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IXODES RICINUS AS A POTENTIAL VECTOR OF *TOXOPLASMA GONDII*

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Abstract: The presence of *Toxoplasma gondii* DNA was detected by polymerase chain reaction (PCR) test in 2 out of 92 *Ixodes ricinus* ticks (2.8%) collected in the woodlands of eastern Poland. This suggests that ticks of this species may be involved in the spread of toxoplasmosis under natural conditions.

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INTRODUCTION

Toxoplasmosis, caused by parasitic protozoan *Toxoplasma gondii*, is an usually asymptomatic zoonotic disease common in man and over 100 species of mammals and birds. In humans, toxoplasmosis may pose a severe medical problem as a congenital infection causing cerebral and ocular damage in newborns, and as an acquired infection in immunocomprised individuals, such as AIDS patients [5, 8].

The sexual reproduction of the parasites has been observed until recently only in intestine of felids. Oral transmission, by consumption of raw meat or food contaminated with cat feces containing *T. gondii* oocysts, is regarded as the only route of primary infection. However, this route hardly explains the common occurrence of *T. gondii* in variety of hosts, such as herbivorous mammals, wild rodents and birds that are unlikely to contract primary infection orally with meat or cat feces. Thus, some additional routes of transmission suggested by earlier authors should be also considered, including infection by skin lesions, and transmission by arthropods [3, 7, 9, 13, 19].

The possible role of arthropods in transmission of *Toxoplasma* is discussed with respect to passive spreading by flies and cockroaches [13], and to active transmission by blood-sucking insects and arachnids. Of the latter, most concern was directed towards ticks (Ixodida) as potential vectors of infection in man and warm-blooded animals. Cases of human toxoplasmosis associated with tick bite were described [2, 14, 15], *Toxoplasma* strains were isolated from naturally infected ticks [3, 9, 11, 15], and possibility of experimental transmission of infection by various tick species was evidenced [6, 7, 19]. Deryło *et al.* [7] have demonstrated experimentally the transmission of *Toxoplasma gondii* infection by nymphs of *Ixodes ricinus* and found microscopically the presence of *T. gondii* tachyzoites and bradyzoites in the tissues of nymphs and females.

Based on above-mentioned findings, we have examined *Ixodes ricinus* ticks collected from the natural habitats in eastern Poland for the presence of *T. gondii* with the use polymerase chain reaction (PCR).

MATERIALS AND METHODS

Area of the study and tick collection. Unfed ticks (adults and nymphs) belonging to one species *Ixodes ricinus* were collected in May 2000 and in May and September 2001 in five woodland localities (Tab. 1) situated on the territory of the Lublin region (eastern Poland). Out of five localities, one (No. 1) was a suburban locality near the city of Lublin, while the other four (Nos. 2-5) were situated on

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"Polesie Lubelskie", a wooded lake-land area spreading for a distance of circa 100 km in a north-eastern direction from Lublin. Ticks were collected by flagging lower vegetation at peripheral areas and inner parts of deciduous and mixed forests, mostly at the edge of forests, along the forest paths and in the mid-forest clearings, such as parking lots or picnic areas. Collected ticks were placed in vials separately (adults), or in pools of 5 specimens (nymphs), killed with hot water and immersed in 70% ethanol for further examination.

Polymerase chain reaction (PCR). DNA was isolated from the examined ticks by lysis in ammonium hydroxide [18]. After removal from alcohol, all specimens were air dried on filter paper, and then each adult individually and nymphs in pools of 5 specimens were immersed in 100 µl of 0.7 M NH_4OH and crushed with pipette tips. The suspensions were boiled for 15-20 min in a heating block at 98ºC in 2 ml Eppendorf sealed vials. Then vial caps were opened and heating was extended for another 15 min to remove ammonia and reduce the volume to 50 µl. The lysates were then stored at -20ºC.

Amplification of *T. gondii* DNA was performed using PCR kit obtained from DNA-GDAŃSK II s.c. (Gdańsk, Poland). Detection of *T. gondii* DNA was based on amplification of gene fragment coding 65 kDa antigen protein in two subsequent reactions with the same pair of primers. Primers, deoxynucleotides and other ingredients of reaction mixture, positive control (genomic DNA of the RH strain of *Toxoplasma gondii*), and a marker were included in the kit.

Polymerase Delta 2 (Fermentas, Lithuania) was used for amplification. The size of the amplified fragment was 262 base pairs. Amplification reactions were carried out in Hot-Shot 25 thermal cycler (DNA-GDAŃSK II s.c., Gdańsk, Poland). Samples were initially denatured for 2 min at 94ºC. Subsequent cycles were at 94ºC for 30 sec (denaturation), 64ºC for 1 min (annealing), and 72ºC for 30 sec (extension). 35 cycles were performed.

For the analysis of PCR amplification products, 13 µl aliquots of reaction mixtures, marker, positive control and negative control (re-distilled water) were applied to 1.5% agarose gels (Basica LE, Prona, EU) with Tris-Borate-EDTA (pH 8.2) as running buffer and electrophoresis was performed for 55 min at 110 V. DNA bands were stained with ethidium bromide and visualised by UV transillumination. Achieved specific products of 262 base pairs were considered as a positive result (Fig. 1).

