

**POLISH PARASITOLOGICAL SOCIETY**

**ANNALS OF  
PARASITOLOGY**

**volume 59 · supplement · 2013**



**PL ISSN 2299-0631**

The XXIIIth Congress of PPS is organized by Executive Board of the Polish Parasitological Society and Wrocław Branch of the Society, and Wrocław University, Wrocław University of Environmental and Life Sciences and Wrocław Medical University



Uniwersytet  
Wrocławski



UNIWERSYTET MEDYCZNY  
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

### *Honorary Patronage*

**Rector of the University of Wrocław**  
**Prof. Marek Bojarski**

**Rector of the Wrocław University of Environmental and Life Sciences**  
**Prof. Roman Kołacz**

**Rector of the Wrocław Medical University**  
**Prof. Marek Ziętek**

### *Organizing Committee*

**Chair: Prof. Anna Okulewicz**  
**Vice-chair: Dr Marcin Popiołek**  
**Secretary: Dr Joanna Hildebrand**  
**Treasurer: Dr Jolanta Piekarska**

### *Committee members*

**Dr Katarzyna Buńkowska-Gawlik**  
**Dr Dorota Kiewra**  
**Prof. Elżbieta Lonc**  
**Dr Agnieszka Percec-Matysiak**  
**Prof. Zenon Sołtysiak**  
**Dr Maria Wesolowska**  
**Dr Grzegorz Zaleśny**

### *Sponsors*

The Organizing Committee for the XXIIIth Congress of the Polish Parasitological Society acknowledges with gratitude the generous support received from the following sponsors:



**EURx**  
Molecular Biology Products



**ABE-IPS**  
books online journals  
[www.abe.pl](http://www.abe.pl)

Print of the Congress materials is supported by Ministry of Science and Higher Education of Poland.

Authors are responsible for supplied abstracts.

**The XXIIIth Congress  
of the Polish Parasitological  
Society**

4-7 September 2013, Szklarska Poręba-Piechowice

**ABSTRACTS**



# **SESSION IX**

## **Epidemiology of the vector-pathogen relationship**



## Detection of *Borrelia burgdorferi* sensu lato, *Babesia* spp. and *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks (Acari: Ixodida) collected from the area of Bieszczadzki National Park. A preliminary study.

Marek Asman<sup>1</sup>, Magdalena Nowak<sup>2</sup>, Joanna Strzelczyk<sup>3</sup>, Piotr Szilman<sup>1</sup>, Ewa Szilman<sup>1</sup>, Krzysztof Siuda<sup>2</sup>, Krzysztof Solarz<sup>1</sup>, Andrzej Wiczkowski<sup>3</sup>

<sup>1</sup>Department of Parasitology, Medical University of Silesia, Jedności 8, 41-218 Sosnowiec

<sup>2</sup>Department of Invertebrate Zoology and Parasitology, Pedagogical University, Podbrzezie 1, 31-054 Cracow

<sup>3</sup>Department of General Medical Biology, Medical University of Silesia, Jordana 19, 41-808 Zabrze Rokitnica; Poland

