Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 19, issue 1, 2009, pp. 69-73

ASPECTS OF TOXOPLASMA TOXOPLASMA-LIKE PARASITISM IN SMALL MAMMAL SPECIES IN TRANSYLVANIA AND THEIR ROLE IN TOXOPLASMA DISSEMINATION

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ABSTRACT. Toxoplasmosis is one of the most severe zoonosis, due to the forms developed in immunodepressed individuals and during trasplacentar transmission, considering also the numerous transmission ways and its prevalence in human populations. The paper shows the presence of Toxoplasma Toxoplasma-like cysts and pseudocysts in different tissues from six species of small mammals captured in different localities and habitats. The presence of oocysts in the feces of four small mammal species proves that these animals can disseminate the unsporulated oocysts in the environment. The cysts from the tissues may be a source of infection for the pigs from the individual households, increasing the contamination risk for humans.

Keywords: small mammals, parasitology, Toxoplasma gondii, dissemination, zoonosis

INTRODUCTION

The name of "Toxoplasma-like" is given to species belonging to Apicomplexa group, which present similarities concerning their morphology, life cycle and lesions caused in the hosts.

Toxoplasma gondii is a protozoan capable of parasiting a large number of animal species. Felids, both domestic and wild, are definitive hosts and contribute to the oocysts dissemination by their feces. All endothermic animals, including humans, may potentially serve as intermediary hosts, sheltering the parasite in different tissues. In most cases, the infection causes only minor health problems in humans (Carme et all, 2002) and a significant percentage of the human population is seropositive to Toxoplasma. In case of transplacentar, in immunodepressed patients and sometimes also in immunocompetent individuals, severe problems appear, associated with some syndromes as encephalitis and neuroretinitis.

In most humans, toxoplasmosis is acquired after birth, by ingestion of oocysts from contaminated environments, by consumption of insufficiently heated meat or by contaminated water.

The importance of each transmission way of toxoplasmosis is difficult to asses, due to the fact that there are no serological methods of differentiate the infections produced by oocysts from those caused by cysts (Boothroyd et all, 2002). Despite this, the impact of toxoplasmosis epidemiology caused by oocysts should not be neglected as it seems that it is associated with the seroprevalence of *T.gondii* in some communities. Oocysts are recognized as a major source of acute toxoplasmosis having as source the soil or water (Bahia-Oliveira et all, 2003). Very likely they are also responsible for a significant part of the infections in animals that might be consumed by humans (Tenter et all, 2000). Oocysts of *T. gondii* persist for a long time in the environment, being disseminated by biotic and abiotic factors (Dumètre et Darde, 2003). Usually the number of oocysts that can be identified in different samples from the environment is low. Besides, they can be mistaken for oocysts of *Hammondia sp.* and *Neospora sp.*, two coccidian that can also be found in the environment (Frenkel et all, 1975, Dubey et all, 2002).

A series of invertebrates and ectothermic vertebrates are considered responsible for the dissemination of oocysts, and unsporulated oocysts can transit the digestive system of homoeothermic animals without causing the infection, and then eliminated (Miller et al. 1972) in the feces, contributing thus to the oocysts dissemination.

Different species of mammals or groups of species can serve as reservoirs for *T. gondii* (Hejllicek et al, 1997).

MATERIALS AND METHODS

The studied material was collected from wild animals in nine stations from three counties (fig. 1): Sibiu (Agnita, Cisnădie and Valea Lotrioarei), Maramureş (Paltin, Repedea, Coşnea, Bardău and Făina forest ranges) and Hunedoara (Bucura Lake in Retezat Mountains).

Captured animals were used for sampling feces and blood; all the small mammals that were analyzed in the laboratory were found dead in the traps. For the histological analysis all organs including the nervous system were sampled. The coproparasitologic analysis was done using Willis method. May Grünwald – Giemsa method was used for colouring the blood and histological samples.

42 coproparasitologic analyses were done and sets of organs were collected from 10 individuals.

