EPIDEMIOLOGICAL SURVEY OF TRICHINELLOSIS IN YUGOSLAVIA

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Abstract. In a long term epidemiological survey that included different hosts for *Trichinella spiralis* among wild and domestic animals, the parasitological and serological screening revealed a high number of positive animals.

The incidence of *T. spiralis* in grazing pigs was high, 21.76% (170 examined) but most of positive animals were lightly infected (0.016–0.02 l/g). While out of 2,394 pigs raised in small private farms, 1.67% were positive with high infection rates, none of the pigs raised on a modern breeding and fattening farm were seropositive. A repeated survey in grazing pigs performed five years later and in the same area, showed the prevalence of seropositive pigs to be still high (22% of 100 examined pigs).

The same data were confirmed by detection of specific anti-*Trichinella* antibodies in Western blotting procedure. One of 2,031 serologically (IgG-ELISA) examined horses from Croatia reacted positively with very high OD values. Different characteristics of biological patterns (RCI, female fecundity, capsule formation, isoenzyme analysis) were found between two well distinguished groups of isolates.

The first group consisted of well defined T1 isolates with patterns described by the Reference Centre for Trichinellosis, Rome, Italy. The data obtained by isoenzyme analysis of the representative sylvatic isolate were comparable to T3 characteristics of isolates from foxes in Italy.

INTRODUCTION

In the last ten years the knowledge of trichinosis in Yugoslavia has increased (Rapic *et al*, 1985). Increased number of outbreaks and interest in epidemiology has contributed to the knowledge of the most important transmission routes of the infection in endangered regions of the country. New immunodiagnostic methods including ELISA have also improved the diagnosis of this zoonosis in pigs and horses.

In this study the following aspects of epidemiological survey for trichinellosis will be presented:

1. Epidemiology of trichinosis in pigs raised under different rearing conditions.
2. Incidence of trichinosis in some wild mammals killed in northwestern region of the country (Croatia, Slovenia).
3. Seroprevalence in horses from small private farms.
4. Biological differences among isolates of *T. spiralis* from rural and sylvatic cycle.

MATERIALS AND METHODS

Antigen

For ELISA and Western blotting procedures, *T. spiralis* was kindly provided by HR Gamble (US Department of Agriculture, Beltsville, MD, USA).

Indirect Micro Elisa

Micro Elisa plates (Titertek, Flow Laboratories) were coated with 100 µl of excretory-secretory antigen per well at a concentration of 5 µg/ml in 0.1M carbonate-bicarbonate buffer (pH 9.6). After overnight incubation the antigen was discarded and the plates were washed with PBS Tween. After washing, 100 µl of 1:20 diluted sera were added in triplicate. The plates were incubated for 30 minutes at room temperature. After washing, 100 µl of peroxidase-labelled goat anti-swine IgG or goat anti-horse IgG (Kirkegaard and Perry Laboratories) diluted 1: 1000 in PBS were added to the wells. The visua-

lization was performed using 5-aminosalicylic acid (Merck) substrate. The plates were incubated at room temperature for 30 minutes and read at 450 nm using a Titertek Uniskan (Flow Laboratories).

Immunoblotting

Proteins were separated by SDS-PAGE in 10% gels using a Midget Electrophoresis Unit (LKB). Proteins were visualized by staining with Coomassie blue R-250 or transferred to nitrocellulose using a Midget Multi Blot Transfer Unit (LKB). Following transfer, blots were blocked in 50 mm TRIS (pH 7.5) containing 150 mm NaCl and 5% non-fat dry milk (wash buffer A). Blots were then incubated sequentially for 24 hours in swine or horse serum (diluted 1:40) in wash buffer A and goat anti-swine or goat anti-horse peroxidase-labeled IgG (diluted 1:1000 with wash buffer A) antibodies. Between and following antibody incubations, blots were washed twice with wash buffer A and twice with wash buffer B (wash buffer A containing 1% Triton × 100 and 0.1% SDS) and a final time in wash buffer A. The blots were developed in 0.18% 4-chloronapthol. (Kirkegaard and Perry Laboratories) and 0.012% hydrogen peroxide in TRIS-buffered saline.

Muscle sample collecting and parasitological examination

Diaphragmatic muscle samples from different hosts (pigs, wild animals) were collected from killed animals during different studies performed during epidemiological surveys in Slovenia and Croatia. Standard procedures for the pepsin-HCl digestion of muscle tissue (diaphragm or tongue) in all parasitological examinations were followed.

Sera collection

Sera collected during seroepidemiological surveys from pigs and horses were frozen until the day an ELISA or immunoblotting was performed.

Infectivity of pigs, mice and rats

Methods used was described by Murrell *et al* (1985). Reproductive capacity index in mice equals the mean number of larvae (10 outbred CD1 mice) recovered from the whole carcass per number of larvae infected. Reproductive capacity index in pigs equals the number of larvae recovered from 100g of diaphragm from an individual pig per number of larvae infected.

In vitro female fecundity in CD1 mice

The test was performed following the protocol of Marti *et al* (1986). The adult females for test were recovered 6 days after infection of 5 outbred CD1 mice (Charles River Laboratories, Italy). The results represent the mean newborn larvae burden in 24 hours from 40 females per isolate.

Histology

Capsule formation was described using different histological methods for capsule collagen visualization (Van Gieson staining, Masson Trichrome staining). The histological procedures were previously described by Marinculic *et al* (1990).