Corresponding author: Marek Asman; e-mail: masman@sum.edu.pl

*Ixodes ricinus* (Acari: Ixodida) is a tick with the greatest medical importance in Poland. This species is the vector of many pathogens of humans and animals. Some of the most dangerous being *Borrelia burgdorferi* sensu lato, *Babesia* spp. and *Anaplasma phagocytophilum*. These pathogens are the etiological agents of various diseases such as: Lyme disease, babesiosis and human granulocytic anaplasmosis. The aim was to assess the exposure to ticks infected with *B. burgdorferi* s. l., *Babesia* spp. and *A. phagocytophilum* in the area of the Bieszczadzki National Park. Ticks were collected in spring 2011 from selected places in the area of the Bieszczadzki National Park by flagging. The ticks were preserved in 70% ethanol and identified under stereomicroscopes. DNA were isolated from 37 randomly selected individuals by the ammonia method. The pathogens in the ticks were detected by PCR. For the identification of pathogens, pairs of primers specific to the flagelline gene (for *B. burgdorferi* s. l.), 18S rDNA gene (for *Babesia* spp.) and 16S rDNA gene (for *A. phagocytophilum*) were used. PCR amplification products were separated on 2% agarose gels and visualized under an ultra violet light. The expected product sizes were: 442 base pairs [bp] for *B. burgdorferi* s. l., 560 bp for *Babesia* spp., 227 bp for *A. phagocytophilum*. A total of 67 *I. ricinus* ticks were collected. The studies showed that 26/37 (70%) of ticks were infected. It was also shown that 7/37 (18.9%) of ticks were infected with *B. burgdorferi* s. l. and 18/37 ticks (48.6%) with *A. phagocytophilum*. *Babesia* spp. were found only in 1 male (2.7%). A co-infection of *B. burgdorferi* s. l. and *A. phagocytophilum* in two females (5.4%) was detected, while co-infection with *A. phagocytophilum* and *Babesia* spp. occurred only in 1 male (2.7%). Co-infections of *Babesia* spp. and *B. burgdorferi* s. l. or with all these pathogens were not found. The results showed a high risk of exposure of humans and animals to infected ticks on the studied area. Moreover, it was shown that the examined ticks were most frequently infected with *A. phagocytophilum*. The study revealed also some co-infections between the examined pathogens. Ticks and tick-borne diseases may be a serious threat within the area of the Bieszczadzki National Park and these studies should be continued.

## ***Anaplasma phagocytophilum* in wild rodent populations from areas of Lower Silesia, Poland**

**Ewa Gajda, Magdalena Woźniak, Katarzyna Buńkowska-Gawlik,  
Agnieszka Perec-Matysiak, Joanna Hildebrand, Martin Kváč<sup>1</sup>**

Department of Parasitology, Institute of Genetics and Microbiology, Faculty of Biological Sciences, University of Wrocław, Poland

<sup>1</sup>Institute of Parasitology, Biology Centre Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

Corresponding author: Joanna Hildebrand; e-mail: joanna.hildebrand@microb.uni.wroc.pl

The Gram-negative, tick-transmitted bacterium *Anaplasma phagocytophilum* is an obligate intracellular pathogen, which propagates inside peripheral blood granulocytes, mainly neutrophils. This bacterium is the causative agent of granulocytic anaplasmosis, recognized as an emerging tick-borne disease, an infection of medical and veterinary importance. The bacterial parasite has been detected in a wide range of animals and in several different *Ixodes* species. This suggests that it is a generalist pathogen, which is capable of exploiting a variety of hosts and vectors. In Europe, the deer population is considered as a main reservoir of bacteria (particularly *Capreolus capreolus* and *Cervus elaphus*), while the role of small wild rodents (e.g. *Apodemus flavicollis*, *Myodes glareolus*) is not clearly assessed. The *msp2* gene encoding major surface protein 2 (MSP2) is specific for *Anaplasma* and has high intraspecific variability. This variability, the consequential phenotypic variability of the protein and its antigenic variation may be important factors which help maintain the parasite in various hosts.

The aim of this study was the detection of *A. phagocytophilum* in spleen samples of wild rodents in order to recognize the role of small mammals in maintaining a source of infection in the Lower Silesia area. A total of 256 small wild rodents belonging to the three species *Apodemus flavicollis* (58), *Apodemus agrarius* (149) and *Myodes glareolus* (49) were captured in 2010, 2011 and 2012 in areas of Lower Silesia (Poland), i.e. Mokry Dwór, Osobowice, the Stawy Milickie reserve and the Ślężański Nature Park. DNA from spleen samples was isolated with a GeneMATRIX Bio-Trace DNA Purification Kit (EURx) according to the producer's instructions and stored at -20°C until the time of analysis. The primer pair *msp2*-3f, *msp2*-3r specific for the fragment of the *msp2* gene with a 334 bp length was used for detection of *A. phagocytophilum* DNA. PCR products were electrophoresed in 1% agarose gels and stained with Midori Green DNA Stain. *A. phagocytophilum* DNA was detected in 49 (19.1%) of 256 small wild mammals. The highest infection rate was demonstrated for *A. agrarius* (21.5%). Bacterial DNA were found in samples from small rodents in all studied regions located in Lower Silesia with the highest prevalence in the Ślężański Nature Park (27.7%). The results indicate that small mammals probably play an important role as reservoir hosts for *A. phagocytophilum* and maintain the circulation of this pathogen in the natural environment.