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Article received: october 2008; published: march 2009

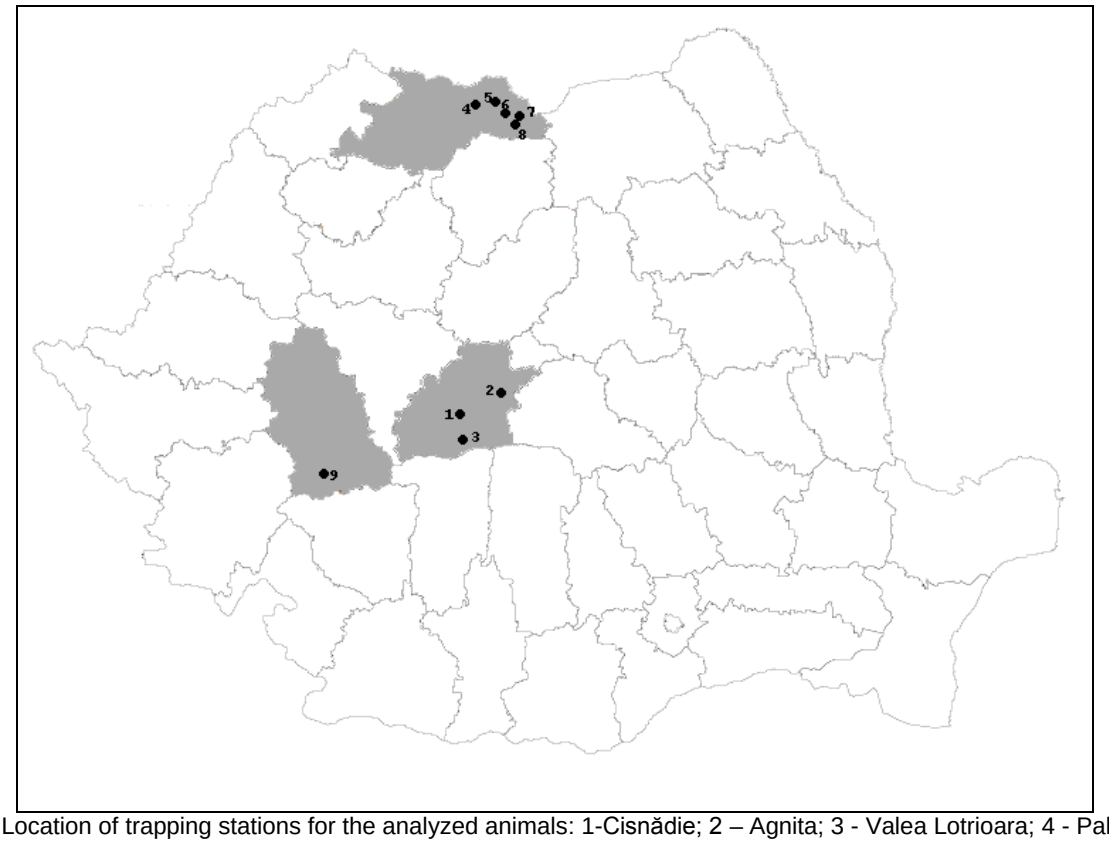

Fig. 1 Location of trapping stations for the analyzed animals: 1-Cisnădie; 2 – Agnita; 3 - Valea Lotrioara; 4 - Paltin; 5 - Repedea; 6 - Coşnea; 7 - Bardău; 8 - Făina; 9 – Bucura Lake

RESULTS AND DISCUSSIONS

Due to the impossibility of differentiation by simple microscopic analysis of oocysts, cysts and pseudocysts belonging to Toxoplasma from those belonging to other species in Apicomplexa group (Neospora, Hammondia, Sarcosporidium, etc.) we will refer to these formations using the term Toxoplasma Toxoplasma – like.

We mention that for species differentiation serologic analysis (with debatable results in some cases, Wreghitt, 1986) and DNA analysis is used.

Six species of small mammals were captured; in four of them were identified oocysts which can be ascribed to Toxoplasma – Toxoplasma-like group: Apodemus agrarius, Clethrionomys glareolus, Apodemus sylvaticus, Apodemus flavicollis. In Microtus agrestis and Sorex araneus no such formations were identified.

Table 1 presents the results of parasitic forms identification in feces and tissues from small mammals.

Table 1

Presence of oocysts of Toxoplasma, Toxoplasma-like in feces, respectively of oocysts and pseudocysts in tissues from small mammals species

No	Species	Toxoplasma Toxoplasma-like oocysts in feces	Toxoplasma T-like cysts and pseudocysts in tissues
1	***Apodemus agrarius***	+	Heart, lung, nervous system
2	*Clethrionomys glareolus*	+	-
3	*Apodemus sylvaticus*	+	-
4	*Apodemus flavicollis*	+	Testicle, pancreas, intestine
5	*Microtus agrestis*	-	-
6	*Sorex araneus*	-	Nervous system

By histological analysis, cysts and pseudocysts were identified in different organs. In Apodemus agrarius cysts were identified in the heart, lung and nervous system, in Apodemus flavicolis cysts were found in the testicle, pancreas and intestine, while in Sorex araneus cysts were found only in the central nervous system (fig. 2-7).

