SHORT COMMUNICATION

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Absence of *Theileria* spp. in ixodid ticks collected from vegetation and animals in eastern Poland

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Abstract

Introduction and Objective. Parasites of the genus *Theileria* are intracellular protozoa that infect the leukocytes and erythrocytes of animals, causing theileriosis. The aim of the study was to examine the presence of *Theileria* spp. in adult ticks and their offspring in the Lublin region of eastern Poland.

Materials and Method. Ticks were collected from vegetation and from wild and domestic animals in Lublin Province, Poland. Engorged females were left in laboratory conditions to lay eggs. Detection of *Theileria* spp. was performed by amplifying a fragment of the 18S rRNA gene.

Results. Overall, 1,000 ticks collected from vegetation, 50 engorged female ticks from animals, and eggs and larvae from 5 females were tested for *Theileria* spp. The parasite was not detected in adult ticks or samples isolated from eggs and larvae.

Conclusions. The results did not confirm the prevalence of *Theileria* in ticks in eastern Poland. The distribution of *Theileria* in ticks and animals in the Lublin macroregion requires further monitoring.

Key words

Poland, ticks, *Ixodes ricinus*, *Dermacentor reticulatus*, *Theileria* spp.

INTRODUCTION

Theileria is a genus of obligate intracellular and haemoparasitic protozoa belonging to the order of Piroplasmida within the phylum of Apicomplexa. The parasites infect leukocytes and erythrocytes, causing theileriosis. The life cycle of *Theileria* involves vertebrate and invertebrate hosts and can infect both wild and domestic animals. They are transmitted by ixodid ticks, which determines the occurrence of *Theileria* in tropical and subtropical regions. Ticks belong to the genera of *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* are considered as a vector of *Theileria*. *Theileria,* together with *Babesia,* are considered the second most frequently parasites found in the blood of mammals, after trypanosomes. This parasite influences livestock production and has a negative impact on the quality of human life [1–7].

The genus of *Theileria* is divided into transforming and non-transforming types of parasites. In the schizont stage, some species of this genus (*T. annulata*, *T. parva*, *T. lestoquardi, T. taurotragi*) could transform the leukocytes of its mammalian host into tumour-like cells which are characterized by immortal hyperproliferation and highly dissemination. A non-transforming species of *Theileria* (*T. equi*, *T. haneyi*, *T. orientalis* and *T. mutans*) persists as intra-erythrocytic piroplasms with transmission via contaminated needles, blood products, or transplacental transmission commonly occurring [3, 7]. The infective sporozoites of *Theileria* are secreted into the feeding site by the tick and infect leukocytes, after which sporozoites multiply by merogony and the realized merozoites invade erythrocytes. During the next tick feeding, the larvae or nymphs ingest a parasite that formulate a zygote after syngamy in the tick gut. The motile kinetes formed by division of the zygote, invade epithelial cells of the tick gut where they remain during the tick moult cycle. The kinetes then move to the haemolymph and infect the salivary glands of the tick. The rapid sporozoite development starts during tick feeding, and infective sporozoites are transmitted in the later feeding stages [8, 9].

Among the *Theileria* genus, some species cause mild infection while others develop into severe clinical disease. The two most important species affecting cattle, responsible for the highest mortality in the herds, are *T. parva* and *T. annulata*. These species are transmitted primarily by ticks from *Hyalomma* genus and *Rhipicephalus appendiculatus* [10]. *T. parva* is a causative agent of East Coast Fever (ECF); *T. annulata* – a tropical theileriosis, and *T. orientalis* cause oriental theileriosis [11–13].

The aim of the study was to assess the prevalence of *Theileria* in ticks collected from vegetation and animals, and in the case of engorged female ticks which have laid eggs, examine them for the presence of the parasites in eggs and larvae.

MATERIALS AND METHODS

Ticks collection and identification. Ticks from vegetation were collected in May, June and September 2022 with the flagging method from three locations in the Lublin Province of eastern Poland – Chełm (51.1431, 23.4712), Cyców Kolonia

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II (51.291944, 23.161111) and Stawek (23.200000, 51.278611). In addition, partially and fully engorged ticks were collected from wild and domestic animals. Ticks removed from dogs and cats were delivered by their owners to the Department of Health Biohazards and Parasitology, Institute of Rural Health in Lublin. Ticks from wild boar and deer were collected in May-April and September-October, respectively, during the legal hunting season in 2022. Animals shot by certified hunters were taken to a collection point where they were made available for post-mortem tick sampling. Live, engorged females were placed in sterile, airtight containers and left in the laboratory to lay eggs. All tick specimens were identified to developmental stage, gender, and species morphologically using a taxonomic key [14].

DNA extraction and molecular analysis. Total DNA from engorged female ticks was extracted with a QIAamp DNA Mini Kit (Qiagen, USA), according to the manufacturer's protocol. DNA from single ticks from vegetation and pulled eggs and larvae (20 specimens per pool) were isolated using the ammonia method, according to Rijpkema et al. [15]. The extracts were stored at -20 °C until use.