## The infection of *Dermacentor reticulatus* ticks with *Babesia canis* and *Anaplasma phagocytophilum* in Chernobyl exclusion zone

Grzegorz Karbowski<sup>1</sup>, Bronislava Vichova<sup>2</sup>, Kateryna Slivinska<sup>3</sup>,  
Joanna Werszko<sup>2</sup>, Julia Didyk<sup>3</sup>, Branislav Pet'ko<sup>2</sup>

<sup>1</sup>W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland

<sup>2</sup>Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01, Košice, Slovakia

<sup>3</sup>I.I.Schmalhausen Institute of Zoology of NASU, B.Chmielnicki 15, 01601 Kiev, Ukraine

Corresponding author: Grzegorz Karbowski; e-mail: grzgrz@twarda.pan.pl

In 1986, after the disaster at the Chernobyl nuclear power plant, a 30 kilometre exclusion zone was established, within which human activity was eliminated. The occurrence of the ticks was evaluated from August to October in the years 2009–2012. The study area was located in preventive zone B, 10–20 km from the disaster point. The ticks were collected using the flagging method, in locations of their prospective appearance. The predominant tick species was *Dermacentor reticulatus*. The collected ticks were fixed in ethanol and transported to the laboratory to diagnose any infections with *Babesia* spp. and *Anaplasma phagocytophilum*. The DNA extraction was performed using ammonium hydroxide. A nested PCR assay, targeting the *groESL* gene was performed for the detection of *A. phagocytophilum*. In the first round, the pair of primers HS1 and HS6 was used. These primers amplify the end of the *groES* gene, intergenic spacer and approximately 2/3 of the *groEL* gene. In the second round, HSVF and HSVR primers that span a 395-bp region of the *groEL* gene were used. DNA samples were tested also for the presence of protozoan pathogens from the genus *Babesia*. The presence of these pathogens was detected by the use of genus-specific BJ1, BN2 primers, which amplify the portion of 18S rRNA. Altogether, 205 *Dermacentor reticulatus* ticks were screened. The prevalence of infection of ticks with *Babesia canis* and *Anaplasma phagocytophilum* was 3.41 and 25.36%, respectively.

Randomly chosen positive samples were subsequently sequenced. Sequencing confirmed the identity of *Anaplasma phagocytophilum* and *Babesia canis canis* in screened DNA samples of *Dermacentor reticulatus*. The results are the first evidence of the *B. canis* and *A. phagocytophilum* zoonotic foci in the Chernobyl exclusion zone, and present the possibility that tick-borne diseases can maintain foci in areas with no human activity. For the first time *A. phagocytophilum* has been detected in questing *D. reticulatus* ticks.

## Biological and medical monitoring of tick-borne disease risk in Wrocław and Lower Silesia, Poland (1998–2013)

Dorota Kiewra, Elżbieta Lonc, Aleksandra Czulowska, Piotr Jawień

Department of Microbial Ecology and Environmental Protection, Institute of Genetics and Microbiology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

Corresponding author: Dorota Kiewra; e-mail: dorota.kiewra@microb.uni.wroc.pl

In the last decades, tick-borne diseases (TBD) have become increasingly important health problems both on the global and local scales. The emergence of new pathogens, as well as their spread by vectors to new areas and habitats requires systematic biological and medical monitoring to assess the risk of TBD over space and time. As Lyme borreliosis (LB) is still the most often recognized TBD and plays a leading role among the occupational diseases, according to reports on cases of infectious diseases and poisonings in Poland ([www.pzh.gov.pl](http://www.pzh.gov.pl)), our study is mainly concentrated on the prevalence and infection of the tick population both in the Wrocław area as well as the selected Lower Silesia regions, previously the Ślązański Landscape Park. Environmental seasonal monitoring conducted regularly since 1998, confirmed that *Ixodes ricinus* is the most important tick species. As in the rest of Europe, it has been found across Lower Silesia in the forests, grasslands, and recently also in the urban areas.