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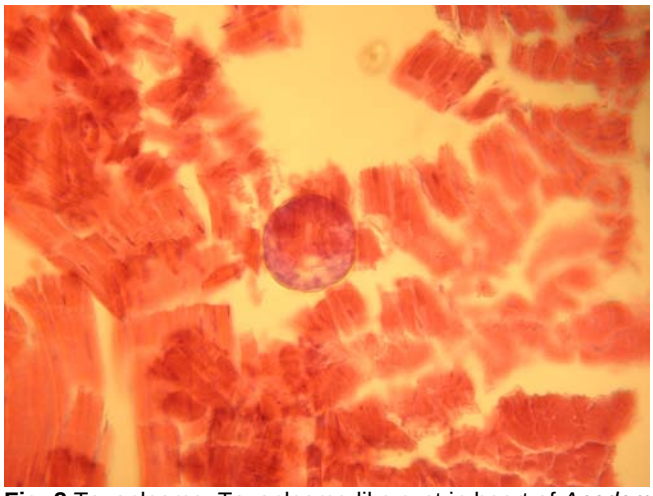

Fig. 2 Toxoplasma, Toxoplasma-like cyst in heart of *Apodemus agrarius*

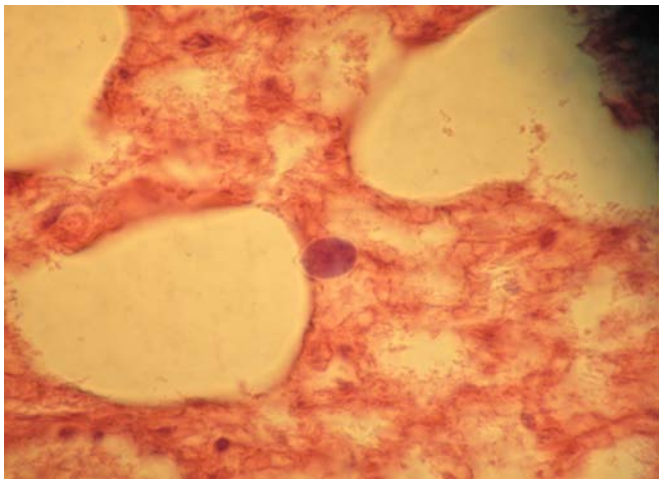

Fig. 3 Toxoplasma, Toxoplasma-like cyst in lung of *Apodemus agrarius*

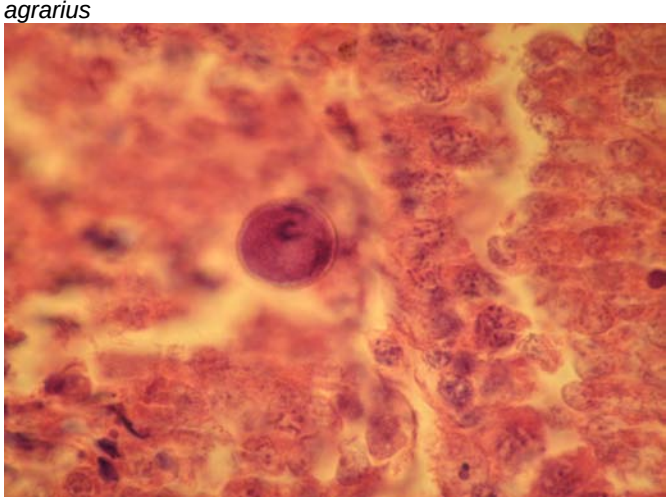

Fig. 4 Toxoplasma, Toxoplasma-like cyst in testicle of *Apodemus flavicollis*

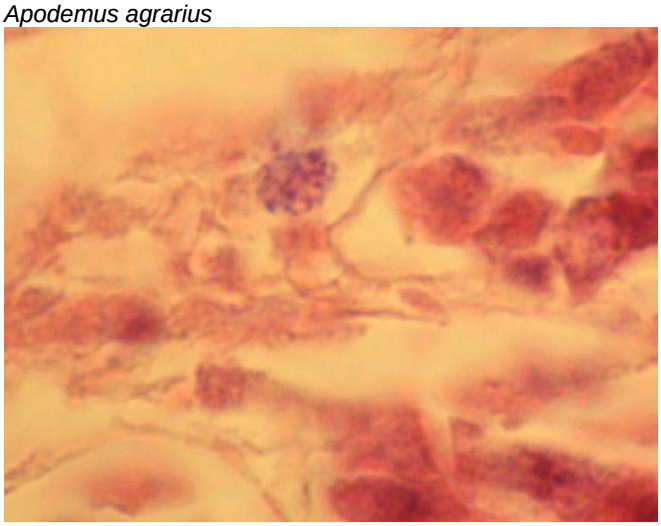

Fig. 5 Toxoplasma, Toxoplasma-like pseudocyst in pancreas of *Apodemus flavicollis*

