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163

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ON THE PRESENCE OF PARASITES IN NUTRIA (MYOCASTOR COYPUS, MOLINA, 1782) LIVING IN THE UMBRIAN TERRITORY (CENTRAL ITALY): BIO-SANITARY EVALUATIONS

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Key words: Nutria (*Myocastor coypus*) - Coprological examinations - Trichinella spp. - Arthropoda - Dermatophytes.

SUMMARY – **«On the presence of parasites in nutria (Myocastor coypus, Molina, 1782) living in the Umbrian territory (central Italy): bio-sanitary evaluation».**

An investigation was carried out to evaluate the presence of parasites in nutria (*Myocastor coypus*, Molina, 1782). Seventy-five (75) animals from

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the surrounding area of Trasimeno lake (Perugia, Italy) were captured during a project aimed at controling their expansion; parasitological and mycological examinations were performed. Particularly, investigations about endoparasites present in faecal and muscular samples and ectoparasites present on the skin, as arthropoda and dermatophytes, were carried out. The following results were found: a) 86.6% of the total examined animals was coprologically positive for coccidian oocysts of the genus *Eimeria* and for eggs of *Strongyloides* spp., present separately or in association. Adult and young animals showed a coprological positivity of 97.9% and 65.3%, respectively, but the positivity degree of both parasitical genera was consistently low. The protozoan parasite *Cryptosporidium* spp. was not found in any faecal samples; b) the nematode *Trichinella* spp. was not found in any muscular samples and; c) both adult and young animals were positive for some Arthropoda, such as *Atopomelidae*, *Trombiculidae* and *Ixodidae*; in addition, members of the Family *Pulicidae* were found on the hair of adult nutria. The discovery of *Ixodes hexagonus*, never reported in Italy but found on nutria of American territories, was also interesting; d) *Trichophyton mentagrophytes* var. *mentagrophytes* is the only dermatophyte species isolated from 14.6% of the examined animals. The spread of this keratinophilic fungus in the environment can be a source of infection for wild and domestic animals and the human population.

RIASSUNTO – Gli Autori hanno condotto uno studio parassitologico su n.75 nutrie (*Myocastor coypus*, Molina, 1782), provenienti dall'area circostante il Lago Trasimeno (Perugia, Italia), catturate nell'ambito di un progetto di controllo della loro diffusione; nello specifico, sono state effettuate indagini riguardanti parassiti presenti in campioni fecali e muscolari ed ectoparassiti (artropodi e dermatofiti) presenti a livello cutaneo. Sono stati ottenuti i seguenti risultati: a) l'86,6% di tutti gli animali esaminati è risultato coprologicamente positivo per oocisti coccidiche del genere *Eimeria* e per uova di *Strongyloides* spp., presenti separatamente o in associazione. Animali adulti e giovani hanno mostrato positività coprologica del 97,9% e del 65,3% rispettivamente, ma il grado di positività dei due generi parassitari è stato costantemente basso. In tutti i campioni fecali la ricerca di *Cryptosporidium* spp. ha sempre dato esito negativo; b) assenza del nematode *Trichinella* spp. nei diversi campioni muscolari esaminati; c) artropodi appartenenti alle Famiglie *Atopomelidae*, *Trombiculidae* e *Ixodidae* sono stati isolati sia in soggetti giovani che adulti; inoltre, esemplari della Famiglia *Pulicidae* sono stati ritrovati sul pelo di nutrie adulte. È interessante il reperimento di *Ixodes hexagonus*, mai riscontrato in Italia, ma rinvenuto soltanto su nutrie del territorio americano; d) *Trichophyton mentagrophytes* var. *mentagrophytes* è risultata l'unica specie dermatofitica isolata dal 14,6% degli animali esaminati. La diffusione di questo fungo cheratinofilo nell'ambiente può rappresentare fonte di infezione per animali sia selvatici che domestici, nonché per la popolazione umana.

