SUSCEPTIBILITY OF NUTRIA (*MYOCASTOR COYPUS*) TO *TRICHINELLA* INFECTION: BIOLOGICAL ASPECTS

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Summary :

Experimental infections with three different species of *Trichinella* in nutria in order to evaluate the susceptibility and the role of these rodents in the spreading of parasitosis in nature were carried out. The nutria is present in many italian wet areas and its distribution is expanding. The nutria meat is utilized as food in different countries and is retained responsible for trichinellosis in man. Two groups of ten animals were infected per os with 500 and 5,000 (n. 10) infective larvae of *T. britovi*; an additional study was arranged with two groups of animals infected with 5,000 larvae of *T. spiralis* and *T. pseudospiralis*, respectively. After 45 days, all animals were slaughtered and samples of different muscles were processed by standard artificial digestion and by routine histological methods. Serological investigations (specific IgG) have been carried out on sera samples by employing a monoclonal blocking ELISA. The animals showed a significant susceptibility to the infection with all species of tested *Trichinella* and immunological reactivity. Data obtained are discussed.

KEY WORDS : *Myocastor coypus*, trichinellosis, experimental infection.

The nutria (*Myocastor coypus*, Molina 1782), a mammal belonging to Rodentia Order and to Capromydae Family, is part of the fauna of the temperate zones of South-America. In Europe such animals were imported at the beginning of the XXth century and bred either for alimentary aims (in countries such as Poland, Rumania, Russia, Germany) or for the production of fur. Nutria, although essentially herbivorous, has an alimentary spectrum fairly broad and certain conditions, like a deficiency in vitamins and proteins, induces it to eat garbage meat, fish, mollusca and become infected with *Trichinella* spp. (Bessonov *et al.*, 1980). Since 1936 (Rubli, 1936) human cases of trichinellosis caused by infected nutria meat have been described.

In this context, considering that the few available data about experimental infections in nutria have been done with *Trichinella spiralis* only (Bessonov *et al.*, 1980; Popescu *et al.*, 1987; Adamczyk *et al.*, 1996), we have performed experimental infections with the species *Trichinella britovi (T. b.), T. spiralis (T. sp.)* and *Trichinella pseudospiralis (T. p.)*. The degree of susceptibility and reactivity of the nutria were investigated through parasitological, immunological and histopathological examinations.

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MATERIALS AND METHODS

ANIMALS

The animals used were captured in the lacustrine area of Trasimeno lake (Umbria, Italy) where a program of capture has been planned with the purpose of containing the coypus population (Velatta, Ragni, 1991). The experiment was carried out on 32 animals, of less than eight months of age, a middle weight of 1.200 Kg and clinically and parasitologically healthy. The animals were given vegetables, fruit and maize during the experimental infection.

PARASITE

The *T. b.* strain had been isolated from foxes in Umbria, Italy, and maintained in Swiss mice; the *T. sp.* strain had been isolated from pigs and kindly provided by Dr. E.J. Ruitenberg – Bilthoven; the *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.

EXPERIMENTAL DESIGN

The *T. b.*, *T. sp.* and *T. p.* larvae were obtained from the infected mice by artificial method and counted in McMaster counting chambers. The animals were orally infected as follows: n. 10 with 500 L of *T. b.* (group 1), n. 10 with 5,000 L of *T. b.* (group 2), n. 5 with 5,000 L of *T. s.* (group 3), n. 5 with 5,000 L of *T. p.* (group 4). Two animals were used as controls. The infected nutria were kept in separate cages. For ten days p.i. nutria faeces were microscopically examined daily. On day 0

and 45 p.i. (end of experimentation) blood samples were taken to determine the immunological reactivity (specific IgG). After 45 days of infection, samples of different muscles were taken from the carcasses of all sacrificed animals and processed by standard artificial digestion (Polidori *et al.*, 1988) and by routine histological methods (Moretti *et al.*, 1987).

IMMUNOLOGICAL 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 described (Marini *et al.*, 1993).

RESULTS

No particular symptoms were observed in any of the experimental animals. On day 13 and 15 p.i., two nutria died: one of them was orally infected with 500 L of *T. b.*, the other one with 5,000 L of *T. p.* Examination of muscle larvae (carried out also by the trichinoscopy method) and the presence in sera of specific IgG antibodies were negative in both animals. No parasites were found in the daily examination of the faeces until 10th day p.i. *M. coypus* was equally susceptible to infection with *T. b.*, *T. sp.* and *T. p.* The results obtained by the *in vitro* digestion of muscular tissue are reported in Table I. In the four infected groups the muscles showing a higher larval burden were diaphragm, masseter and tongue. A statistically significant correlation (group 1 and 2) was observed between the number of larvae administered and the larval counts found after the artificial digestion in the muscular group examined. At an equal infective dose some statistically significant differences between the infective capability of the three *Trichinella* strains were found in numerous muscles, as illustrated in Table I. Biological tests in mice performed with the larvae of *T. b.*, *T. sp.* and *T. p.* obtained by the *in vitro* digestion of nutria muscles gave positive results. The serological study showed immunological reactivity in all animals infected with the three different strains of *Trichinella* (Table II). The significant histopathological changes were noticed in group no 2 and no 3: the massive inflammatory reaction around the numerous cysts was characterized by the presence of lymphocytes and numerous eosinophils; some affected fibers showed evidence of degeneration, interposed to centres of

