3) A very unlikely case was defined as any person with some or no pathognomonic symptoms and negative immunoserological test results by at least two methods.

The identification of the source of infection was performed by interviewing individuals with suspicion of infection by means of a questionnaire which was completed at serum sample collection to gather data including source and type of products consumed and eating habits with high risk for trichinellosis infection. Sanitary inspections of the village butchery and farms as well as food sample analyses were performed by national and provincial authorities. The results obtained showed that the products involved in this outbreak were pork meat and/or pork products (salami, raw type sausage), bought in a well-known butchery in Pueblo General Belgrano. According to the patients' records, the first cases detected ($n = 10$) occurred due to the consumption of pork products (salami) and eaten in two labour meetings on July 2nd and 8th, 2010. All individuals presented with symptoms within 23–31 days after ingestion.

Forty-three out of the 64 patients with suspicion of trichinellosis were diagnosed in a public hospital (Hospital Centenario de Gualeguaychú) and 21 in a private laboratory. Some patients received antiparasitic treatment (albendazole or mebendazole) and anti-inflammatory drugs. Other patients received anti-inflammatory drugs only. Twenty-six out of the 64 patients received antiparasitic treatment (albendazole or mebendazole) and anti-inflammatory treatment; 7 out of the 64 patients received anti-inflammatory treatment alone. None of the patients needed hospitalization and their condition improved without any complication.

In terms of the control measures of the outbreak studied here, different authorities from several areas such as food science medicine and veterinary worked together and issued an epidemiological alert to the area, implementing sanitary measures, i.e. elaboration of a research protocol and a patient-assistance algorithm, population awareness, inspection of premises and analysis of raw type sausage. Besides, the owners of pork production facilities were prohibited from selling, slaughtering and/or transporting the animals until the sanitary situation was controlled. The selling of pork meat and meat products was discontinued, inspection and interdiction of suspected farms were conducted and lectures were given to the community and health professionals.

2.3. Parasitological studies

Ninety grams of pork-meat product (salami) that led to the onset of the infection were examined by peptic digestion according to the technique described by Gamble et al. (2000). Previously, the fat-free meat sample was hydrated for 2 h before the assay.

Larvae were preserved in absolute ethanol and sent to the International Trichinella Reference Centre at the Istituto Superiore di Sanità (Rome, Italy) to be identified at species level by Multiplex PCR according to the protocol published (Pozio and La Rosa, 2003).

2.4. Serum samples

Serum samples from 52 patients were collected during the acute phase of infection (15–45 days post infection: dpi). Two or three serological samples were drawn from most of the patients with suspicion of infection, at intervals of around 15 days. All samples were obtained by venous puncture, then aliquoted and stored at −20 °C until use.

2.5. Laboratory studies

The following complementary laboratory tests were performed: white blood cell count in Turk's solution (reference range [RR]: 4000–9000 cells/mm^3), percentage of eosinophils by May–Grünwald Giemsa stain (RR: 0–5%) and determination of serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) by kinetic methods (Roche, USA) (RR at 37 °C: CPK, 5–167 UI/l in women, 5–190 UI/l in men; LDH, 240–480 UI/l).

2.6. Immunoserological studies

Specific anti-*Trichinella* antibodies in human serum were detected using frozen sections of ML mounted on microscope slides by IIF (Costantino et al., 2001) and using the excretory/secretory products of ML (ESP-ML) by ELISA (Costantino et al., 2001; Calcagno et al., 2005) and/or Western blot (WB) (Nuñez et al., 2000).

3. Results

3.1. Patients and epidemiological characteristics of the outbreak

Sixty-four individuals with suspicion of trichinellosis were recorded throughout the outbreak period. The group of patients analyzed was composed of 38 men (59%) and 26 women (41%). Two of these women were pregnant, in their second trimester at the moment of the ingestion. Babies were born within the expected date of delivery and they were completely healthy. The average age was 38.5 ± 15.6 years (range: 3–72 years). Patients were grouped by age in: children (3–12 years; $n = 5$, 8%), teenagers (13–19 years; $n = 5$, 8%), adults (20–60 years; $n = 52$, 81%) and older than 60 years ($n = 2$, 3%).

According to the above-mentioned criteria, the 64 individuals were classified into: 49 confirmed cases for