In the years 1998–2012, a prevalence of tick specimens infected with *Borrelia burgdorferi* s. l. was found in all eleven examined districts: Wrocław, Dzierżoniów, Świdnica, Wałbrzych, Środa Śląska, Trzebnica, Oleśnica, Lubań, Milicz, Oława, and Legnica. The minimal infection rates of *I. ricinus* collected in 2012 in twelve sites located in four districts (Lubań, Milicz, Oława and Legnica) varied from 15.8% for nymphs to 37.0% for adults, respectively. At the same year, over 470 cases of LB (16.2/100000) were reported at Lower Silesia, including 149 hospitalized people (31.6%), and over 70 patients in the Wrocław area. In addition, during the last monitoring (2011–2012) *I. ricinus* specimens infected with *Anaplasma phagocytophilum* and *Babesia microti* were found in four districts (Lubań, Milicz, Oława, and Legnica).

The results of environmental tick monitoring, laboratory and epidemiological results stored in a digital database visualized by the use of a geographic information system (GIS) revealed the differential *I. ricinus* population and the potential areas of newly emerging *Dermacentor reticulatus* tick species. TBD risk evaluation can be seen by GIS mapping of both biological and medical data using the most legible symbols and colours, which creates opportunities both for comprehensive analysis and for the purposes of ecological education.

This work was partly supported by National Science Centre (NCN), Poland (Grant No. N N404 014 740).

## **The occurrence of nematode *Setaria tundra* in roe deer from Małopolska province – preliminary studies**

**Jerzy Kowal, Sławomir Kornaś, Paweł Nosal, Maciej Lesiak, Marta Basiaga, Filip Klimczak**

Department of Zoology and Ecology, University of Agriculture in Cracow, Mickiewicza Av. 24/28, 30-059 Cracow, Poland

Corresponding author: Sławomir Kornaś; e-mail: skornas@ur.krakow.pl

*Setaria tundra* is a filarioid nematode transmitted by mosquitoes (from the genera *Anopheles*, *Aedes* and *Culex*), sporadically noted in Poland. The synurbanisation of some wild ungulates, as well as climate changes observed in recent years, could affect the possibility of infection, which is connected with the development of the parasite in arthropod vectors living in disturbed environments.

The study was conducted to recognize the presence of *Setaria* sp. in roe deer populations inhabiting different environments. Parasitological dissections of 46 roe deer were performed. The animals either originated from the area of the Cracow agglomeration, or forests and arable lands near the city.

During autopsy, nematode specimens (from one up to 12 per host) were found only in the abdominal cavity of roe deer from the urban area. All parasites were classified to the *Setaria tundra* species, on the basis of its morphology and dimensions. The infection observed was probably associated with the specific environmental conditions of the urban area.

## **Anaplasmataceae infections including the first detection of ‘*Candidatus Neoehrlichia mikurensis*’ in *Ixodes ricinus* ticks from urban and rural forest areas of Poland**

**Maciej Kowalec<sup>1</sup>, Renata Welc-Falęciak<sup>1</sup>, Grzegorz Karbowski<sup>2</sup>,  
Tomasz Szewczyk<sup>1</sup>, Anna Bajer<sup>1</sup>, Edward Siński<sup>1</sup>**

<sup>1</sup>Department of Parasitology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw

<sup>2</sup>Laboratory of Unicellular Parasite, The Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw; Poland

*Ixodes ricinus* ticks are vectors of pathogenic microorganisms, for instance *Anaplasma phagocytophilum* and ‘*Candidatus Neoehrlichia mikurensis*’. The former is a well-known causative agent of Human Granulocytic Anaplasmosis (HGA), while the latter has just recently been recognized as a human pathogen. Both belong to the Anaplasmataceae family together with *Ehrlichia muris*, which is a murine pathogen also transmitted by *I. ricinus*. However, Anaplasmataceae play the role of natural endosymbionts in ticks.