The species of the engorged ticks was confirmed by amplification of the mitochondrial 16S rRNA (rDNA) gene fragment of 460bp with primers 16S (5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3'), and 16S2 (5'- CCG GTC TGA ACT CAG ATC AAG T-3'), according to Black and Piesman [16] with modification [17]. Amplification products were separated electrophoretically in 2% agarose gels stained with ethidium bromide and visualized with Ingenius Syngene Bio Imaging (Syngene, GB). All PCR amplicons were then subjected to sequencing with the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA) using a BigDye® Terminator v3.1 Cycle Sequencing Kit and Big Dye XTerminator Purification Kit (Applied Biosystems, USA). The nucleotide sequences were compared with data stored in GenBank using the Basic Local Alignment Search Tool (BLAST).

The ticks were screened for the presence of *Theileria* spp. using PCR targeting the 18S rRNA gene fragment of 1100 bp, according to Li et al. [18]. The amplicons were subjected to agarose gel electrophoresis (2%) to determine the presence or absence of specific PCR products and to confirm or exclude the presence of *Theileria* DNA in the tested ticks.

RESULTS

In total, 1,000 ticks were collected from vegetation – 800 adult *I. ricinus* ticks (500 females and 300 males) and 200 adult *D. reticulatus* ticks (150 females and 50 males). Additionally, 50 engorged female ticks were collected from animals – 4 dogs, 3 cats, 6 wild boar, 4 cervids (2 roe deer and 2 red deer). Molecular testing confirmed identification of 2 species of ticks: *D. reticulatus* (MH645514.1; KX881100.1; OR936111.1) and *I. ricinus* (KF197133.1; MF370648.1; KJ414456.1) (Tab. 1).

Of the 50 female ticks collected from animals, only 5 laid eggs (10%), all of which belonged to the *D. reticulatus* species (12.5%) (Tab. 2). In total, *D. reticulatus* females laid 1,180 eggs, of which 27.12% hatched into larvae (320 specimens). The average number of eggs laid was 236 (between 180–280), and in the case of hatched larvae, the average number was 80 (between 20–120). The females that laid eggs fed on dogs, wild boar and cervids, all of which were collected in the spring months (April and May), which corresponds to the optimal time for egg laying by female ticks. The average time from the start of egg laying to the end was about 7 days. The time in which the larvae hatched was 7–10 days.

Table 1. Tick species collected from wild and domestic animals in Lublin Province, Poland

Host	Tick species	No. of collected female ticks	Sampling city
Dog	*Dermacentor reticulatus*	9	Lublin/Chełm/Cyców
Dog	*Ixodes ricinus*	6	Lublin/Chełm
Cat	*Ixodes ricinus*	4	Lublin/Chełm
Deer	*Dermacentor reticulatus*	13	Chełm
Wild boar	*Dermacentor reticulatus*	18	Cyców

The presence of *Theileria* spp. DNA was not detected in samples isolated from *I. ricinus* and *D. reticulatus* collected from vegetation. The pathogen was also not found in the female ticks removed from animals, nor in samples isolated from eggs and larvae.

DISCUSSION

Ixodes ricinus and *D. reticulatus* are the 2 most common tick species in Europe. Both tick species are vectors of many pathogens that cause diseases in humans and animals, among others apicomplexan protozoa *Theileria* and *Babesia*. The risk of exposure to tick-borne diseases is determined by several factors, including the prevalence of pathogens in the tick population in the study area. In this study, DNA of *Theileria* spp. was not detected in any of the 1,050 collected ticks; it can be therefore assumed that the risk of theileriosis in the study area is low. So far, few studies have demonstrated the presence of *Theileria* in *I. ricinus* and *D. reticulatus*, especially from vegetation. In Poland, Adamska and Skotarczak [19] examined *I. ricinus* ticks collected from horses and vegetation. Similarly to the results of the current study, they did not detect the piroplasm DNA. In an earlier study conducted in 2008, Skotarczak et al. [20] found DNA of *Theileria* in *I. ricinus* ticks in the western part of the country with a prevalence of 5.3% in ticks collected from game animals, and 4% collected from vegetation. In north-eastern Poland, Wondim et al. [21] confirmed the presence of the parasites in both tick species collected from the environment – in 9.59% of *I. ricinus* and 9.85% of *D. reticulatus*.

