SHORT REPORT: IDENTIFICATION OF THE LIKELY ETIOLOGIC AGENT OF HUMAN TRICHINELLOSION IN SICILY (ITALY) BETWEEN 1933 AND 1946

EDOARDO POZIO AND GIUSEPPE LA ROSA

Laboratory of Parasitology, Istituto Superiore di Sanità, Rome, Italy

Abstract. Larvae of Trichinella sp. collected in 1961 from muscle samples of the last infected domestic pig slaughtered in Sicily (Italy), and preserved in absolute ethyl alcohol until 1997, have been identified as Trichinella spiralis by the polymerase chain reaction. This finding explains the severity of the clinical picture, resulting in death, and the high number of fatal cases (20 of 184, 11%) observed in three Sicilian outbreaks between 1933 and 1945, caused by the consumption of sausages from pigs originating from the same focus of 1961. The relationship between the human outbreaks and the infected pig of 1961 is supported by epidemiologic data that show that Sicily was and is Trichinella-free, with the exception of the above reported focus.

In Italy since 1887, when the first human case of trichinellosis was described during a post-mortem examination, 1,483 infections have been reported in humans. Twenty-two infections were fatal and all occurred before 1946. In 1917, two persons died in an outbreak involving 20 persons in northern Italy who consumed infected pork during World War I. The other 20 fatal cases occurred in Sicily in three outbreaks involving 184 persons between 1933 and 1945. The last outbreak of trichinellosis in Sicily occurred in 1946, but no deaths were reported (Table 1). Epidemiologic investigations carried out from 1933 to 1946 showed that the source of infection was sausages from pigs bred on family farms in a village, Montemaggiore Belsito, near Palermo (Sicily). The porcine trichinellosis focus in Montemaggiore Belsito came to an end only in 1961, when the last infected pig was found at slaughtering. At that time, muscle tissue samples from the pig were collected and preserved in absolute ethanol for teaching purposes. By chance, 36 years later, we have come into possession of these muscle samples, allowing us to identify the etiologic agent of the Sicilian trichinellosis outbreaks and to explain the high mortality rate observed.

Muscle samples were cut into small pieces (approximately 2 × 2 mm). Muscle larvae were collected by hand with the aid of two needles under a dissection microscope at 40–50×. They were washed 10 times in water and stored separately in 1-ml vials at −30°C. For crude DNA preparation, individual muscle larvae were recovered in 14 μl of Tris-HCl, pH 7.6, overlaid with mineral oil, heated at 90°C for 10 min, treated with 100 μg/ml of proteinase K at 55°C for 3 hr, and heated again at 90°C for 10 min. The amplification experiments using the polymerase chain reaction (PCR) were carried out both with the random oligonucleotide primer AGCGCTGTAGAAGATGAAAGAT according to the protocol for the random amplified polymorphic DNA (RAPD) analysis of Bandi and others, and with one of two primers (forward CTCCACTTACGCAATGCAACG, and reverse ACCACCAACGGCAACTGCTA) specific for Trichinella spiralis according to the protocol of Wu and others. The amplification experiments were carried out three times separately for each larva. Muscle larvae from two reference strains, T. spiralis (code ISS3; Trichinella Reference Center, Istituto Superiore di Sanità) and T. britovi (code ISS2), were used for comparison. Reference larvae of T. britovi were not included in the electrophoresis of PCR amplification products because the primer pairs used do not amplify any sequence of this species.

Forty-seven muscle larvae were collected from the muscle tissue samples of the Sicilian pig. After electrophoresis of the RAPD amplification products, muscle larvae from the Sicilian pig did not show any reproducible pattern. In contrast, after electrophoresis of PCR amplification products made using the specific primers, six muscle larvae (12.8%) showed the specific band for T. spiralis of approximately 444 basepairs, similar to the muscle larvae from the T. spiralis reference strain (Figure 1).

The RAPD pattern showed several nonreproducible bands in 12.8% of the samples or no bands in 87.2% of the samples; we have frequently observed this phenomenon when the DNA of muscle larvae is partially or completely degraded. In this case, no comparison can be made between the pattern under study and that of the reference muscle larvae. This is one of the limitations of RAPD analysis.

