TRICHINELLA SPP. IN OSTRICH MEAT: A PUBLIC HEALTH RISK?

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Summary :

In the present work the biological behaviour of *T. spiralis* and *T. pseudospiralis* in ostriches is reported. Oral infections were performed in eight ostriches with two infective doses (10,000 and 80,000 larvae) for each species of *Trichinella*. On day 0, 30 and 60 p.i. blood samples were collected to assay the serum changes concerning specific muscle enzyme activities and total proteins. The immunological study, to determine specific IgG in sera, was conducted employing a monoclonal blocking ELISA. From the carcasses of sacrificed animals, samples of various muscle tissues were examined by the digestion method and by standard histopathologic procedures. The study showed a low susceptibility of the ostriches to *T. pseudospiralis*; preferential sites of larval distribution were muscle tissues of the legs. *T. spiralis* could be found in muscle tissues only when a high number of larvae were inoculated. Immunological reactivity was found only in animals infected with higher doses of *T. pseudospiralis*.

KEY WORDS : ostriches, *T. spiralis*, *T. pseudospiralis*, muscle larvae, ELISA.

Today ostrich breeding is an important animal management sector all over the world (South Africa, United States, Canada, Australia, Europe, China) for the high quality of the meat.

No or little data are available on the ostrich's role as carrier of zoonotic parasites and no data are reported about natural and experimental infections with *Trichinella* spp. Thus, the infectivity of *T. spiralis* and *T. pseudospiralis* to ostriches were investigated in this study.

MATERIALS AND METHODS

ANIMALS

Ten ostriches, aged twenty-five days, clinically and parasitologically healthy, were used. The animals were given commercial feed (a mixture of maize and lucern).

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PARASITE

The *T. pseudospiralis* (*T. p.*) strain used was kindly provided by Dr E. Pozio (Istituto Superiore di Sanità, Roma) and since then has been maintained in Swiss mice. The *T. spiralis* (*T. sp.*) strain had been isolated from pigs in Denmark (kindly provided by Dr E.J. Ruitenberg) and maintained in Swiss mice.

EXPERIMENTAL DESIGN

T. p. and *T. sp.* larvae were obtained from the infected mice by artificial digestion and counted in McMaster counting chambers. Oral infections were performed in eight ostriches with two infective doses for each species of *Trichinella* as follows:

- two infected with 10,000 larvae of *T. p.*; two infected with 10,000 larvae of *T. sp.*;
- two infected with 80,000 larvae of *T. p.*; two infected with 80,000 larvae of *T. sp.*

Two animals were used as controls. For ten days p.i. the faeces were observed daily to evidence the presence of *T. p.* and *T. sp.* larvae. On day 0, 30, 60 p.i. blood samples were collected to determine (by standard techniques) the white blood composition, the serum changes of specific muscle enzyme activities (CK, LDH, GOT) and of total proteins, the presence in sera of specific IgG antibodies. The animals were sacrificed on day 60 p.i. and samples of various muscle tissues were examined by digestion method (20 grams for muscle sample) (Polidori *et al.*, 1988) and by standard histopathologic procedures (Moretti *et al.*, 1987).

SEROLOGICAL TEST

Sera were tested by a competitive inhibition assay using a monoclonal antibody (Mab Pg 6 B1) specific for a 47-53 KDa antigen of *Trichinella* previously employed and described (Piergili Fioretti *et al.*, 1996). Samples with an inhibition equal to or greater than 50 % were considered positive. To select the optimal percentage of inhibition, indicative of specific infection, the test was first performed on 50 serum samples from *Trichinella* free ostriches (collected at slaugther house from animals negative to trichinoscopy).

RESULTS

No clinical symptoms were observed in any of the animals; no changes in body temperature were observed; the increase in body weight was also normal.

PARASITOLOGICAL STUDY

Very low numbers of larvae were recovered from faeces, but only for two days p.i. The results obtained by digestion of the muscle tissues are summarized in Table I.

The infectivity of the two *Trichinella* species differed. For *T. pseudospiralis,* muscle larvae were recovered from all animals. The larval burden obtained per gram of muscle was very low and without significative differences (χ^2 test) between the animals infected with 10,000 larvae or with 80,000 larvae.

For *T. spiralis,* only in the experimental group receiving high parasitic doses (80,000 larvae) larvae were able to produce light infections. Biological tests in mice performed with the larvae of *T. p.* and *T. sp.* obtained by the digestion of ostrich muscles gave positive results.

HAEMATOCHEMICAL INVESTIGATIONS

No significant variations of haematological values were observed. are reported in Table II. Only muscle enzyme activities showing a light increase (CK and GOT) according to the muscular invasion are reported in Table II. The increase was seen on 60th day p.i.

HISTOPATHOLOGICAL STUDY

In relation to the low number of larvae recovered in the muscle tissues, very rare histopathological lesions due to the parasites were found. The most prominent lesion observed in *T. p.* infected ostriches was a basophilic transformation of muscle fibers with loss of their cross-striation and loss of their affinity for eosin. No cysts were formed.

Only one larva of *T. sp.* was found, typically encapsulated by the development of granulation tissue containing eosinophil leukocytes.