Introduction

The nutria (*Myocastor coypus*, Molina, 1782) is a large semiaquatic rodent (Family *Myocastoridae*) indigenous to South America (10). Free-ranging populations are present in Europe, largely as the result of escapes and releases from fur farming operations. They were first introduced in Italy in 1928 (18) and they are now present in wild living conditions in most wetlands of Northern and Central Italy, representing a serious economic and environmental problem (39): this is also reported in other geographical areas (17, 11, 7, 1).

A high reproductive rate, the lack of natural predators (first of all versus adult nutria), winter seasons not particularly cold and a great degree of adaptability, contributed to the birth of permanent colonies, near to moist areas, as the sides of not whirling rivers, but also in not conventional places for their biology, as in Italy for example, along the cold waters with gravelly bottom of the Brenta river, or in the brackish areas of the Venice lagoon (34, 36, 41).

In these areas, nutria are perceived to cause serious damage to agriculture and wetlands: their burrows damage draining systems in rice-fields and rivers, they devastate crops and native plant communities and compete with the native and migrating birds in protected areas, with the risk of an impact on the natural biocenosis and, particularly, on the marshy fitocenosis (6).

The consistence and the dynamics of the nutria population have been gradually developing during the last years in Umbria too; naturalized populations are living in a large area, including 26% of the hydrographic network of the Region (43). To contain this animal population, the Umbria Government, similarly to other Italian Regional Governments, performed eradication campaigns.

In this context, thanks to the availability of some captured specimens, an investigation was carried out to evaluate the endo-ectoparasitological status of this animal species, with the aim of increasing the knowledge, poor in the national scientific literature (15, 3, 39, 36, 6, 26).

Materials and Methods

Animals: 75 nutria (49 adults and 26 young animals of about 6 months) were provided by the Province Veterinary Service,

Department of Environment Defense; the animals, belonging to the surrounding areas of Trasimeno Lake, were captured following the project to control their expansion in the hydrographic network of Perugia Province. Both adult (31 females and 18 males, with a mean weight of about 7 kg) and young (15 females and 11 males, with a mean weight of about 1.5 kg) animals were in a good state of health.

Coprological examinations: faecal samples, collected from the rectum of each dead animal, were examined both macroscopically and through qualitative coproscopic tests, according to the standard procedures of copromicroscopy (39). Particularly, a) faecal flotation technique (saturated Sodium chloride, specific gravity 1.18-1.2) for recovery of Cestoda and Nematoda eggs and protozoan oocysts and cysts; b) faecal flotation and sedimentation technique for Trematoda eggs; c) Baermann technique for lungworm larvae isolation; d) faecal concentration with Sheather's sugar solution and Ziehl-Nielsen staining modified method for the detection of *Cryptosporidium* spp.: e) quantitative evaluations of eggs (n. of eggs/gram of faeces) using the Mc Master technique, were performed. The faecal samples of nutria positive for coccidia were dissolved in potassium dichromate solution (final concentration 2.5% v/v) in a Petri dish and maintained at room temperature to allow sporulation of the oocysts. Observations on the size, morphology, sporulation time, presence or absence of definite bio-morphological elements (as micropyle, number of sporocysts, number of sporozoites, etc.) of the oocysts were used for speciation.

Detection of Trichinella spp.: in order to detect *Trichinella* larvae (L1) in different muscular samples (diaphragm, masseter, tongue), collected from all subjects, the artificial digestion method (with HCl and pepsine) "Trichomatic 35" was utilized (32).

Ectoparasitological examinations: arthropod specimens found on the skin and the hair of the 75 examined animals, were collected about one hour after capture and fixed in 80% alcohol. Some of these were dipped into lactic acid for 48 hours and then put on a slide in Berlese solution, to find out morpho-structural features useful for their taxonomic identification.

Mycological examinations: samples of fur and dandruff, collected from all subjects in order to estimate the prevalence of dermatophytes, were infixed in Petri dishes with Sabouraud's agar containing cycloheximide (0.1-0.4mg/ml) and an antibacterial antibiotic (chloramphenicol: 0.05 mg/ml). The plates were subsequently incubated at 26-28°C for 3 weeks and observed for

fungal growth. The positive plates for fungal development were observed both macro- and microscopically. A fragment of the colony was stained with lactophenol blue cotton in order to identify the genus and the species of the fungus (35).