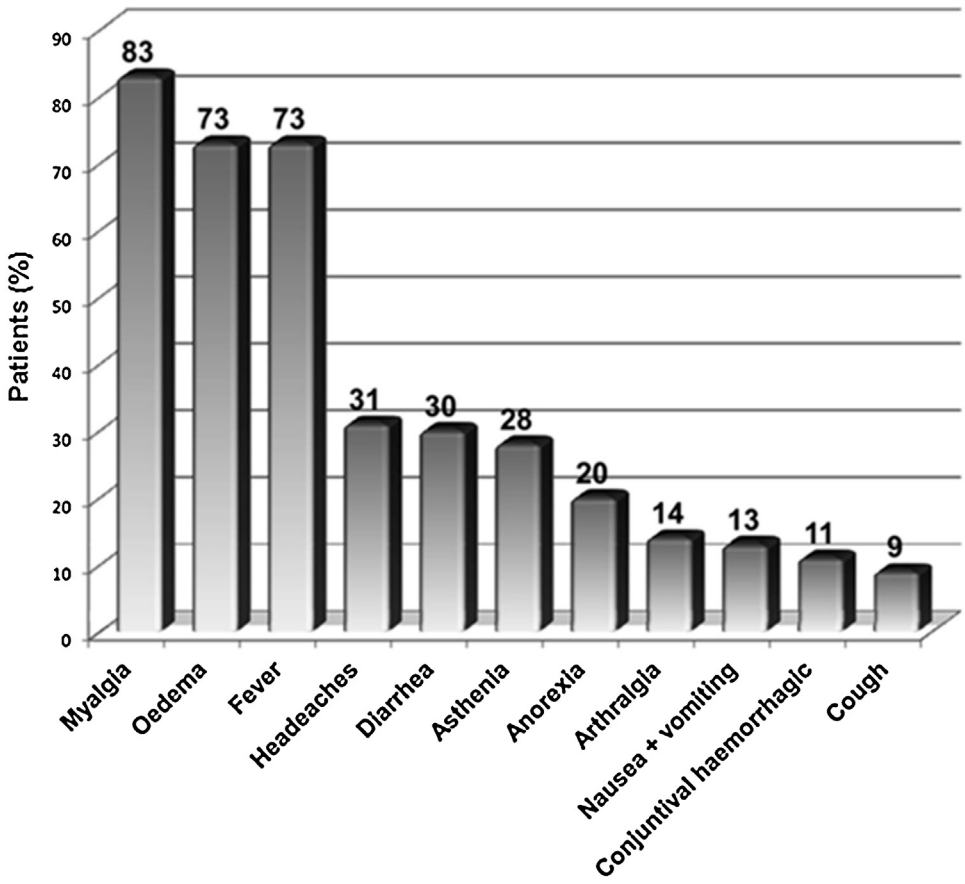

Fig. 2. Main clinical manifestations during the acute phase of infection ($n = 64$).

trichinellosis, including one pregnant woman, 12 probable cases and 3 very improbable cases, including the other pregnant woman whose only sign was presented with a high white blood cell count (15,900/mm^3).

The average incubation period of infection was 11 days (range: 8–27 days), with a median of 9 days. Incubation was calculated according to the 33 patients with confirmed infection since, the rest of the patients, clinical records included only data confirming the date of initial symptoms but not the date of consumption.

The primary case (first chronological case in the transmission chain) corresponded to a 10-year-old male, a native of Pueblo General Belgrano, who – on July 10 – presented with the frequent disease signs and symptoms: myalgia, oedema of eyelids, diarrhea, fever and headache. The patient had ingested salami.

The index cases (first case confirmed of the illness identified by the surveillance system) were two: a 44-year-old woman who had presented with myalgia, oedema of eyelids, fever and headache since July 22; this woman had ingested pork meat from the butcher's which was later determined to be the carrier of the infected meat, and a 53-year-old woman, native of Pueblo General Belgrano, who had presented with myalgia, fever and headache on July 29. The woman reported having ingested pork meat, and the diagnosis was confirmed by laboratory tests. All the patients improved without complications and none of them needed hospitalization.

3.2. Clinical observations

The most frequent signs and symptoms presented by the patients were myalgias (83%), oedema (73%), fever (73%), diarrhea (30%) among others (Fig. 2). Two patients were confirmed by serological tests despite being asymptomatic.

3.3. Parasitological studies

The average parasite load of the pork product analyzed by artificial digestion method was 1.1 ML/g of muscle. The isolated ML was *Trichinella spiralis*, as determined by Multiplex PCR.

3.4. Laboratory studies

Of the 35 patients with white blood cell counts, 66% ($n = 23$) presented with leukocytosis, with an average value of 10,688 ± 3239 cells/mm^3. While in 39 patients with eosinophil counts, 90% ($n = 35$) presented with eosinophilia, with an average percentage of 23 ± 12%.

Out of the 24 patients with serum CPK activity detection, 14 (58%) showed an increased activity with an average value of 465.5 ± 376.9 UI/l. Serum LDH activity was assessed in 21 patients, and 43% ($n = 9$) presented elevated values, with an average value of 463.7 ± 170.5 UI/l.

3.5. Immunoserological studies

Fifty-three patients were evaluated by IIF; 49 of them (92%) had positive results at the following time: 15–20 dpi, 78% (38/49); 30 dpi, 16% (8/49); and 60 dpi, 6% (3/49); while 17 (68%) out of 25 patients who were evaluated by ELISA showed anti-*Trichinella* antibodies at 30 dpi.

WB was performed only in those individuals ($n = 4$) with positive IIF and negative ELISA or close to the cut-off, and did not reveal specific bands for *Trichinella*. However, the infection was confirmed in 2 out of 4 individuals according to symptoms and epidemiological data.

4. Discussion

In this study we report and studied the first endemic outbreak of human trichinellosis occurred in an area of Argentina historically regarded as *Trichinella*-free.

