Article available at http://www.parasite-journal.org or http://dx.doi.org/10.1051/parasite/200108s2249

SWINE TRICHINELLOSIS IN SLAUGHTERHOUSES OF THE METROPOLITAN AREA OF TOLUCA

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Summary :

In order to determine the prevalence of *Trichinella spiralis* infections in abattoirs of the metropolitan area of Toluca where pigs from commercial farms as well as backyard pigs are slaughtered, 539 swine diaphragm tissue samples were collected and examined by trichinoscopy and artificial digestion. Serum samples from the same animals were analyzed by ELISA using somatic and excretory/secretory antigens, and by Western blot analysis. *T. spiralis* muscle larvae were not found by trichinoscopy or artificial digestion. However, specific antibodies were detected by ELISA and confirmed by Western blotting in 12.4 % of the serum samples examined. Analysis of risk factors showed no association of seropositive results with sex. However, significant higher risk was observed in swine seven to 12 months old and in backyard pigs, compared with pigs from commercial farms.

KEY WORDS : Trichinellosis, swine, seroprevalence, risk factors.

Trichinellosis is considered endemic in the metropolitan area of Toluca, State of Mexico, where several human cases were documented from 1978 to 1982 and two outbreaks involving 18 persons occurred in 1983. Thirty eight more cases, confirmed by biopsy were reported from 1985 to 1986 and 15 were officially notified from 1992 to 1997 in this area (Martínez-Marañón *et al.*, 1985; González *et al.*, 1991; Ortega-Pierres *et al.*, 2000). In addition, a frequency of 14.8 % of *Trichinella* infections was determined in human cadavers taken to the Forensic Service in Toluca (González *et al.*, 1991). In all cases, consumption of infected pork meat was considered as the source of infection for humans.

On the other hand, *Trichinella spiralis* infections in backyard pigs from this area have also been demonstrated both by ELISA and Western blot analysis and by direct detection of the parasite in muscle tissue samples by artificial digestion. In these studies a seroprevalence of 24 % was determined (Arriaga *et al.*, 1989) and muscle larvae (ML) were detected in three of 50 (6 %) pigs examined at slaughter (Arriaga *et al.*, 1991). Nevertheless, data on prevalence of swine trichinellosis in abattoirs of this area is limited. In 1992 the Health Institute of the State of Mexico (ISEM) started a program of Control and Epidemiologic Survaillance of trichinellosis, based on inspection by trichinoscopy at the municipal abattoir of Toluca where pigs from commercial farms as well as backyard pigs are slaughtered. During 1993 and 1994, a total of 151,900 carcasses were examined and in two animals *Trichinella* ML were detected. Since then, positive animals have not been notified.

In this study, in addition to trichinoscopy, artificial digestion and immunoenzymatic techniques were used to determine the prevalence of *T. spiralis* infections in these abattoirs. Association of the presence of specific antibodies against *T. spiralis* with sex, age and type of farm was also investigated.

MATERIAL AND METHODS

TISSUE AND SERUM SAMPLES

A total of 539 swine diaphragm tissue and serum samples were collected. One hundred of the animals were backyard pigs and the rest came from commercial farms.

ARTIFICIAL DIGESTION AND TRICHINOSCOPY

Tissue samples (10 g) were examined by artificial digestion with 1 % pepsine-HCL and trichinoscopy, according to standard procedures.

ANTIGENS

Somatic (SOM) antigen was prepared as described before (Parkhouse *et al.*, 1981) and excretory/secretory (E/S) antigens were obtained according to Gamble *et al.* (1983).

ELISA

Serum samples were diluted 1:50 and analyzed by ELISA using the method described by Arriaga *et al.* (1989) with some modifications. Cut off values were

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determined as the average optical density (O.D.) value obtained with serum samples from non infected swine plus two standard deviation. ELISA-positive samples were confirmed by Western blot as described before (Arriaga *et al.*, 1989).

Epidemiologic analysis

EPIDAT (APHIS) was used to determine associations between variables and carry out risk analysis by sex, age and type of farm.

	Positive	%	Negative	%	Total
ELISA-SOM[a]	75	13.9	464	86.1	539
ELISA-E/S[a]	63	11.7	476	88.3	539
Western Blot	67	12.4	472	87.6	539

[a] Cut off value for ELISA was determined as the average OD value obtained with serum sample from 20 non infected swine plus two standard deviation. This values were 0.5 for ELISA-SOM and 0.6 for ELISA E/S.

Table 1. Percentage of samples positive by ELISA and Western Blot.

RESULTS

Trichinella ML were not detected by artificial digestion or trichinoscopy in any of the samples examined. However, 13.9 % (75/539) of the serum samples were positive in ELISA using SOM and 11.7 % (63/539) were positive using E/S antigens (Table I). Sixty seven out of 539 (12.4 %) samples, 63 of them positive in ELISA using E/S antigens and four positive in ELISA only with SOM antigen, were confirmed by Western blot analysis since they showed characteristic recognition of TSL-1 antigens. Representative patterns of antigenic recognition by these serum samples are shown in Figure 1. Distribution of seropositive results by sex, age and type of farm is presented in Table II. Higher seroprevalence rates were obtained with backyard pigs and with pigs 7-12 months old. Statistical relative risk analysis showed no association with respect to sex. However, higher risk was observed in swine 7-12 months of age (O.R. 6.49) and in backyard pigs (O.R. 2.47) as shown in Table III.