Data obtained were analyzed using the Fisher's Exact Test, in order to determine statistical significance.

Results

Coprological examinations: 86.6% of the total examined samples resulted coprologically positive (Table 1): coccidian oocysts of the genus *Eimeria* and eggs of *Strongyloides* spp., observed separately or in association, characterized the faecal parasitic fauna (Table 2). In adult animals, separately and in association, coccidian oocysts were present in the 46.9% and *Strongyloides* eggs in the 93.8% of the total examined subjects. In young animals, separately and in association, coccidian oocysts were present in the 46.1% and *Strongyloides* eggs in the 50% of the total examined subjects.

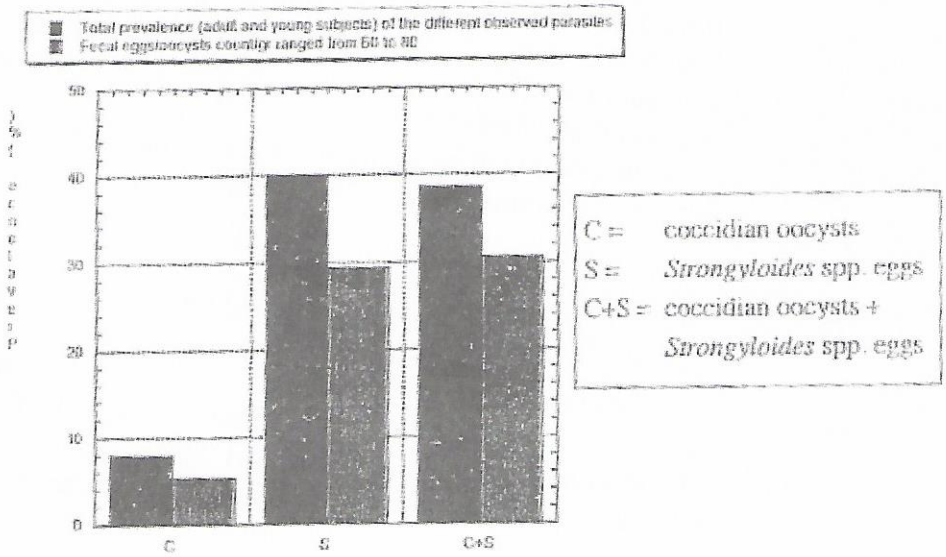
The positivity degree of the two parasitical genera, both singularly or in association, was constantly low, except for a few samples; only two faecal samples, belonging to adult animals, were positive for strongyles eggs, in association with coccidian oocysts and *Strongyloides* eggs. All oocysts were of the genus *Eimeria* and the morpho-metric analysis were homogeneous in all examined samples: sporulated oocysts were characterized by an oval-round-

TABLE 1.
Copropositive animals and relative prevalence.

	N° of examined animals	N° of copropositive animals (Prevalence %)
Adult subjects	49	48 (97.9%) *
Young subjects	26	17 (65.3%)
Total	75	65 (86.6%)

* Statistically significant difference (Fisher's Exact Test, $P < 0.001$)

TABLE 2.
Coprological results and relative prevalence.



shaped, yellow-brownish colour, a thick and double stratified wall with "pitted" surface, a mean size of 19.4 x 24.4 μm (range of 18.5-21 x 22.2-25.9 μm), absence of micropyle, 4 sporocysts inside in a seed-shape and a sporulation time of 6-7 days (Fig. 1, 2, 3) (at the 6th day more than 50% of the oocysts were sporulating).

Strongyloides spp. eggs were in the larval phase, characterized by a thin and flat wall and a mean size of 60.9 x 30.4 μm (range of 58.1-62.4 x 25-33.2 μm) (Fig. 4).

In all faecal samples the search for *Cryptosporidium* spp. oocysts resulted negative.

Detection of Trichinella spp.: in the different muscular samples examined the investigation of nematode *Trichinella* spp. was negative.