In Europe between 2017–2022, 8 species of *Theileria* (*T. annulata, T. buffeli, T. capreoli, T. cervi, T. equi, T. lestoquardi, T. orientalis, T. ovis*) and *Theileria* sp. were described in 19 species of tick belonging to the genera *Dermacentor, Heamaphysalis, Hyalomma, Ixodes* and *Rhipicephalus* collected from a host and the environment [22]. The results of the current study are in accordance with the study by Andersson et al. [23] in Romania, in which *Theileria* spp. was not detected in any *I. ricinus* and *D. reticulatus* collected from animals. Similarity, in Slovakia, *Theileria* sp. was not detected in any of *I. ricinus* ticks but was found in only one *Haemaphysalis concinna* tick collected from red fox [24]. Also, Habib et al. [25] did not identify any *Theileria* spp. in *I. ricinus* ticks collected from chamois and mouflon in mountainous areas of France. However, in Italy,

Table 2. Egg-laying process in females and larvae hatching of the species *Dermacentor reticulatus*

Host	No. of egg-laying females / total number of female ticks (%)	Total number of eggs laid	No. of eggs laid by one female. Value: average / (min – max)	No. of eggs for testing / number of hatched larvae for testing	No. of larvae hatched by one female. Value: average / (min – max)	Month the female started laying eggs	No. of days from start of egg laying to completion. Value: average / (min – max)	No. of days from the start of larvae hatching to the end. Value: average / (min – max)	Weight of egg-laying female (grams). Value: average / (min – max)
Dog	2/9 (22,2%)	420	210/180-240	320/100	50/0-100	April /May	7/5-9	7/0-7	0,3702/0,3675–0,3734
Boar	2/18 (11,1%)	560	280/280-280	360/200	100/80-120	April /May	8/8-8	8,5/8-9	0,2934/0,2486–0,3379
Cervidae	1/13 (7,7%)	200	200/200-200	180/20	20/20-20	May	8/8-8	10/10/10	0,2144/0,2144–0,2144
TOTAL	**5/40 (12,5%)**	**1180**	**236/180-280**	**860/320**	**80/20–120**	**April /May**	**7,6/5–9**	**8,5/7–10**	**0,3083/0,2144–0,3734**

the different species within the genus *Theileria,* including *T. buffeli/ sergenti/ orientalis*, *T. cervi*, *T. equi*, *T. ovis*, were found in pools of *I. ricinus* collected from dogs, with the Minimum Infection Rate (MIR) estimated to be 0.2% – 2.5% [26].

The main reservoir hosts of *Theileria* are ruminants, but some non-ruminant animal, such as horses, are shown to be hosts for the parasite. The infection rate of ticks depends, among others, on the prevalence of pathogens in animal hosts. *Theileria* spp. DNA was found in red deer and roe deer in the western and northwestern part of Poland, with prevalence ranging from 24.6% – 88%, and in horses – 1.3–7.2%) [20, 27–29].

Little we know about the infection rate of *Theileria* in wild animals in the study area of eastern Poland, although single research results indicate the presence of *T. equi* in horses in the region [30]. The very low prevalence of the pathogen in ticks may be due to the low level of infection of the animal reservoir, which requires confirmation in further studies.

Ticks are a vector for many pathogens, including viruses, bacteria and parasites, that can be transmitted via various routes in ticks. The main transmission routes are horizontal, transstadial, transovarial, venereal, and co-feeding [31]. Transovarial transmission was considered to be a distinguishing feature of the protozoa *Babesia* from *Theileria*, where earlier results had excluded transovarial transmission of *Theileria* and only confirm transstadial transmission, which means that pathogens ingested by larvae or nymph of a tick are transferred to a new animal by nymphal stage or adult tick [10, 32–34].

The phase of zygote development differs between *Babesia* and *Theileria*. In *Babesia* the zygotes multiply which results in the invasion of numerous organs of the tick, including the ovaries. In this way, the infection passes through the ovary and the egg to the next tick generation. In *Theileria,* the zygotes do not multiply but invade the haemolymph of the tick where they proceed to the salivary glands [32, 33]. These results confirm the reports conducted on *Haemaphysalis qinghaiensis* and *Hyalomma anatolicum* anatolicum ticks feeding on small ruminants, where no transovarial transmission was observed, only transstadial transmission of the protozoa *Theileria* sp. [35]. Other studies conducted in New Zealand on *H. anatolicum* ticks demonstrated the lack of transovarial transmission of *T. annulata* protozoa [36]. Later studies produced different results and confirmed the possibility of transovarial transmission. Kakati et al. [37] observed the presence of *T. orientalis* in eggs laid by infected females of *Rhipicephalus* (*Boophilus*) ticks, obtained by Giemsa-stained blood smear and PCR. In another study, the larvae and egg pools obtained from the infected females were positive in PCR, and confirmed the presence of transovarial transmissions of *T. annulata* in *H. anatolicum* tick [38].

The results obtained in the current study did not confirm the occurrence of *Theileria* in ticks collected from vegetation and animals, nor were *Theileria* detected in any of the tested pools of eggs and larvae. The distribution of *Theileria* spp. in the population of ticks in the Lublin macroregion as well as the occurrence of this protozoa in animals that could serve as a reservoir of the pathogen, require further monitoring. The possibility of transovarial transmission of *Theileria* in tick population was confirmed in some studies while other studies exclude this route. Future studies aimed at determining the transovarial transmission of different species of *Theileria* in ticks from different genera will provide knowledge regarding the risk of infection during larvae feeding.

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