Isoenzyme analysis

Isoenzyme analysis with 10 different gene enzyme systems was performed by the Reference Centre for Trichinellosis (Istituto Superiore di Sanita, Rome, Italy). The procedure was detailed by Pozio (1987).

RESULTS

Data obtained in our survey for Trichinellosis are presented in Tables 1–6.

DISCUSSION

Preliminary investigations demonstrated that sylvatic trichinosis is distributed at the same rate in both tested regions. This is especially true for data obtained in prevalence studies in foxes and wild boars. The presence of *T. spiralis* in the roe deer was not previously reported. The results also show that the high prevalence of *T. spiralis* infection among pigs raised under seasonal grazing conditions is probably due to close contact with sylvatic hosts of trichinosis. The fact that infection was very low is probably the result of a low *T. spiralis* infection by the potential sylvatic biotype source. The prevalence of possible natural trichinosis in grazing pigs was also determined by the presence of specific

Table 1

Trichinellosis in some wild mammals in Yugoslavia.

Host	No. samples examined	% Infected samples
Fox	201*	5.47
	48**	6.30
Lynx	17*	47.05
Badger	12*	25.00
Brown bear	6*	16.67
	8**	0
Wild boar	23*	4.34
	78*	7.89
Roe deer	3**	66.66

* Animals killed in Slovenia.
** Animals killed in Croatia.

Table 2

Epizootiological survey for trichinosis in pigs raised under different conditions.

Group	No. animals examined	Seropositive	Infection rate %
1*	170	21.76	0.016–0.02
2**	2394	1.67	1–256
3***	560	0	0

* Grazing pigs.
** Pigs raised on small private farms.
*** Pigs raised on a modern breeding and fattening farm.

IgG antibodies. Using the mentioned criteria 22% (100 examined) pigs were *Trichinella* seropositive.

Immunoblotting results with some positive sera confirm the real presence of specific IgG antibodies to excretory-secretory antigen of *T. spiralis*. Since positive animals were still kept under fattening conditions we were not able to define their real parasitological status. To further investigate the feasibility of these findings circulating antigen detection should be also performed (Ivanoska *et al*, 1988). Our findings concerning the seropositive horse was not clear since parasitological examination of the animal was not performed. The possibility of cross reaction with other horse nematodes is not excluded.

Different biological patterns of tested isolates clearly segregated two distinguished groups. The first group consisted mostly of pig isolates with high infectivity for pigs and mice that is also in correlation with data shown by Murrell (1985). The female fecundity in mice for same pig isolates was high, too. All isolates induced very rapid capsule formation and, according to the criteria established by the Reference Centre for Trichinellosis, they should be defined as T1

Table 3

IgG humoral response in grazing pigs (optical density values*).

Animal no.	Seropositive	Highly suspect
6001	0.384	
6003	0.481	
6005	0.502	
6009		0.310
6017	0.405	
6022		0.312
6031	0.428	
6042	0.464	
6101	0.361	
6108	0.399	
6109	0.415	
6110	0.349	
6121	0.351	
6128	0.430	
6131	0.391	
6134	0.484	
6174	0.402	
6175	0.480	
7143	0.384	
7154	0.452	
7187	0.432	
7653	0.390	
Normal controls**	0.099–0.123	

* Mean optical density (OD) values of three replicates per animal. The criterion for determining positive test result was the OD values >3 times the OD of the normal serum pool. In this study, OD values >2 times the normal serum control OD were considered highly suspect.

** Normal pig serum controls consisted of pools of 40 serum samples from farm raised pigs found to be noninfected.

isolates.

The second group of isolates with very low fecundity and infectivity induce slow formation of capsule what is especially true for isolate #10 from a wild boar. In order to compare the biology patterns with the genetic structure further isoenzyme analysis should be performed.

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Table 4

IgG humoral response of the seropositive horse (optical density values*).

Suspected horse	0.602
Positive control	0.710
Negative control (50 sera)	0.105–0.190

* Mean OD values of three replicates per animal.

Table 5

Biological patterns of different *Trichinella* isolates in Yugoslavia.

Isolate host	Infective to rats	RCI pigs	RCI mice
1 YUVFZGRR1 Rat	Yes	1.88–1.95	74.5
2 YUVFZGSSD1 Pig	Yes	0.94–1.26	22.5
3 YUVFZGSSD2 Pig	Yes	ND	34.2
4 YUVFZGSSD3 Pig	Yes	ND	81.8
5 YUVFZGSSD4 Pig	Yes	2.02–3.70	165.0
6 YUVFZGVV1 Fox	No	ND	17.5
7 YUVFZGVV2 Fox	No	ND	25.0
8 YUVFZGVV3 Fox	No	ND	20.6
9 YUVFZGMM1 Badger	No	0.002–0.14	3.3
10 YUVFZGSSF1 Wild Boar	No	0.16–2.97	16.8
11 YUVFZGSSF2 Wild Boar	No	0.012–0.035	7.8

Table 6

Biological patterns of different *Trichinella* isolates from Yugoslavia.

Isolate	Female fecundity	Capsule formation	Isoenzyme type
1	45.20	Fast*	
2	ND	Fast	
3	30.15	Fast	
4	52.15	Fast	
5	67.50	Fast	
6	11.25	Slow**	
7	9.15	Slow	
8	ND	Slow	
9	ND	Slow	
10	1.90	Slow***	T3
11	ND	Slow	

* Capsule around larvae completely formed 50 days after infection.

** Capsule around larvae completely formed after 50 days after infection (50-100 days).

*** Most larvae non-encysted until 100 days after infection.

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