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EXPERIMENTAL TRICHINELLOSIS IN FALLOW-DEER (*DAMA DAMA L.*)

MORETTI A.*, PIERGILI-FIORETTI D.*, GRELLONI V.**, ANTOGNONI M.T.***, LEONARDI L.* & TACCONI G.*

Summary :

Herbivora can play a very important role in spreading trichinellosis, as showed by the massive epidemics in man, caused by the consumption of horse meat in the last years. In this context, the present study has been undertaken to verify, through an experimental infection, the susceptibility, together with other biological parameters, of fallow-deer to *Trichinella* infection. The four animals, 8-9 months of age and 18-25 Kg body weight, were orally infected with low doses of *Trichinella britovi* and *T. pseudospiralis* (2,000 larvae/animal). After day 30 p.i., the animals were necropsied and, using artificial digestion methods, larval burden of *Trichinella* in muscle tissues was determined. Histopathological, serological (IgG monoclonal blocking ELISA) and biochemical data were assessed during the experiment. The results showed the susceptibility of fallow-deer to *T. britovi* and *T. pseudospiralis* infection; under the same inoculum size, the number of larvae/g was higher in group infected with *T. britovi*. The animals showed a higher immunological response to *T. pseudospiralis* infection. The results are discussed.

KEY WORDS : fallow-deer (*Dama dama L.*), *T. pseudospiralis*, *T. britovi*, susceptibility.

Atypical hosts such as herbivora may play a very important role in spreading trichinellosis. In particular, some changes in the epizootic and epidemic situation of trichinellosis, during the last years, were found to be caused by horse responsible for the transmission of parasitosis in man (Dupouy-Camet,1996). As regards wild herbivora, natural infections due to *Trichinella* spp. have been reported in roe-deer (Marinculic, 2000); in camel (Bommer *et al.*, 1980); in hare (Rausch *et al.*, 1956; Pozio, 1988); in rein-deer (Kirichek, Abramov, 1980; Bessonov, 1981) and supposed in deer (Currier *et al.*, 1983). No data are reported about natural or experimental *Trichinella* infection in fallow-deer. Fallow-deer farming for meat production on grassland has increased in importance in some central european countries during the last decade.

In Italy, fallow-deer are usually extensively farmed in order to utilize otherwise unproductive areas.

Breedings of fallow-deer are particularly concentrated in Central Italy (Umbria and Tuscany) and Piedmont (Salghetti, 1991) where the consumption is also higher. In Umbria there are 3,076 animals (21 breedings) of which 83 % are bred for the production of meat (Mattiello *et al.*, 1993). The qualitative and nutritional characteristics (crude protein 20.7 %, lipid 1.27 %, gross energy 90-95 Kcal/100g) make this meat extremely competitive compared to that of other animal species (Duranti *et al.*, 1994).

In this context, the present study was undertaken to investigate, through an experimental infection with low larval doses, the susceptibility of fallow-deer to *Trichinella* infection and, consequently, the potential risk of infestation for human population linked to ingestion of meat.

MATERIALS AND METHODS

ANIMALS

Five fallow-deer, bred for meat production in Umbria, females, 8-9 months of age and about 18-25 Kg body weight were used. All animals were clinically and parasitologically healthy and were fed with a standard diet (hay and maize) and water *ad libitum*.

PARASITE

The used *T. britovi (T .b.)* strain has been isolated from foxes in Umbria and maintained in Swiss mice (Baldelli,1963). The *T. pseudospiralis (T .p.)* strain was kindly provided by Dr. E. Pozio (Istituto Superiore di Sanità, Roma) and since then has been maintained by us in Swiss mice.

EXPERIMENTAL DESIGN

T. b. and *T. p.* larvae were obtained from the infected mice by artificial digestion and counted in McMaster

* Dipartimento di Scienze Biopatologiche Veterinarie, Università di Perugia, S. Costanzo n° 4, 06100 Perugia, Italy.
** Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, G. Salvemini, 06100 Perugia, Italy.
*** Dipartimento di Diagnostica, Patologia e Clinica Veterinaria, Università di Perugia, S. Costanzo n° 4, 06100 Perugia, Italy.
Correspondence: A. Moretti.
Tel.: + 39 (075) 5857742 – Fax: +39 (075) 5857743.
E-mail: rprof2f2@unipg.it

counting chambers. By an oro-gastric tube, two animals (no 1, 2) were infected with *T. b.* larvae (2,000 L/animal), while the remaining animals (no 3, 4) were infected with *T. p.* larvae using the same way and infective dose. One animal was used as control. For ten days p.i. the faeces were observed daily. After 30 days of infection all animals were painlessly killed. From the carcasses of the sacrificed animals, samples of various muscle tissues were obtained in order to obtain the degree of muscular establishment and the histopathological reactions by means of acid-pepsin artificial method (Polidori *et al.*, 1988) and by standard histopathologic procedures (Moretti *et al.*, 1987), respectively. The infectivity of *Trichinella* larvae in positive samples was examined in white mice killed on 40th day p.i.
Blood samples to determine the serum changes of specific muscle enzyme activities (LDH, GOT, CK) and of total proteins and to detect the presence of specific IgG were collected on day 0 and day 30 p.i.

