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THE TRICHINELLOSIS RISK IN UMBRIA, ITALY. A SEROEPIDEMIOLOGICAL STUDY IN 1000 NORMAL SUBJECTS

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ABSTRACT: The Umbria region of Italy is peculiar in that two risk factors for human trichinellosis are concomitant: the great number of wild boars hunted and the frequent habit of breeding domestic pigs. To evaluate the risk of infection in humans, about 1000 people from different centers in the Umbria region were interviewed as to their alimentary habits and then studied serologically by screening tests for *Trichinella*-specific antibodies. The results show that, though 81% of people usually consume domestic pork meat, and 46% of those usually consume wild animal (mainly wild boar) meat, only 1.39% of sera resulted positive for antibodies with screening tests. This positivity was not confirmed by a Competitive Inhibition Assay, whose results were more specific. The search for antibodies against some *Enterobacteriaceae* showed a high positivity rate in the sera that was positive in the screening tests, suggesting a cross-reaction between the nematode and these bacteria. The very low prevalence of *Trichinella*-specific antibodies in normal subjects studied could be due to the fact that 76.5% of these declared that they only ate well-cooked meat.

KEY WORDS: Trichinellosis, seroepidemiological study, competitive inhibition assay, Umbria, Italy.

INTRODUCTION

Trichinellosis has a world distribution and there are few geographic areas where it is rare or absent (MORTENSEN, 1995).

In Italy trichinellosis, together with echinococcosis/hydatidosis, is the most prevalent helminthic infection (POZIO, 1991). In particular, in the Umbria region there is the concomitant presence of two risk factors for human trichinellosis: 1) a great number of wild boars hunted (in one year more than 5000 heads are hunted in the Umbria region alone) in the presence of a relevant sylvatic cycle; 2) the very frequent habit of slaughtering domestic pigs.

The sylvatic cycle in Umbria mainly involves foxes, which represent the main reservoir of the parasite in the Central Appenine area. In a particular area of the Umbria region, near Vallo di Nera-Norcia-Cascia, 33% of the foxes examined in the past were found to be infected by *Trichinella* (BALDELLI & FRESCURA, 1963), and similar results were obtained more recently by PIERGILI-FIORETTI, SPACCHETTI & POLIDORI (1986). The sylvatic cycle also involves wolves and wild boars. The wild boar in Europe, and also in Italy, is an important reservoir of the nematode *Trichinella* and it is a frequent zoonotic source of human infection, in particular where hunting is a traditional sport (CAMPBELL *et al.*, 1988; WORLEY *et al.*, 1994). It was supposed that the parasite passed from foxes or other wild animals to wild boars, at least partially as a result of the hunters' habit of discarding animal carcass remnants which were scavenged by boars (WORLEY *et al.*, 1994). Furthermore, in this region domestic pig breeding is very frequent (13403 pigs slaughtered in 1992 and 12399 in 1993). In these conditions the risk of passage from the sylvatic cycle to rodents and then to swine is very high, rats being a possible link between the sylvatic and the domestic cycles (MURRELL & BRUSCHI, 1994). Despite this situation, outbreaks of trichinellosis are quite rare. Following one in 1953 involving 9 people, the most recent was in 1988, in the Polino area, involving 48 people who had consumed sausages made with raw wild boar meat (FRONGILLO *et al.*, 1992). The *Trichinella* species responsible was *T. britovi*, autochtonous in Italy, and known to produce a mild symptomatology (POZIO *et al.*, 1993). In fact, only 42% of infected people in the above-described outbreak had clinical symptomatology.

The discrepancy between the high risk of infection and the very rare clinical cases could be explained by the existence of low-grade infections completely asymptomatic in humans, and by the fact that *T. britovi* has a low fecundity (POZIO *et al.*, 1992).

To verify this hypothesis, we planned a seroepidemiological study on about 1000 normal subjects, with no clinical history of trichinellosis, living in different parts of the region, evaluating their alimentary habits, the prevalence of *Trichinella*-specific antibodies in their sera, and indirectly possible contact with the parasite. Sera resulting positive in screening tests were also evaluated by a confirm test, to exclude possible cross-reactions. Similar studies were performed in 900 normal subjects in The Netherlands (VAN KNAPEN & FRANCHIMONT, 1983) and in the Italian Lombardy region on 982 blood donors (DI MATTEO *et al.*, 1992). Unfortunately in both cases no confirm test was used and no information is available about the alimentary habits of serologically evaluated subjects for correlation with serological data.