Ectoparasitological examinations: n. 39 (79.5%) of adult animals and n.8 (30.7%) of young animals were positive for the following arthropoda: *Listrophoroides* spp. (*Listrophoroidea, Atopomelidae*) in 47% of adults and 19% of young animals; *Neotrombicula* spp. (*Trombiculidae*) in 49% of adults and 31% of the young animals; *Ixodes ricinus* and *Ixodes* (*Pholeoixodes*) *hexagonus*

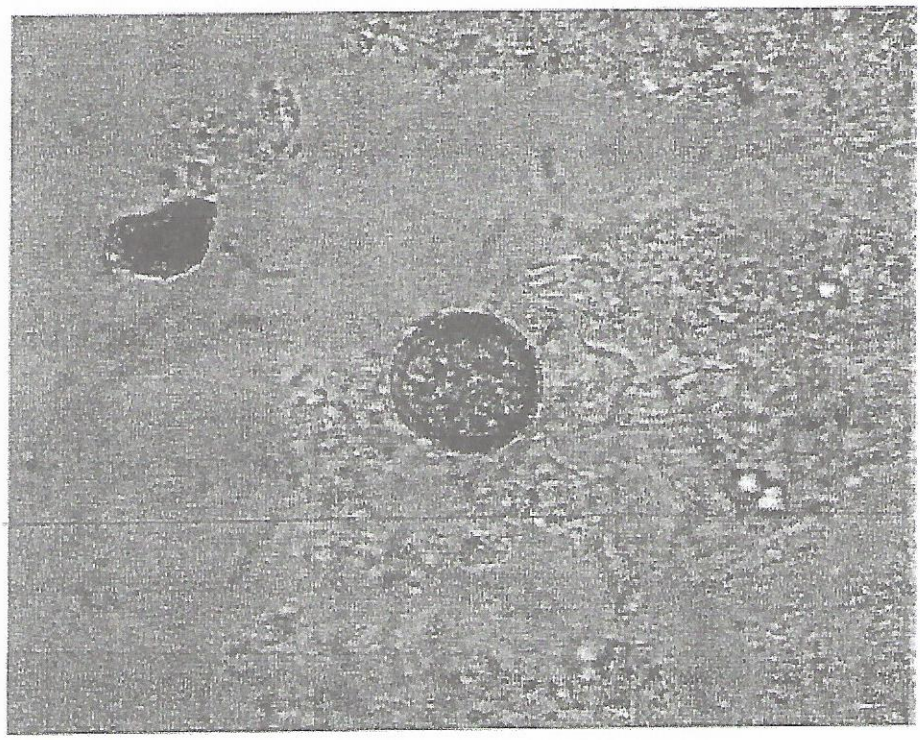


Figure 1 - Not-sporulated coccidian oocyst (40x).

(*Ixodidae*) in 8% and 4% of adults, respectively; *Pulex irritans* (*Pulicidae*) in 10% of adults.

Neotrombicula spp. was always found in the larval stage, most of all in the auricle, between September and November; *Listrophoroides* spp., isolated during the whole year, particularly along the dorsum and the scapular region of the animals. *I. ricinus* and *I. hexagonus* (Fig. 5) were observed in adult animals, as nymph or adult stages, only in October. The only flea recovered during the period October-November was *P. irritans* (males and females), present on adult animals.

Mycological examinations: the presence of dermatophytes was observed in 11 animals (14.6%) (8 young and 3 adults) of the total subjects examined; *Trichophyton mentagrophytes* var. *mentagrophytes* was the fungus isolated in these asymptomatic animals. Microscopically abundant globose microconidia in grapelike clusters, some macroconidia and several spiral hyphae were usually observed.