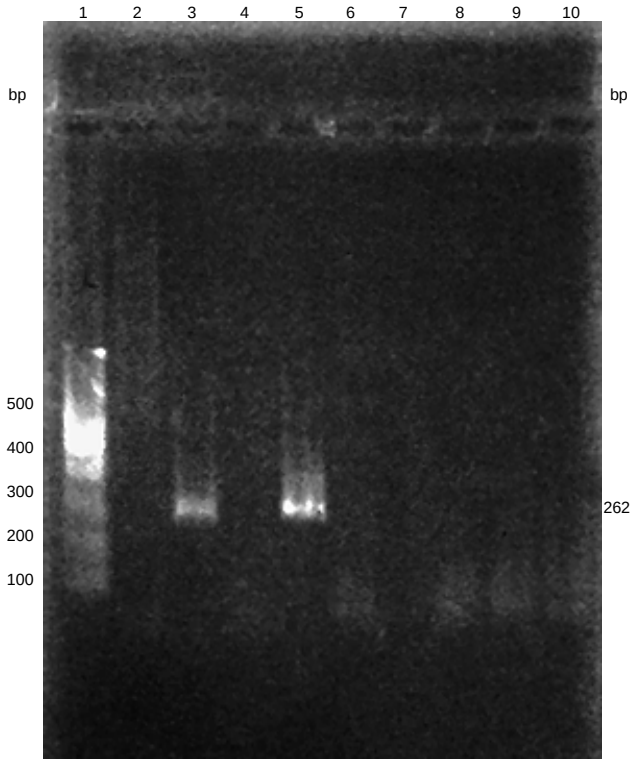

Figure 1. PCR amplification of *Toxoplasma gondii* in *Ixodes ricinus* lysates demonstrated by agarose gel electrophoresis after ethidium bromide staining. Lane 1: M1 marker; Lane 2: negative control; Lane 3: positive control; Lane 5: tick lysate with positive amplification; Lanes 4 and 6-10: tick lysates with negative amplification.

RESULTS

The presence of the *Toxoplasma gondii* DNA was found in two *Ixodes ricinus* females by clearly expressed PCR reactions (Fig. 1). The infected ticks were collected from only one locality, a mid-forest parking lot in Sobibór woodland. The frequency of the *T. gondii* infection in *I. ricinus* females was 6.1%, none infection was found in males and nymphs. The total infection rate with *T. gondii* parasites among *I. ricinus* ticks collected in eastern Poland was 2.2% (Tab. 1).

Table 1. Frequency of occurrence of *Toxoplasma gondii* DNA in *Ixodes ricinus* ticks collected in five localities of the Lublin region (eastern Poland), as assessed by polymerase chain reaction (PCR). N.t. = not tested

Locality				Examined ticks (positive/examined/percent)			
No.	Name	Description	Time of collection	Nymphs	Males	Females	Total
1	Dąbrowa near Lublin	Mixed forest (pine, oak, birch)	May 2001	0/5/0	0/5/0	0/7/0	0/17/0
2	Urszulin	Deciduous forest (oak)	September 2001	0/10/0	0/4/0	0/5/0	0/19/0
3	Parczew	Mixed forest (pine, oak)	May 2000	0/10/0	0/5/0	0/5/0	0/20/0
4	Sobibór	Parking lot surrounded by mixed forest (oak, pine)	May 2001	N.t.	0/15/0	2/16/12.5	2/31/6.5
5	Włodawa	Deciduous forest (oak)	September 2001	N.t.	0/5/0	N.t.	0/5/0

DISCUSSION

Detection of the *Toxoplasma gondii* DNA in *Ixodes ricinus* ticks suggests a possibility of tick transmission as a new way of spreading this parasite in nature. This confirms earlier reports [2, 3, 6, 7, 9, 11, 15, 19] indicating ticks as potential vectors of toxoplasmosis.

Ixodes ricinus is the most common tick species in Europe [16]. Thus, the ability of transmission of *T. gondii* by this species would be of significant epidemiological importance. This could explain, at least in part, the high incidence of seropositive reactions in direct agglutination test for toxoplamosis (68.6%) found by Sroka [17] among forestry workers in the Sobibór area. This author has also described a case of clinical toxoplasmosis in a forester from this area [17]. A high, exceeding 50%, prevalence of anti-*Toxoplasma* antibodies among cows in eastern Poland [17] seems to indicate that besides the oral route of *T. gondii* infection, an additional route of infection should be considered, possibly by tick-borne transmission. *I. ricinus* ticks are common in woodlands of eastern Poland where they play an important epidemiological role as vectors of Lyme borreliosis and tick-borne encephalitis [4].

So far, our hypothesis on the possible role of ticks in epidemiology of toxoplasmosis must be regarded with some caution, as some authors were not successful in isolation of *T. gondii* from ticks collected in natural habitats or in experimental transmission of the infection by these arachnids [1, 10, 12]. Also, it is unknown which stage(s) of *Ixodes ricinus* might be involved in the transmission of *T. gondii* and how the life cycle of parasites looks in the tick body. In this work, *T. gondii* DNA was found only in the adult female ticks but in an earlier study, Deryło *et al.* [7] evidenced the transmission of *Toxoplasma* by intermittent blood sucking of *I. ricinus* nymphs, but not larvae and females. However, in the present work, no nymphs were found in the locality where infected *I. ricinus* females were collected. Thus, further studies are needed for elucidation of all the questions concerning possible transmission of *Toxoplasma gondii* by *Ixodes ricinus* ticks.

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