MATERIAL AND METHODS

Recruitment of subjects: Male and female subjects with no clinical history of trichinellosis, age range from 0 to 60+ years, arrived at collection Centres for routine analyses (Table 1). They were interviewed and an anamnestic-epidemiological questionnaire compiled for each, with particular attention as to alimentary risk habits (wild animal and in particular wild boar meat, domestic pig meat, horse meat, raw or poorly cooked meat, visits abroad).

Sera collection: Between March 1, 1993 and January 31, 1995, 1017 sera were collected from 1002 subjects in 10 Centres chosen to cover most of the Umbria region (Amelia, Cascia, Gualdo, Gubbio, Norcia, Orvieto, Passignano, Perugia, Spoleto and Terni). After collection, sera were stored at –20° C until used.

Serological screening tests: *Trichinella*-specific antibodies were detected in all the 1017 sera, using commercially available immunoenzymatic tests, such as Prowell *Trichinella spiralis* (296 sera), *Trichinella* serology-LMD Laboratories, Inc. (63 sera), Trichinosis Biolife (406 sera) and commercially available indirect haemagglutination tests (252 sera) such as ANI™ Trichine Ab. In all these cases parasitic antigen is represented by excretory/secretory (E/S) antigen from muscle larvae. Sera were first evaluated in pools of 5 sera, at optimal dilutions. When a pool resulted positive all corresponding sera were evaluated one by one again. Sera were considered positive when the ratio (R) between the optical density and the test cut-off was > 1 (BRUSCHI, POZIO & TASSI, 1990).

Serological confirm test: To confirm the positive results obtained at screening, a competitive inhibition assay (CIA) using a monoclonal antibody (PG6B1) specific for a 49-53 Kda antigen of *Trichinella* (MARINI *et al.*, 1993), previously described (MARINI *et al.*, 1994) was used. Briefly, polystirene plates (Nunc, Polysorp) were incubated with the monoclonal antibody as catcher diluted 1:500 in 0.1 M carbonate-bicarbonate buffer pH 9,6 overnight at 4° C. After washing with 0.01 M phosphate-buffered saline (PBS) added to Tween 20 at 0.05% (PBST), 100 µl of the E/S antigen from *Trichinella* muscle larvae was incubated for 2 hours at 37° C, diluted in PBST added to sheep serum at 2% (PBSTS) to obtain a concentration of 7 µg proteins/ml, which was optimal after checkerboard titration. After washing with PBST, plates were incubated with 100 µl human sera from normal subjects or from trichinellosis patients as positive controls (giving a 100% inhibition) and diluted 1:5 in PBSTS. Plates were repeatedly washed, then peroxidase-conjugated monoclonal antibody PG6B1 diluted 1:500 in PBSTS was added. After further washings, complexes were revealed by adding a solution of o-phenylenediamine (Sigma) and hydrogen peroxide. After 15 minutes the reaction was stopped with H_2SO_4 0.5 M and plates were read at 492nm, with a Titertek Multiskan (Flow).

Age	0-14	15-60	>60	Total
No. males	15	248	153	416
%	3,6	59,6	36,8	41,5
No. females	16	372	198	586
%	2,7	63,5	33,8	58,5
Total	31	620	351	1002
%	3,1	61,9	3,5	100

Table 1.– Age and sex of the studied population.

The inhibition rate was calculated according to the following formula:

$$\frac{\text{O.D. of the negative control} - \text{O.D. tested serum}}{\text{O.D. of negative control} - \text{O.D. of positive control}} \times 100$$

where O.D. is optical density.

Samples with an inhibition equal to or greater than 50% were considered positive, according to results obtained from 245 negative sera (206 from swine and 39 from humans) and 12 positive sera (from experimentally infected swine).

Microbiological tests: Sera testing positive with screening tests were also evaluated for the presence of antibodies against *Salmonella typhi*, according to Widal-Wright modified for O and H antigens and against *Brucella abortus*, using a commercially available kit (Febrile Antigène kit Slide Test, Sclavo).

RESULTS

Epidemiological study

Data obtained from analysis of the questionnaires show that 81% of the people contacted frequently ate pork meat from non-industrialized breedings, 46% wild animal meat, 30% wild boar meat and about 14% horse meat. However, 76,5% of the people interviewed declared they ate only well cooked-meat (Fig. 1 and 2).

Serological study

Only 14 (11 males and 3 females) of 1002 subjects showed positive in the screening test (1,39%). Two were evaluated with Prowell test, 11 with the Trichinosis Biolife and 1 with the ANI™ Trichine Ab. The origin and the alimentary habits of these subjects are reported in Table 2.