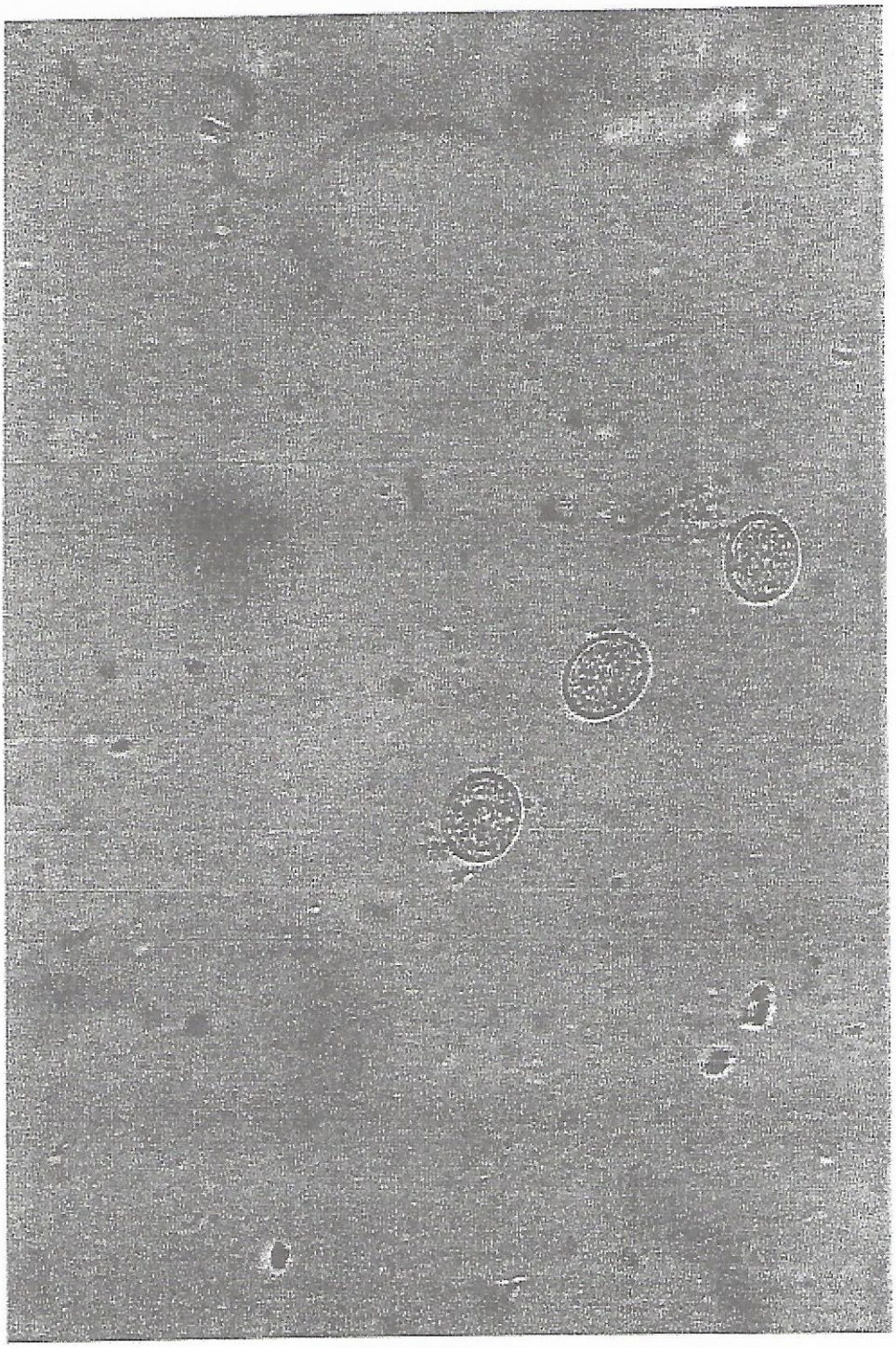


Figure 2 - Not-sporulated coccidian oocyst (25x).

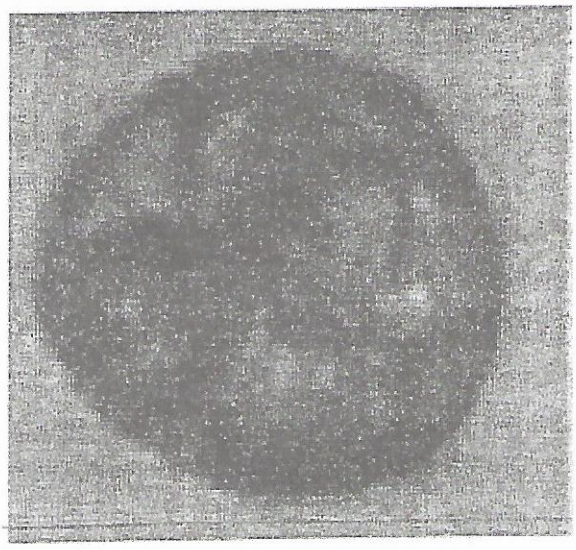


Figure 3 - Sporulated oocyst of genus *Eimeria* (100x).