IMMUNOLOGICAL TEST

Serum IgG was determined on days 0 and 30 p.i. employing a monoclonal blocking ELISA previously described (Marini *et al.*, 1993).

RESULTS

No particular symptoms were observed in any of the experimental animals which remained healthy. Daily observations of the faeces gave negative results in all infected animals.
Muscle larvae were found in all infected animals. The fallow-deer are susceptible either to *T. b.* or to *T. p.* even if, under the same inoculum size, the lowest number of larvae per gram (lpg) was found in all muscles of the animals infected with *T. p.* (Table I).

	T. britovi*	***T. pseudospiralis*****
Masseter	169	4.5
Diaphragm	42.5	1
Lingua	20	1
M. longissimus dorsi	7	1
M. deltoideus	15	0.5
M. extensor carpi radialis	6.5	1.5
M. biceps femoris	9.5	1.5
M. fibularis longus	13	0
M. intercostales	12	0.5
M. iuguli	7	0.5
M. rectus abdominis	5.5	0.5
M. gastrocnemius	13	2

* = mean values referable to no 1, 2 animals; ** = mean values referable to no 3, 4 animals.
Samples weighing 20 g from each muscle were digested.

Table I. – Number of larvae recovered per gram of different muscular regions in *T. britovi* and *T. pseudospiralis* infected fallow-deer on day 30 p.i.

The most parasitized muscle locations were masseters, diaphragm and tongue in the animals infected with *T. b.*, while masseter was the most positive site in the animals infected with *T. p.* Muscle larvae of *T. b.* and *T. p.* obtained from fallow-deer after 30 days p.i. remained infective for mice. The serological study showed immunological reactivity in all animals: on day 30 p.i. a higher immunological response (specific IgG) was observed in the animals infected with *T. p.* (titers 1/80 and 1/160) compared with the ones infected with *T. b.* (titers 1/10 and 1/20). Regarding the trend of specific haematochemical investigations, no significant changes 30 days p.i. were observed in any of the infected animals.
The significant histopathological finding in the animals infected with *T. b.* was the presence of parasitic cysts in the skeletal muscles surrounded by an inflammatory infiltrate of moderate entity, prevalently characterized by lymphocytes and rare plasmacells, macrophages and eosinophilic leukocytes. The affected fibers appeared to be thinner with voluminous nuclei and some of them showed degenerative phenomena. In the animals infected with *T. p.* a weak inflammatory reaction with eosinophils, lymphocytes, macrophages and rare epithelioid cells was observed. No cysts formed.

DISCUSSION

The results obtained allow to conclude that:
1. the muscular establishment of *T. b.* and *T. p.* larvae and the increase of antibody levels confirm the host character of the fallow-deer to the parasite, as other herbivorous animals. Our aim to verify the susceptibility of fallow-deer to *T. b.* and *T. p.* infection by using a low infective dose (2,000 L/animal) gave as a result a positive response, confirmed by the fact that all animals became infected;
2. in the animals infected with *T. b.* the preferential sites of larval distribution are, in decreasing order, the muscle tissues of the masseter (the larvae of both species of *Trichinella* showed a high affinity for this muscular region) diaphragm and tongue. It is difficult to explain the great difference concerning the lower number of larvae recovered per gram in animals infected with *T. p.* compared with those infected with *T. b.* It is probable that:
a) the biological phases of development of the parasite are delayed by a hostile surrounding for *T. p.* (perhaps this can involve a lower fertility of females);
b) the experimentation time (30 days) is too short to cause a massive invasion from *T. p.* in a particular host as fallow-deer;
3. the animals infected with *T .p.*, showing a low parasitic burden, showed a higher titre of specific IgG than

the one observed in animals infected with *T .b.*(a longer experimentation time would have permitted to detect the real kinetics of both *Trichinella* species). The monoclonal blocking ELISA proves to be a very sensitive and valid method for mass screening in epidemiological surveys;

4. the histopathological findings performed on infected animals are like those observed in the animals presenting natural trichinellosis. The moderate inflammatory reaction observed with *T. p.* are in accordance with Bessonov, 1996;

5. it might be assumed that, under some circumstances, atypical hosts can play an epidemiological role in spreading trichinellosis: in this context, the fallow-deer must be added to the list of potential hosts receptive to *Trichinella* infection.

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