DISCUSSION

In this study, a seroprevalence of trichinellosis of 12 % was determined in swine slaughtered in the area of Toluca, although no *Trichinella* ML could be detected in these animals either by trichinoscopy or artificial digestion. Although cross reactivity with

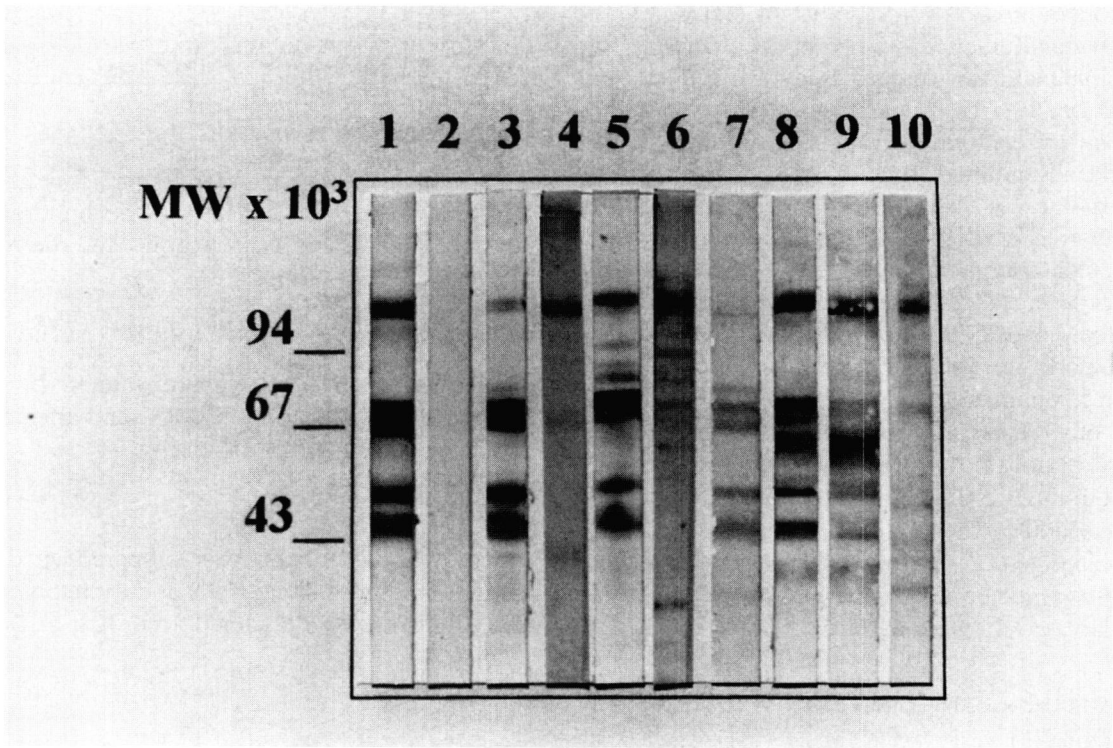

Fig. 1. – Representative patterns of antigenic recognition obtained by Western blot analysis with serum samples from slaughtered pigs. Lane 1: serum sample from experimentally infected swine. Lane 2: serum sample from non infected control. Lanes 3-10: serum samples positive in ELISA using E/S antigens.

Sex	Sample	%	Positive	Rate (x 100)
Male	282	54.5	37	0.13
Female	257	45.5	30	0.11
Total	539	100.0	67	–
Age (months)	**Sample**	**%**	**Positive**	**Rate (x 100)**
0-6	232	43.0	26	0.11
7-12	178	33.0	37	0.20
13-24	103	19.1	4	0.04
> 25	26	4.9	0	0.0
Total	539	100.0	67	–
Farm type	**Sample**	**%**	**Positive**	**Rate (x 100)**
Backyard	100	18.6	22	0.22
Commercial	439	81.4	45	0.10
Total	539	100.0	67	–

Table II. – Epidemiological Analysis of Western blot results.

Sex	Odds Ratio Confidence Interval	Risk Ratio Confidence Interval	χ^2
Male	1.14	1.12	0.26
	0.66 < OR < 1.97	0.72 < RR < 1.76	
Female	1.0	1.0	
Age (months)	**Odds Ratio Confidence Interval**	**Risk Ratio Confidence Interval**	**χ^2**
0-6	3.12	2.89	4.69
	1.00 < OR < 10.87	1.03 < RR < 8.06	
7-12	6.49	5.35	14.96
	2.12 < OR < 22.2	1.96 < RR < 14.6	
13-24	1.0	1.0	–
Farm type	**Odds Ratio Confidence Interval**	**Risk Ratio Confidence Interval**	**χ^2**
Backyard	2.47	2.15	10.33
	1.35 < OR < 4.5	1.35 < RR < 3.4	
Commercial	1.0	1.0	–

P < 0.05.
Confidence level 95 %.

Table III. – Risk Analysis of Western blot results.

other nematodes or bacteria infecting swine could explain some of the positive results obtained in ELISA using crude extracts of the parasite, the use of E/S antigens greatly improves the specificity of the test (Gamble *et al.*, 1983; Arriaga *et al.*, 1989). Besides, the fact that serum samples positive in ELISA using E/S showed reactivity mainly with TSL-1 antigens suggests that these are specific reactions. These results are in accordance with the high percentage of seropositive backyard pigs that has been previously determined in this region (Arriaga *et al.*, 1989). Furthermore, the seroprevalence found in this area agrees with the frequency of 14.8 % that has been reported in human cadavers (González *et al.*, 1991). Failure to detect ML by artificial digestion could probably be explained by low parasite burdens, below the limit of detection of this technique. In this case, recovery of ML could be improved by increasing the sample size.

As expected, the percentage of seropositive animals was higher among backyard pigs than in swine from commercial farms and stresses the need of adequate farming conditions for the control of trichinellosis. The finding of higher risk in swine in the age group of 7-12 months was interesting since these were animals that for different reasons did not gain weight, thus took longer to be ready for the slaughterhouse. Association of trichinellosis with other diseases could probably explain this finding and should be investigated.

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