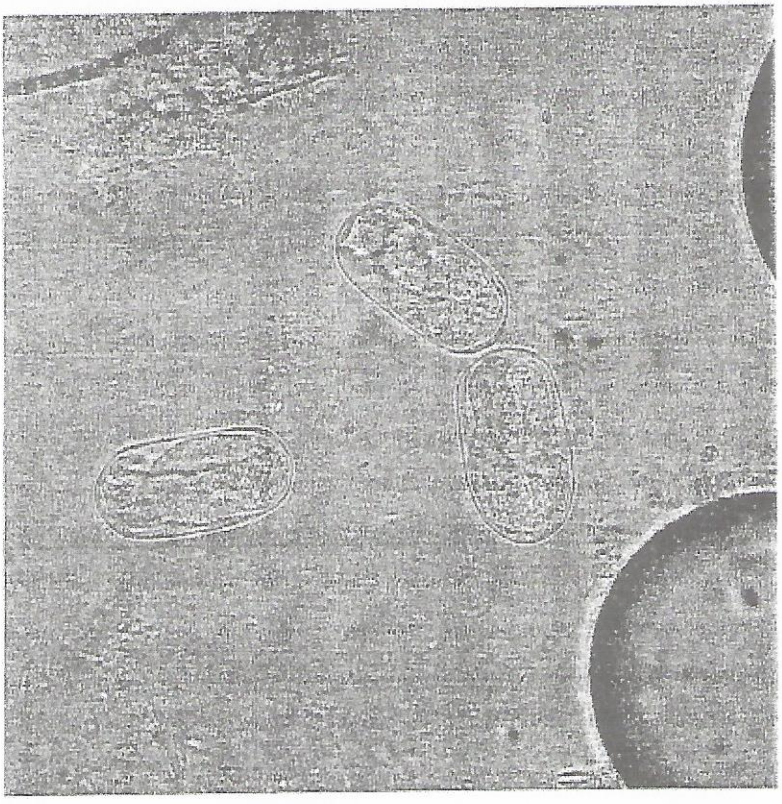


Figure 4 - *Strongyloides* spp. eggs with larva inside (40x).

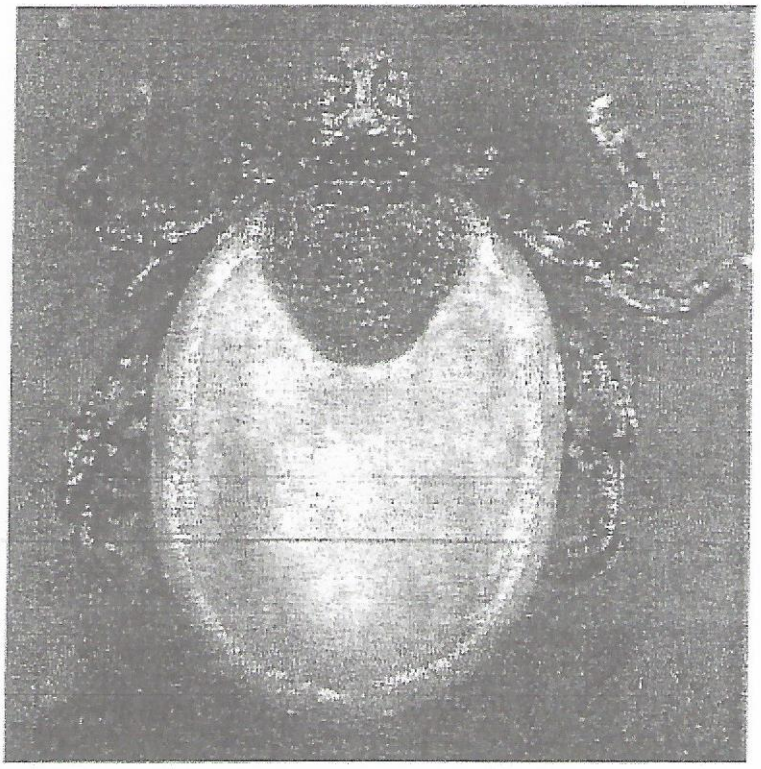


Figure 5 - *Ixodes exagonus* (female).