Though 3 of 14 subjects declared they ate poorly cooked meat, no difference was observed in the risk habits of this group and the other 986 subjects. In the CIA test 11 of of 14 sera were negative, and 3 doubtful (51% of inhibtion) (see Table 3). An immunoblot analysis revealed that these sera did not recognize any *Trichinella* antigen (data not shown). In 13 of 14 sera showing positive in the screening phase, it was possible to check the presence of antibodies against some *Enterobacteriaceae* known to give cross-reactions with *T. spiralis* (RODRIGUEZ-OSORIO, GOMEZ-GARCIA & CAMPOS BUENO, 1977). The results (Table 3) show that 11 of 13 were positive for antibodies against *Salmonella paratyphi* A-B and *S. typhi*.

DISCUSSION

The results indicate a high incidence of both wild boar and non-industrialized pork meat consumption in the Umbria region. These habits are frequent all over the re-

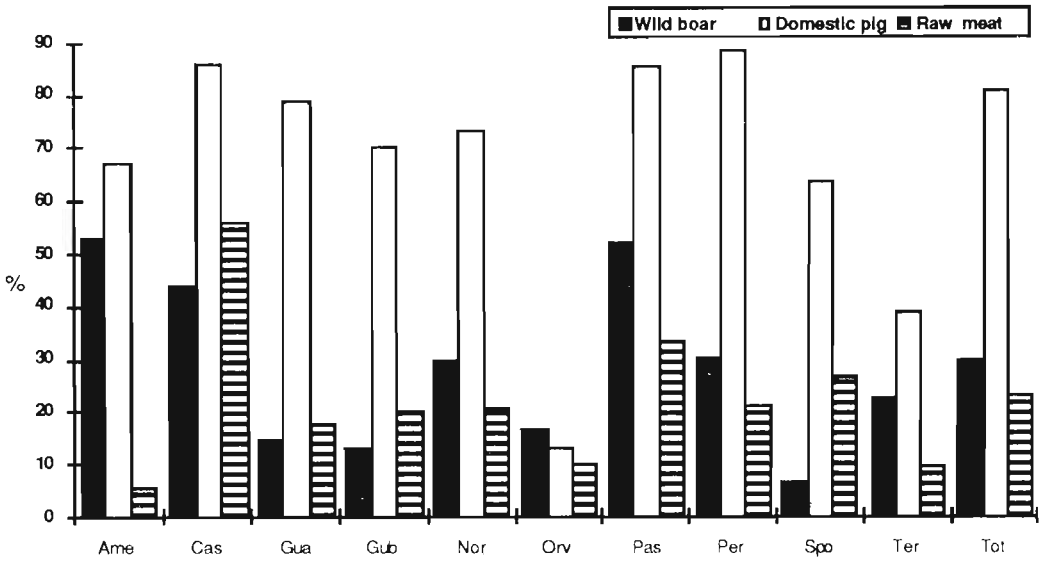

Fig. 1.– Wild boar, domestic pig and raw meat as risk factors for trichinellosis in all population studied. Ame = Amelia; Cas = Cascia; Gua = Gualdo; Gub = Gubbio; Nor = Norcia; Orv = Orvieto; Pas = Passignano; Per = Perugia; Spo = Spoleto; Ter = Terni.