At the present time, the epidemiology of trichinellosis in Italy is characterized by the presence of only the sylvatic cycle of T. britovi in the red fox (Vulpes vulpes). Trichinella spiralis has been observed in Italy only in imported animals (two horses and a wild boar), and in a wild red fox shot near the border of France. Between 1958 and 1987, more than 2,600 red foxes and approximately 100 mustelides were examined for Trichinella infection in Sicily, but all examined animals were negative. These data suggest that Sicily, like other Mediterranean islands (Sardinia, Corsica), is Trichinella-free. The discovery that T. spiralis existed in Sicily explains the finding of this parasite in synanthropic rodents in the focus of porcine trichinellosis. In fact, the sylvatic species T. britovi, the only one present on the mainland of Italy, is unable to be maintained in a rodent population.

The identification of the etiologic agent of the Sicilian outbreaks as T. spiralis explains the severity of the clinical picture, resulting in the death of 11% (20 of 184) of the infected persons. The causes of death were bronchopulmonary complications and severe myocarditis. In a review of 41 fatal cases of trichinellosis in humans, post-mortem examination revealed three major causes of death, including myocarditis and pneumonitis, in addition to encephalitis. The identification of the etiologic agent of the Sicilian outbreaks as T. spiralis is also consistent with our experience that the clinical picture is more severe in T. spiralis than in T. britovi infections in humans. The mortality rate observed in the Sicilian outbreaks (average = 11%, range = 6–15%) is also high in comparison with the mortality rate observed.
in epidemics that occurred in other parts of the world in the same decades. One possible explanation for this high mortality rate could be the poor health conditions that prevailed from 1942 to 1945 due to the war. In fact, the mortality rate observed in the first outbreak, which occurred before the war, was 6%, compared to the 10–15% rate observed in the outbreaks that occurred during the war, and no deaths were observed in the last outbreak, which occurred one year after the war had ended.

What was the origin of the presence of *T. spiralis* in Sicily? The most reasonable explanation is that pork or pork products (sausages, salami) infected with *T. spiralis* were brought to Sicily from persons who had emigrated to the United States and then returned home. It is well known that in the 1930s, hundreds of thousands of persons emigrated from Sicily to the United States, where domestic trichinellosis was a common infection in pigs. Leftovers of this infected food could have been ingested by domestic pigs, establishing a domestic focus on this island. Another explanation could be the importing of infected pork or pork products from a European country (France, Germany, Spain, etc.) in which the domestic cycle occurred. Further explanations, though unlikely, could be that synanthropic rats ate the corpse of an infected emigrant who returned to Sicily before dying, or an infected emigrant who returned to Sicily and was killed by the Mafia and given to pigs as food.

Finally, it is of interest that the DNA of larvae in muscle tissues preserved in absolute ethanol for 36 years was still undamaged, at least in part, allowing the identification by PCR of the parasite at the species level.

Acknowledgments: We thank Marco Amati for technical assistance, and Dr. Stefano Riili who provided the pork samples preserved in ethanol since 1961.

Financial support: This work was partly supported by funds from the U.S. Department of Agriculture, Agricultural Research Service (Beltsville, MD).

Authors’ address: Edoardo Pozio and Giuseppe La Rosa, Laboratory of Parasitology, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.

REFERENCES


**TABLE 1**

Outbreaks of human trichinellosis in Sicily (Italy)*

<table>
<thead>
<tr>
<th>Year</th>
<th>Locality of the human outbreak (province)</th>
<th>Deaths/infected persons (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1933</td>
<td>Casteltermini (Agrigento)</td>
<td>5/80 (6)</td>
</tr>
<tr>
<td>1942</td>
<td>Villafrati (Palermo)</td>
<td>2/20 (10)</td>
</tr>
<tr>
<td>1945</td>
<td>Montemaggiore Belsito (Palermo)</td>
<td>13/84 (15)</td>
</tr>
<tr>
<td>1946</td>
<td>Caccamo (Palermo)</td>
<td>0/15</td>
</tr>
</tbody>
</table>

* No other human infections were reported before or after these epidemics in Sicily. The breeding site of the infected pigs that were sources of human infection was always the village of Montemaggiore Belzio near Palermo.

**FIGURE 1.** Identification of *Trichinella* larvae collected from muscle samples of the infected Sicilian pig slaughtered in 1961 by the polymerase chain reaction using the primer pairs specific for *Trichinella spiralis* according to the protocol of Wu and others. Lanes 1, 10, and 18, patterns derived from the reference strain of *T. spiralis*; lanes 2–9 and 11–17, polymerase chain reaction on the unknown larvae. Only larvae in lanes 5 and 13 show the specific band of 444 basepairs. Since no bands were observed in lanes 2–4, 6–9, 11, 12, and 14–17, the DNA of these larvae was assumed to be degraded. Lane M is a molecular weight marker (100-basepair ladder; Pharmacia, Uppsala, Sweden). kb = kilobases.