Discussion and Conclusions

A sanitary surveillance of wild animals, through epidemiological studies, is particularly important to know the role and significance of the environmental spreading of pathogens. Current knowledge on the parasitological status of nutria comes mainly from studies carried out in Northern and Eastern Europe, where the presence of farms with many subjects allowed a lot of pathological investigations and bio-epidemiological researches (25, 41, 45). Farmed and feral nutria show a variable parasitical fauna, including *Protozoa*, such as genera *Isospora*, *Eimeria*, *Cryptosporidium*, *Trichomonas*, and different genera of *Trematoda*, *Cestoda* and *Nematoda* (30, 37); but coccidia of *Eimeria* spp. genus and nematoda of *Strongyloides* spp. genus have a more marked presence.

Since 1937, when Obitz and Wadowski (28) first described

clinical coccidian infections in nutria caused by *Eimeria coypi*, other Authors carried out investigations in order to obtain etiological data and a morpho-biological characterizations of the coccidian isolated species, which seem to have different geographical spreading (19). No complete or definitive data were obtained, due to the slight differences about the dimensional values or the sporulation time of oocysts, and the presence of numerous synonyms, given by the different Authors along the years to the different isolated species, created difficulties in the etiological definition. In an interesting work, Scheuring (1996) (38) describes the morpho-biological characteristics of the main coccidian oocysts, isolated from nutria, belonging both to *Isospora* (*Isospora* spp.) and *Eimeria* genera (*E. seideli*, *E. coypi*, *E. myocastoris*, *E. myopotami*, *E. fluviatilis*, *E. nutriae*, *E. quiyarum*, *E. obitzwadowski*; these are *Eimeria* species with a different pathogenicity degree, frequently associated in natural infections). The Author considers coccidiosis as the main invasive disease of young farmed animals, with a high mortality degree (*E. coypi*, known in Poland since 1937, is considered a highly pathogen species, and the finding of ≥400.000 oocysts/gram/faeces induces death in young animals of about 8 weeks old). An epidemiological study of the same Author carried out on n.1059 farmed subjects, apparently healthy, revealed the presence of 19.5% of coccidia and 28.5% of worms (particularly, 20.3% of *Trichuris myocastoris*, 13.7% of *Strongyloides myopotami*, 0.17% of *Trichostrongylus* spp.).

Data obtained in this study, compared with similar ones obtained during other Italian investigations, allow us to say that:

1. adult and young animals show a coprological positivity of 97.9% and 65.3%, respectively. The parasitical fauna is represented, in both animals categories, by coccidian oocysts of *Eimeria* genus (in the 8% of nutria as single infection and in the 38.6% in association with *Strongyloides* spp. eggs) and *Strongyloides* spp. eggs (in the 40% of nutria as single infection), with a low positivity degree. For what concerns morpho-biological characteristics of sporulated oocysts, according to Scheuring's data (1996) (38), we consider *E. myopotami* the species present in all examined samples. Further investigations are necessary to confirm, in wild nutria, this coccidian monospecificity and the low degree of parasitical density also in other Italian geographic areas. *Strongyloides myopotami* (4), previously described by Enigk as *Strongyloides chapini*, is considered the species linked to *Myocastor coypus* (21, 8). As regards, the experimental Little's study (1965) (20) on human

volunteer is interesting because the infective larvae of *S. myopotami* can cause of dermatitis in human.