Muscle groups	Group 1 *T. britovi* 500 L x L/g		Group 2 *T. britovi* 5,000 L x L/g	Group 3 *T. spiralis* 5,000 L x L/g	Group 4 *T. pseudospiralis* 5,000 L x L/g	
Diaphragma	853	$P < 0.05$*	2570	247	1005	$P < 0.05$**
Lingua	500	$P < 0.001$	5340	2082	717.5	$P < 0.001$
Masseter	297.8	$P < 0.001$	7095	888	3337.5	$P < 0.01$
Larynx	180	$P < 0.001$	5858	1106	2347.5	$P < 0.01$
Oesophagus	200.7	$P < 0.001$	1044	546	387.5	$P < 0.01$
Musculi intercostales	371.1	$P < 0.01$	1860	269	597.5	$P < 0.05$
Musculus brachiocephalicus	181.1	$P < 0.01$	2901	1210	735	N.S.
Musculi abdominis	200.7	$P < 0.01$	3058	986	260	N.S.
Musculus praesp. scapulae	229.7	$P < 0.001$	2520	1376	452.5	$P < 0.05$
Musculus gastrocnemius	308	$P < 0.01$	1950	1076	1032.5	N.S.
Musculus longissimus dorsi	302.3	$P < 0.05$	2472	156.4	840	N.S.
Musculi femoris	299.8	$P < 0.001$	3055	1232	1657.5	$P < 0.05$
Musculi caudalis	340.3	$P < 0.001$	2412	426	1002.5	$P < 0.01$

* = statistical analysis (χ^2 test) between group 1 and 2; ** = statistical analysis (χ^2 test) between groups 2, 3, 4; N.S. = not significant.

Table I. – Number of larvae recovered per gram of muscle (mean values) of different muscular regions in infected animals on 45th day p.i.

Groups examined		Antibody titres 1/80	1/160	1/320	1/640	1/1280	1/2560	1,5120
T. britovi	500 L	xx	xx	xxx	xx			
	5,000 L			x	xx	xxx	xx	xx
T. spiralis	5,000 L				x	xx	xx	
T. pseudospiralis	5,000 L	xxx	x					

x = no positive animals (two died during the experiment).

Table II. – Antibody titres obtained on day 45 p.i. with a monoclonal blocking (IgG) ELISA in the four groups infected.

necrosis and fibrosis. The tests performed on control group resulted to be negative.

DISCUSSION

The results obtained allow to summarize some conclusive remarks:

1. all animals became infected;
2. the diaphragm muscles, masseter and tongue were the more intensively infected by the three strains of *Trichinella* larvae and therefore these muscles should be the first to be controlled during the veterinary-sanitary examination of nutria carcasses;
3. under the same dose-inoculum (5,000 L), the number of larvae (mean values) per gram of muscle on 45 day p.i. was higher in *T. b.* compared to the other two strains;
4. the biological examinations carried out on the two animals that died during the experimentation on day 13-15 p.i. allow to point out that, in accordance to Popescu *et al.* (1987) and Scheuring (1999), larvae require more time to invade different muscles and to induce an immunological response;
5. the monoclonal blocking ELISA is a very sensitive and valid method for epizoological and diagnostic researches on trichinellosis. The immunological reaction is correlated to the larval burden present in muscular mass;
6. the histopathological results obtained on the four experimentally infected groups were similar to those observed in the animals with spontaneous trichinellosis;
7. Nowakowski (1988) and Scheuring (1999) have suggested possible ways of infection of nutria either in breeding farms or in its natural habitat: a) possible ingestion of rodents; the experimental infection carried out by Adamczyk *et al.* (1996) showed that the nutria has no difficulty and no disdain for ingesting triturated meat of infected mice; b) ingestion of potentially infected food and/or swallowing of carcasses of animals when there are promiscuous breeding of nutria and other animals such as foxes and minks (the nutria can sometimes move freely under the cages of these animals); c) possible ingestion of contaminated cereals by *modus agendi* of some breeders that put the cereals into the meat grinders "in order to clean" them; d) ingestion of potentially infected meat or fish meal (industrial additives) or scraps of home cooking that some breeders in Poland and in Russia are in the habit of doing to integrate nutria's feeding. It is not casual that all data of spontaneous infection on nutria with following human transmission are available from Polish and Russian territory and all related to animals coming from promiscuous and of great size breedings (Gavrilynk, 1979; Pietrowski, 1984; Giczela, 1985; Traeger, 1985; Nowakowski, 1988).

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