The aim of the study was to estimate the prevalence of Anaplasmataceae bacteria infection in ticks from forests under different levels of anthropopression, and to make a preliminary evaluation of genetic diversity in the obtained isolates.

Overall, a total of 1589 ticks were collected in the year 2011. A molecular study was performed on 1325 ticks, including 405 individuals from the city forests of Warsaw and 920 from the national/landscape parks (Kampinoski National Park, Białowiecki National Park, Mazurski Landscape Park). Ticks were tested for the presence of Anaplasmataceae bacterial DNA with PCR using the *groESL* heat shock operon fragment. For molecular phylogenetics, *groESL* and *16S rRNA* gene were used. The prevalence of Anaplasmataceae in *I. ricinus* ticks was 2%, although the prevalence was 4% in city forests, and only 1% in national/landscape parks. Zoonotic and non-zoonotic strains of *A. phagocytophilum* were detected. Additionally, for the first time in Poland, ‘*Candidatus Neoehrlichia mikurensis*’ and *Ehrlichia muris* were detected in questing *I. ricinus* ticks. The prevalence of infection and genetic diversity of *A. phagocytophilum* in *I. ricinus* ticks differ between areas under low and high level of anthropopression, however, more detailed further ecoepidemiological studies of Anaplasmataceae are needed.

The study was supported by the Ministry of Science and Higher Education, Grant No. NN404795240.

## The coexistence of pathogenic microparasites *Babesia* spp. and ‘*Candidatus* Neoehrlichia mikurensis’ in *Ixodes ricinus* ticks from forests under different levels of anthropopression

Maciej Kowalec<sup>1</sup>, Renata Welc-Falęciak<sup>1</sup>, Grzegorz Karbowski<sup>2</sup>,  
Tomasz Szewczyk<sup>1</sup>, Anna Bajer<sup>1</sup>, Edward Siński<sup>1</sup>

<sup>1</sup>Department of Parasitology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw

<sup>2</sup>Laboratory of Unicellular Parasite, The Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw

*Ixodes ricinus* ticks are vectors of many pathogenic microorganisms, for instance *Babesia* spp. (protista) and ‘*Candidatus* Neoehrlichia mikurensis’ (bacteria). The former are well-known causative agents of babesiosis and tick parasites, while the latter was just recently recognized as a human pathogen and is believed to be an endosymbiont of the ticks together with other Anaplasmataceae bacteria.

The aim of the study was to compare the occurrence and coexistence of *Babesia* spp. and ‘*Candidatus* Neoehrlichia mikurensis’ in *I. ricinus* ticks from forest areas under low and high anthropopression levels by estimating the prevalence of infection and co-infection with these pathogens.

Ticks collected in 2012 in the city forests of Warsaw (Bielański Forest and Kabacki Forest) and the national/landscape parks (Kampinoski National Park, Białowiecki National Park, Mazurski Landscape Park) were tested by PCR for the presence of *Babesia* spp. and ‘*Ca. N. mikurensis*’ DNA using the *18S* and *16S rRNA* genes, respectively. Additionally, phylogenetic analyses were performed using same genes.

Presenting data of prevalence and co-infection together with preliminary molecular characteristics sheds light on the potential transmission of the new emerging pathogen ‘*Ca. N. mikurensis*’ and *Babesia* spp. in Poland. Additionally, the comparison of the coexistence of these microorganisms with different ecological statuses in *I. ricinus* ticks from forests under different levels of anthropopression is a starting point for further ecoepidemiological investigations.