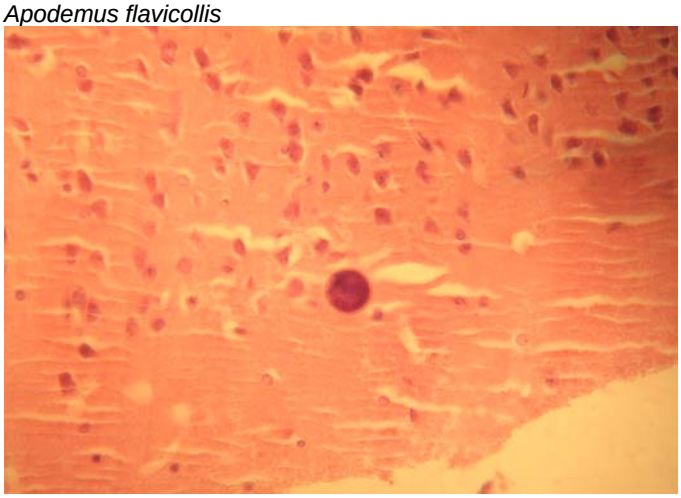

Fig. 6 Toxoplasma, Toxoplasma-like cyst in nervous system of *Apodemus agrarius*

Fig. 7 Toxoplasma, Toxoplasma-like cyst in intestine of *Apodemus agrarius*

The presence of parasite forms in the tissues of 3 from the 6 captured small mammal species is most likely the result of the low number of analyzed samples. Usually the presence of cysts in the testes - recorded in Apodemus flavicollis - is not common (Baretto et al, 2008), and studies on humans show that it is not necessary correlated with sexual transmission of the parasite (Janitschke and Nürnberger 1975).

No correlation could have been established between the degree of infestation and the capture place of the analyzed individuals. Thus, in animals collected from the vicinity of human settlements was not observed a

more intense parasitation. This fact is probably due also to the low number of samples, which did not allow the calculation of infestation prevalence in different species.

The presence of oocysts in feces can be explained by the ingestion of oocysts by rodents in the first days after their elimination by the definitive host. The unsporulated oocysts can transit the digestive tube of the animals without causing an infestation. This way, the small mammals can contribute to the dissemination of the parasite in the environment and the expansion of the area infested by domestic or wild felids.

An indirect implication of rodent parasitation in transmitting the parasite to humans seems to be via suinae. Rodents represent one of the main causes of toxoplasmosis in domestic pigs. Using DNA analysis of heart and brain Kijlstra et all. (2008) found for T. gondii a prevalence of 10,3% in Rattus norvegicus, 6.5% in Mus musculus, 14.3% in Apodemus sylvaticus and 13.6% in Crocidura russula, emphasizing the role of these small mammals in the transmission of T. gondii to pigs and the importance of rodent control in order to get uninfected meat.

Serologic studies show different prevalence of the infection in rodents, depending on area and species. Thus, in Peromyscus sp. it may reach 26% (Dabritz et all, 2008), 6.4% in different species of small mammals from Norway (Spoodler et all, 2007) and 1,49% in Apodemus agrarius from Korea (Jeon and Yong, 2000).

CONCLUSIONS

Small mammals are reservoirs and vectors of several infectious and parasitic diseases. Among these toxoplasmosis represent one of the zoonosis with most severe medical and social implications. Small mammals contribute to the parasites' dissemination in two ways: they expand the contaminated area by elimination of unsporulated oocysts in the feces and on the other hand, they are part of the trophic chain, being consumed by numerous carnivores. Thus, tissular cysts and pseudocysts may cause contamination of animals on other trophic levels, reaching in the end the humans.

The finding of Toxoplasma – Toxoplasma like cysts in the testes, even if it does not necessarily indicate a sexually transmission, is one of the rare cases found in mammals.

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