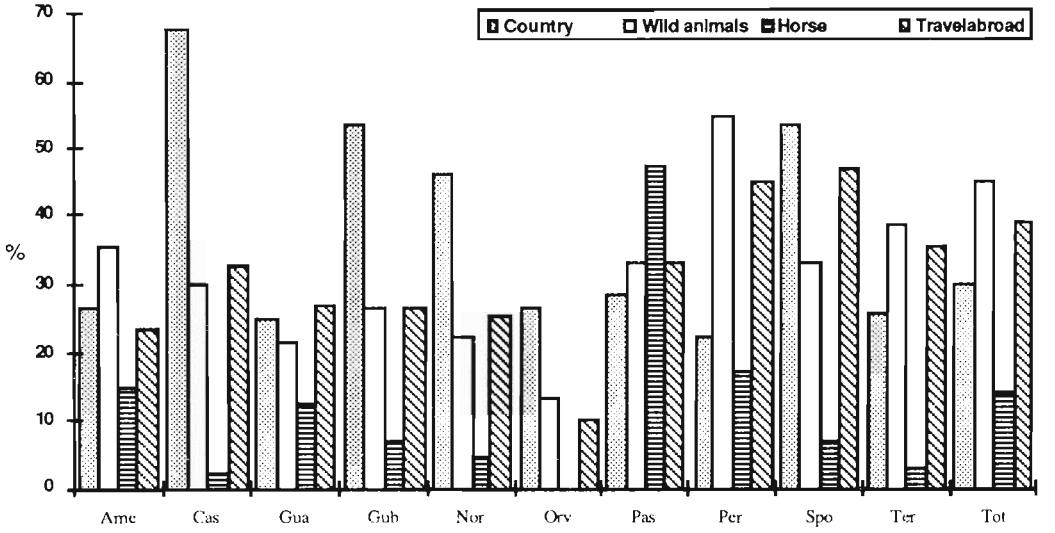

Fig. 2.– Country, wild animals, horse and travel abroad as risk factors for trichinellosis in all population studied. Ame = Amelia; Cas = Cascia; Gua = Gualdo; Gub = Gubbio; Nor = Norcia; Orv = Orvieto; Pas = Passignano; Per = Perugia; Spo = Spoleto; Ter = Terni.

gion, irrespective of the area examined. However, there is a common habit of consuming well-cooked meat and this is a reliable system of preventing infections. In fact, despite the high risk of coming into contact with the parasite, only 1,39% of evaluated subjects had antibodies directed against *Trichinella*. Our results are in good agreement with those obtained by van KNAPEN & FRANCHIMONT (1983), who found no positive sera in 900 people when specific IgG were examined, and 6 of 424 (1,4%) when specific IgM were evaluated. It was hypothesized that subjects with specific IgM were sub-clinically infected because a sporadic consumption of pork meat infected at low grade. In The Netherlands the risk of trichinellosis is quite low since most of the pigs are bred industrially, but there is a sylvatic cycle involving rats (6,6% maximum infected) and foxes (3,7% maximum infected) (FRANCHIMONT, VAN KNAPEN & KREMERS, 1994). Among 982 blood donors, DI MATTEO *et al.* (1992) found only two subjects (who frequently consumed horse meat) who were positive for *Trichinella*-antibodies, corresponding to 0,2% in the Lombardy region (Italy).

It is worth noting that our sera, although positive in the screening test, were not at a CIA test, suggesting a possi-

	Centres														
	Perugia							Cascia			Gualdo	Amelia	Norcia		Total
Subjects	1	2	3	4	5	6	7	8	9	10	11	12	13	14	14
Country habitat			+				+		+		+			+	5
Wild boar meat				+	+	+		+		+		+			6
Wild animal meat					+	+				+					4
Domestic pig meat	+	+	+	+	+	+	+	+	+	+		+	+	+	13
Horse meat						+						+			2
Little-cooked meat	+		+				+								3
Travel broad	+	+			+								+		4

Table 2.– Risk factors for trichinellosis in subjects resulting positive in screening tests.

Sera positive in screening tests	ELISA (R*)	CIA results	Reaction to *Salmonela*	Reaction to *Brucella*
1	1,2	25	1:160	–
2	1,6	23	1:50	–
3	1,2	51	1:50	–
4	2,1	30	1:50	–
5	n.d.**	29	n.d.	–
6	2,3	30	1:50	–
7	1,2	22	1:80	–
8	1,2	25	1:50	–
9	3,0	28	–	–
10	2,5	22	–	–
11	1,2	51	1:50	–
12	1,1	51	1:80	–
13	2,6	29	1:160	–
14	3,6	30	1:80	–

Table 3.– Serological tests used and results. *: R = optical density/cut-off; **: n.d. = positivite from agglutination test.

ble cross-reaction with other infective agents. This hypothesis is confirmed by the results obtained with the Widal-Wright reactions, which were positive in most cases, confirming the previously described cross-reactions between the genus *Trichinella* and some *Enterobacteriaceae* (RODRIGUEZ-OSORIO, GOMEZ-GARCIA & CAMPOS BUENO, 1977). These subjects were probably affected previously by *Salmonella* asymptomatic infections or were immunized by vaccination. The epidemiological study also revealed that no substantial difference in risk factors was present between the positive subjects and the rest of the studied population, even if the 14 subjects came from areas with higher risk factor rates for trichinellosis, and 13 of these declared they ate meat from domestic pigs and 3 often ate poorly-cooked meat.

Many hypotheses can be made to explain the very low prevalence of antibodies specific for *Trichinella*. The habit of eating well-cooked meat is very common among the studied population. This gives protection against infected wild animals. Regarding the risk of infection from consumption of domestic pork meat, it is important to note that sylvatic *Trichinella* species are poorly infective for swine (MURRELL *et al.*, 1985). This fact could probably be a barrier between the sylvatic and domestic cycles and therefore explain why the presence of the risk factor from domestic breed swine has no significant implication on human health.

Finally, our study shows that it is necessary to be careful, when using commercial kits for the diagnosis of human trichinellosis, in evaluating positive results, especially in sporadic cases of infection, since it is possible to have false positive.

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