The study was supported by the Ministry of Science and Higher Education through the Faculty of Biology, University of Warsaw intramural grant DSM.

## **Bacterial pathogens isolated from german cockroaches (*Blattella germanica* L.) collected in dwellings – preliminary study**

**Agnieszka Królasik<sup>1</sup>, Ewa Mikulak<sup>1</sup>, Aleksandra Gliniewicz<sup>1</sup>,  
Grażyna Młynarczyk<sup>2</sup>, Kornelia Dobrzaniecka<sup>2</sup>**

<sup>1</sup>National Institute of Public Health – National Institute of Hygiene, Chocimska 24, 00-791 Warsaw, Poland

<sup>2</sup>Chair and Department of Medical Microbiology, Medical University, Chalubinskiego 5, 02-004 Warsaw, Poland

Corresponding author: Aleksandra Gliniewicz; e-mail: agliniewicz@pzh.gov.pl

In Poland little data has been published about presence and role of German cockroaches in multiapartment dwellings. In our preliminary study, the bacterial flora (aerobic bacteria and fungi) from external body surfaces of German cockroaches caught in private apartments located in buildings in Warsaw was examined. Identification of isolated bacteria was performed by the use of standard biochemical and serological assays, by commercial biochemical tests including ID 32 C, Rapid ID 32 Strep, ID 32 Staph API NE and the Vitek 2 compact system.

The results of the study show that on the body surfaces of German cockroaches caught in private apartments, a rich and varied bacterial flora was found. A total of 77 bacteria strains were isolated, among them Gram (+) cocci: *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Enterococcus avium*, and *Enterococcus faecalis*; Gram(-) rods belonging to the Enterobacteriaceae family – *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens* and *Klebsiella oxytoca*, were also found, as was *Stenotrophomonas maltophilia* from the group of non-fermentative Gram(-) bacteria. The susceptibility to antibiotics and chemiotherapeutics of selected strains belonging to infection-causing species was examined.

The results showed the presence of ESBL resistance mechanisms and extended levels of beta-lactamases AmpC-type in *S. maltophilia*, *E. cloacae* and in one of isolated strains of *C. freundii*. Such strains, resistant to antibiotics, may be reservoirs of resistance genes, which can be transmitted into other bacteria. The presence of such pathogens on the body surface of German cockroaches, which are very mobile insects, might create conditions for their dissemination throughout the environment and on its inhabitants. Such bacteria can constitute an epidemiological and therapeutical hazard when patients colonized with them are admitted into the hospital environment.

## ***Ixodes* and *Dermacentor* ticks as parasites of pets in the Wrocław area**

**Elżbieta Lonc, Nina Król, Dorota Kiewra**

Department of Microbial Ecology and Environmental Protection, Institute of Genetics and Microbiology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

Corresponding author: Elżbieta Lonc; e-mail: elzbieta.lonc@microb.uni.wroc.pl

In urban areas, dogs and cats can be very important hosts of ectoparasites (ticks, fleas or lice) which can also play an important role as vector. Due to direct contact with their animals, and indirectly via pathogens transmitted by parasites, owners and their families are exposed to arthropod-borne diseases, especially tick-borne diseases (TBD). Five Polish species of ixodid fauna are recognized as parasites of dogs and cats, *Ixodes ricinus*, *I. hexagonus*, *I. crenulatus*, *I. rugicollis* and *Dermacentor reticulatus*, and another species from the Mediterranean area, *Rhipicephalus sanguineus*, is rarely found.