The causative agent detected was *T. spiralis*, the etiologic agent responsible for most human infections and deaths globally (Pozio, 2007).

Until the occurrence of this outbreak, the importance of trichinellosis in the area studied was minor because it was deliberately neglected by the authorities due to the fact that there were no official records of cases in humans, pigs and/or game animals and the scientific results of surveillance were not considered.

The epidemiology of the human outbreak shows that a domestic pig was the source. The fact that the infected pig belonged to a farm located really close to an open-air garbage dump suggests that it acts as a reservoir of infection. In Argentina, pigs frequently roam freely and might consume infected rodents, other dead vertebrates and/or discarded infected meat. The presence of rats infected with *T. spiralis* has been already reported as the source of infection for pigs and wild animals by many authors (Venturiello et al., 1993; Gómez Villafañe et al., 2004; Scialfa et al., 2010) and represents one of the risk factors for spreading the infection (Takumi et al., 2009). This demonstrates the importance of conducting preventive measures in relation to pig exploitation such as pig raising/breeding in enclosures and information on the presence of trichinellosis in the area.

The results of the present study and those presented by other authors (Bolpe et al., 2001; Ribicich et al., 2005) indicate that in Argentina, *Trichinella* infection is endemic in pigs.

Likewise, our laboratory group has recently demonstrated the presence of pigs and wild boars infected with low density of larvae, ranging from 0.01 to 2.4 ML/g of meat, in the area where the outbreak took place (Costantino et al., 2009; Cohen et al., 2010). These values are in line with the parasitic load found in the food that originated the outbreak.

One of the features of this outbreak was the presence of symptoms compatible with trichinellosis (Fig. 2) in a high percentage of individuals as well as the short incubation period although the parasite burden of the pork products consumed was low (1.1 ML/g). This might be due to the fact that the individuals belonged to an area with low exposure to *Trichinella*, and for that reason, they were not previously sensitive to the parasite antigen, developing a strong immune response in relation with the pathology (Lyons et al., 2009; McSorley and Maizels, 2012).

The areas regarded as *Trichinella*-free that reveal the presence of the parasite when subjected to epidemiological and surveillance studies (Costantino et al., 2009; Cohen et al., 2010; Barlasina et al., 2009) could be the result of cases of human trichinellosis in which the prevalence is probably underestimated due to wrong diagnosis, since its signs and symptoms can be mistaken for other pathologies (Dupouy-Camet and Bruschi, 2007). This could be enhanced by the lack of diagnostic techniques for livestock as well as laboratories aimed at food control and sanitary studies of meat particularly accessible in rural areas. Besides, porcine outbreaks might remain unrecorded for fear of economic consequences (Pozio, 2007; Murrell and Pozio, 2011).

Although the total number of infected individuals was low, this overwhelmed both the public and the private sanitary systems in the area. There were patients that missed the opportunity to receive an effective treatment on time because albendazole stocks were sold out and/or due to late consultation.

Despite the importance of food-borne diseases (FBD), the number of professionals specialized in this arena is scarce. In this case, the involvement of a physician specialized in infectious diseases into the health system made the early detection of infection possible. Although previous research studies demonstrated the presence of *T. spiralis* infection in the area and the early detection of the first case reported by a physician (10th July), the implementation of sanitary measures was achieved quite late as they were carried out one month after (10th August) the primary case was detected. The first baseline visit to the butchery implicated in this outbreak was done during the first days of August by local authorities.

The serological detection of anti-*Trichinella* antibodies could not be performed in all suspected cases. At the hospital level, only one IS technique, IIF, was performed. At the private laboratory level, the outpatients' diagnosis was made by at least two IS methods. According to our experience, 2 out of 3 positive results confirm the diagnosis (Costantino et al., 2001; Venturiello et al., 2009). False-negative results represent a frequent feature when the sample is collected at the very beginning of the infection, especially when only one IS method is applied. Therefore, in these cases, a second or third blood sample must be drawn during the convalescent period aimed at determining seroconversion.

The WB test, considered by many authors as the confirmatory trichinellosis diagnostic approach (Year et al., 2003; Gómez-Morales et al., 2012), could not detect the infection in some patients ($n = 4$), where only two of them could be confirmed by IIF, symptoms and epidemiological

data. These results allowed us to reassert the importance of performing at least two IS methods due to the fact that they not always achieve 100% certainty.

It is worth mentioning that the regional economic activity was affected, from sellers of pork meat (e.g. butcher's) and meat products to other economic activities in the area (e.g. tourism).

These results emphasize the need for further sanitary education and better control of animal husbandry especially focusing on swine production on a small scale, family farms and hunters.

5. Conclusion

In this study we highlight the importance of an urgent need to implement interdisciplinary and inter-institutional programs aimed to achieve a major control that guarantees food safety, epidemiological surveys and an effective response in case of an outbreak in countries where the infection is present.

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Ethical approval

N/A; the work was carried out in accordance with the code of ethics of the Declaration of Helsinki for experiments involving humans.

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