Similar coprological researches on wild nutria have been carried out in Italy and they revealed a poor parasitical intestinal presence: 1) on 73 animals captured along the delta of Po river (Rovigo) (3) coccidia of genus *Eimeria* (8.2%) and gastro-enteric nematoda (5.4%) were demonstrated; 2) Soldati et al. (1998) (40), in Modena's area, obtained: 4.06% of *Eimeria* spp., 80.49% of *Strongyloides* spp. eggs, 6.5% of *Trichostrongylus* spp. and 25.2% of serological positivity for *Toxoplasma gondii*; 3) Bollo et al. (2003) (6) reported a histopathological study carried out on nutria of Piedmont area, where a positivity of 17.1% for hepatic coccidiosis was demonstrated. Coccidia were histopathologically seen into the proliferated biliary epithelium inflamed areas;

2. with respect to the sporozoan *Cryptosporidium* spp., another intestinal parasite, our investigations were negative; *Cryptosporidium* spp. was actually isolated from nutria coming from Romania (9) and Poland (29) in very young subjects (14-35 days old). Particularly, studies based on cross-infection experiments demonstrated the possibility for *Cryptosporidium parvum* of infection of healthy nutria with oocysts coming from infected lambs and vice versa. Further studies are necessary because cryptosporidiosis is a zoonotic disease and domestic and wild animals should be regarded as important carriers for humans. Of 10 valid species of *Cryptosporidium*, only *C. parvum* is widespread in humans and other animals; faecal-oral transmission of the oocyst stage has resulted in outbreaks through contamination of drinking water, food and recreational waters (12);

3. the research of *Trichinella* spp. in the muscular samples gave negative response. Similar data were obtained by Arcangeli et al. (1997) (3) and Soldati et al. (1998) (40). Nutria is experimentally receptive to *T. spiralis*, *T. britovi* and *T. pseudospiralis* (5, 33, 2, 24). In Poland and Russia, nutria are farmed for alimentary production too and clinical cases of human trichinellosis were reported (14, 31, 43, 16). The parasite has an elevated biological plasticity characterized by a broad range of receptive hosts (carnivora, omnivora, herbivora, birds, insectivora, fishes) and interesting different geographical areas (23);

4. in addition, regarding the ectoparasitological investigations, it is interesting the discovery of *I. hexagonus*, never reported in Italian nutria, as results of the references considered by us; this tick was found only on American nutria (27). This is a triphasical,

monotrope and pholeophiilic parasite of rodents, some lagomorphes (*Lepus europaeus*) and insectivora (*Erinaceus* and *Sorex*), and carnivora, as the fox and the weasel, on which its stages were found during the whole year, except for the hottest months. The tick can parasite humans and have a spread from forest to suburban areas; it can survive into caves or in other places, especially lived by foxes and rodents. *I. hexagonus* life cycle naturally lasts about 2 or 3 years. Its pathogen role is also important: in fact, *Rickettsia conori* and other Rock Mountains Fever Rickettsiae were isolated from this tick in France and Austria. Besides, Manilla (1998) (22) demonstrated in Germany the presence of *I. hexagonus* specimens, infected with some *Borrelia* spp. belonging to *burgdorferi*'s group: they were able to realize both trans-stadium and trans-ovarian infection;

5. since the sanitary implications, interesting is the isolation of *Trichophyton mentagrophytes* var. *mentagrophytes* in the 14.6% of the examined animals. The same species was isolated (11.1%) during a similar research in Italy (13). The environmental spreading of this zoophilic fungus occurs usually through intermediary earth-dwelling carriers, such as mice and small mammals, animals of extraordinary epizootic and epidemic importance. Wild animals may be an indirect source of infection, since the infected hairs shed from the body may contaminate dwelling places and working areas. Both domestic animals, breaded for zootechnic productions, and humans, can be involved, under favourable conditions, in clinical pictures. Zoophilic strains, as *T. mentagrophytes* var. *mentagrophytes*, which usually cause a minor or subclinical infection in animals, will evoke a severe inflammatory response in humans.

Most epidemiological and pathological investigations have involved farm-raised nutria, while there are only few reports in wild ones. A continuous sanitary surveillance in wild animals is recommendable, because they could be potential sources of infection for domestic livestock and humans living in the same habitat, through uncommon epidemiological circuits.

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