

## Streszczenie w j. angielskim

**Introduction:** Babesiosis and tick-borne encephalitis are emerging vector-borne diseases associated with favourable habitats of ticks and their hosts. Ticks are blood-feeding parasites that carry a wide range of pathogens, including the agents that cause Lyme disease, tick-borne encephalitis, anaplasmosis, and babesiosis. *Ixodidae* (hard) ticks are known to transmit the majority of tick-borne pathogens to humans and animals. *Ixodes ricinus* ticks are considered the most relevant tick species to transmit pathogens of Tick-Borne Diseases (TBDs) in Europe; however, other ticks, such as *Dermacentor reticulatus*, are also linked to the spread of TBDs. Babesiosis is an intraerythrocytic infection caused by *Babesia* spp. parasites. Tick-borne encephalitis is an infectious disease of the central nervous system caused by Tick-borne encephalitis virus (TBEV). Both babesiosis and tick-borne encephalitis are mainly transmitted through tick bite. In Poland, close to half of the TBE cases were reported from the Podlaskie region over the past 20 years. *Babesia* spp. in Africa is mainly limited between animals and ticks however there are few cases with substantial evidence of babesiosis. Ethiopia is known for its high prevalence of malaria which shares most of its features with babesiosis. This study intended to investigate the strains of *Babesia* spp. in Ethiopia and eventually will contribute to the existing understanding of the two diseases.

**Aims:** This study aimed to detect and molecularly characterize *Babesia* spp. and tick-borne encephalitis virus in ticks from Poland and Ethiopia. The detailed aims were:

1. To detect and characterize *Babesia* spp. and TBEV using PCR assays in ticks collected from Poland and Ethiopia.
2. To investigate the prevalence and strain variation between *Babesia* spp. of Poland and Ethiopia.
3. To analyse the potential predictors (temperature, humidity, developmental stage, and species) of tick-borne pathogens detection in ticks collected from Poland and Ethiopia.

**Material and Method:** The ticks used in this study were collected from Knyszyn forest landscape park in Poland's north-east region, as well as multiple sites in Ethiopia. The ecology of tick collection sites had the entire essentials for ticks' shelter. DNA was extracted from ticks collected in both Poland and Ethiopia while RNA was extracted from ticks collected in Poland only. Ticks were crashed and nucleic acid from each tick was collected independently. After detecting *Babesia* spp. and TBEV using conventional PCR and qualitative RT-PCR methods respectively, positive samples were sequenced. The

association between the developmental stage of ticks, ambient temperature during tick collection, species, and sex of ticks and TBEV detection rate was conducted.

**Results:** A total of 995 (727 from Poland and 268 from Ethiopia) ticks were collected and examined to detect whether ticks were infected with TBEV and/or *Babesia* spp.. Eighty-five (9.51%) of ticks out of 894 ticks were positive for *Babesia* spp. Among the 626 ticks from Poland 61 (9.74%) and out of the 268 ticks from Ethiopia, 24 (8.96%) of them were positive for *Babesia* spp. Seven (1.17%) out of the 601 ticks had co-infection of *Babesia* spp. and TBEV while 86 (14.31%) of ticks had mono-infection. The prevalence of *Babesia* spp. in ticks has shown a staggering pattern. In this study, the overall prevalence of *Babesia* spp. in both tick species was 9.51% with 9.59% in *I. ricinus* and *D. reticulatus* 9.83%. In a study conducted in northern Poland the overall prevalence of *Babesia* spp. was 10.6% with 7.7% in *I. ricinus* and 18.9% in *D. reticulatus* tick. *Babesia* spp. has been also been detected in 2.0% *I. ricinus* and 7.0% of *D. reticulatus* ticks collected from a forest in north-eastern Poland which is close to the study area where this study has been conducted. Sequencing analysis of *Babesia* spp. showed *B. microti* in 70.59% (60/85) of the samples with a mean homology of 87.56% that ranges between 82.29-100%. Apart from *B. microti*, *Theileria velifera* 8.24% (7/85), *B. capreoli* 4.71% (4/85), *B. venatorum* and *B. canis* each 3.53% (3/85), and *Theileria mutans* 2.35% (2/85) were also detected via sequencing. A multivariable logistic regression has shown, *I. ricinus* was 93.7% less likely to have TBEV compared to *D. reticulatus* species of ticks ( $p=0.007$ ). Ticks collected during an ambient temperature of 15-17°C were 95.8% less likely to have been infected with TBEV compared to those collected under a temperature of less than 15°C ( $p\leq 0.001$ ). As for the developmental stage of ticks adjusted for species and temperature, adult ticks (OR=23.66) were more likely to have been infected with TBEV ( $p\leq 0.001$ ). The prevalence of the *Babesia* strain varies across parts of the world where *B. divergens* is common in Europe while *B. microti* is common in the United States. This study and other studies that used samples from ticks and humans have reported *B. microti* as the most frequently detected strain of *Babesia* spp. in the eastern and north-eastern parts of Poland. All TBEV were the European subtype.

**Conclusions:** *Babesia microti* was the commonest strain detected in ticks collected from both Poland and Ethiopia. *Theileria* spp. were detected from both countries' ticks. All TBEVs detected in ticks from Poland were European subtypes. The temperature during tick collection, species of ticks and their developmental stages determine the detection rate of TBEV. The detection rate of *Babesia* spp. and TBEV in ticks is increasing in recent years

which needs further investigation on the interaction of ticks and hosts. Public health interventions including promoting vaccine campaigns are relevant to prevent the growing incidence of TBDs.