SEROLOGICAL STUDY

The antibody kinetics are shown in Figure 1. A light increase of specific IgG antibodies was observed only in the ostriches infected with *T. p.* No differences in

Muscle tissue examined	Infective dose: 10,000 larvae		Infective dose: 80,000 larvae	
	T. pseudospiralis n. larvae/gr.	*T. spiralis* n. larvae/gr.	*T. pseudospiralis* n. larvae/gr.	*T. spiralis* n. larvae/gr.
Fibularis longus	0.75	–	9.6*	1.5
Gastrocnemius	12	–	16.6*	0.5
Biceps femoralis	5	–	9.85*	–
Iliotibialis lateralis	10.56	–	8.7*	0.5
Triceps brachii	6.5	–	12.7*	0.15
Rectus lateralis capitis	9.5	–	10.1	3.5
Longus colli ventralis	3	–	3.8	4.2
Pectoralis	1.4	–	6	1.4
Heart	–	–	–	–
Tongue	–	–	–	–
Rectus ventralis capitis	3.3	–	5.3	–

* = $p < 0.01$ χ^2 test) between animals infected with 80.000 larvae of *T. spiralis and T. pseudospiralis.*

Table I. – Mean number of larvae recovered per gram of different muscular regions in *T. spiralis* and *T. pseudospiralis* infected ostriches.

Days post infection	Infective dose: 10,000 larvae				Infective dose: 80,000 larvae			
	T. pseudospiralis		*T. spiralis*		*T. pseudospiralis*		*T. spiralis*	
	CK	GOT	CK	GOT	CK	GOT	CK	GOT
30	967	238	567	251	1020	263	935	258
60	1677	561	1052	261	1740	592	1215	354

Range of normal values: CK = 688-1235 IU/l; GOT = 243-251 IU/l.

Table II. – Serum creatine kinase (CK) and glutammic oxalacetic transaminase (GOT) mean levels (IU/l) in *T. pseudospiralis* and *T. spiralis* infected ostriches.

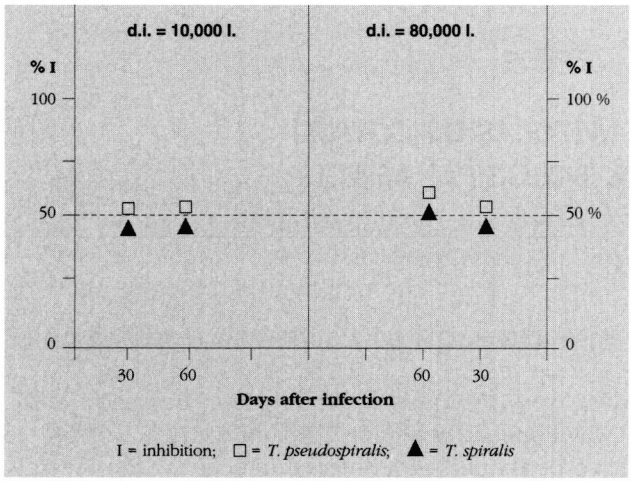

Fig. 1. – Antibody kinetics (monoclonal blocking ELISA) in *T. pseudospiralis* and *T. spiralis* infected ostriches.

relation to the different infective doses were seen. The obtained data are in accordance with the degree or the absence of muscle invasion.

DISCUSSION

Owing to the broad host spectrum of *Trichinella* spp. the objective in this study was to test the possibility of transmission of this parasite due to the consumption of ostrich meat.

The ostrich is a peculiar animal for the physiological characteristics of both birds and herbivora and for its omnivorous diet (it prefers not only grass, berries, seeds, leaves, but also small vertebrates, bugs and other arthropods). The results obtained allow some conclusive remarks: 1) ostriches, like birds and herbivora, are receptive to the infective *T. p.* species but the larval burden in the muscular tissues was very low and independent of inoculum size. Preferential sites of larval distribution were muscular tissues of the legs (*M. gastrocnemius*, *M. ileo-tibialis lateralis*) and of the head (*M. rectus lateralis capitis*); 2) ostriches, like other birds, are not receptive to *T. sp.*, the most pathogenous species for mammals. Only high parasitic doses (80,000 larvae) were able to produce a light infection with presence of larvae in the muscle; but these larvae are evoking the same histopathological picture as in herbivora; 3) according to the parasitological results, the tested muscular enzyme activities during the haematochemical investigations didn't show any significant changes. The same results were obtained as regards the antibody kinetics, as the study has only evidenced a light increase of specific IgG in animals infected with *T. p.* The low infectivity of *T. p* is probably related to the particular digestive system and the physiological characteristics of this animal, as the body temperature that is much lower than in other birds and similar to the horse's one (37°-39° C). Differently from other birds, ostrich gizzard has a muscular layer so strong that, together with the little stones ingested, assumes the function of food grinding. This grinding function is extremely efficient in the larvae killing so only few larvae are able to evolve to adult worms and moreover intestinal secretions are present in great quantity and digestion time is up to 36 hours. These factors can explain the ostrich low receptivity to *T. p.* infection.

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