In own research carried out from April to December 2012, a total of 366 tick specimens were collected from 215 pets (33 cats and 182 dogs) in twelve Wrocław veterinary clinics. The average intensity of tick infestation was 2.8 for cats (range from 1 to 22) and 1.5 for dogs (1–23). Among three recognized tick species, *I. ricinus* was dominant species (94.8%) whereas 4.1% and 1.1% of collected ticks were *I. hexagonus* and *D. reticulatus*, respectively. In total, 347 individuals of *I. ricinus* (292 females, 27 males, 9 nymphs and 19 larvae) were collected, and 15 specimens of *I. hexagonus* (11 females and 4 nymphs) both from dogs and cats. Only four *D. reticulatus* specimens (3 females and one male) were obtained in May and September. They were collected from a Miniature Schnauzer, Bernese Mountain Dog, Yorkshire Terrier, and a mongrel. Due to the emergence of this new tick vector in the Wrocław area, there is a potential risk of the endemic infection of *Babesia canis*, which is mainly transmitted by *D. reticulatus*. The highest *Ixodes* tick infestation of pets was recorded in May (almost 50% of all collected ticks) but the majority (10 from 15 specimens) of *I. hexagonus* were observed in summer, from late June to August.

According to pet owners (147 questionnaires) tick repellents for pets were used by half of dog owners (54.4%) and only by one third (27.3%) of cat owners. Parks, paths along the streets and water places, rivers and ponds, were the most frequently chosen areas for dog walking. This abiotic data, together with the biological data of ticks collected from pets in the Wrocław area were visualized by using the Arc View GIS computer program.

## Description of *Setaria tundra* invasive larvae in a mosquito vector in Poland

Aleksander Masny, Wioletta Rozej-Bielicka, Elżbieta Gołąb

Department of Medical Parasitology, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland

Corresponding author: Aleksander Masny; e-mail: amasny@gmx.com

Mosquitoes are competent vectors for a number of filarial species belonging to the Onchocercidae family. Microfilaria of these species reach the L3 invasive stage in mosquitoes and the L3 larvae are located in the head of the insects. The mosquitoes can transmit the infective larvae to various vertebrates, among them carnivorous and herbivorous mammals. The aim of the study was to find vectors of filarial parasites in the mosquito population from the Mazovia province.

Mosquito samples were collected in the years 2010–2012. The insects were divided and the heads were analyzed separately from the rest of the body. Primer pairs universal for a number of filarial parasites were designed and used for PCR screening of mosquitoes: all those primer pairs amplified fragments of the cytochrome oxidase subunit I gene. PCR products were sequenced and the obtained sequences were analyzed. To identify the species of mosquito, PCR amplification of the internal transcribed spacer (ITS2) of the ribosomal DNA (rDNA) of the infected mosquitoes was performed. The PCR products were subjected to electrophoresis, isolated from the gel and sequenced.

Filarial DNA was detected in the head of a single mosquito from a sample collected in 2010. The NCBI BLAST analysis revealed that the nucleotide sequence of the PCR product was identical to a fragment of *Setaria tundra* COI gene. The DNA sequence amplified from the genomic DNA of the infected mosquito matched the ITS2 sequence of *Aedes vexans*.

The detection of *S. tundra* invasive larvae in mosquitoes proves that the parasite is present in Mazovia. It is the first description of the *Setaria tundra* vector in Poland.

The research was supported by NCN grant no. N N404 256840.



## **Real time PCR based assay for xenomonitoring of filarial parasites – detection of *Setaria tundra* and *Dirofilaria repens* in mosquitoes from central Poland**

**Aleksander Masny, Wioletta-Rozej Bielicka, Elżbieta Gołąb**

Department of Medical Parasitology, National Institute of Public Health–National Institute of Hygiene, Warsaw

Corresponding author: Aleksander Masny; e-mail: amasny@gmx.com

In recent years there has been a focus on human and animal dirofilariosis in Europe. During the last two decades, an expansion of *Dirofilaria repens* into the new territories has been described. In Poland, both animal and human *D. repens* dirofilariosis was shown to be autochthonous, however, little was known about the abundance of the parasites in the population of the mosquito vectors. Xenomonitoring of filaria in mosquitoes could be used to assess if the parasites were present in the local population of their hosts, eliminating the need for collecting vertebrate blood samples. The applicability of Real Time PCR-based xenomonitoring in the detection of *D. repens* in the mosquito populations inhabiting a region of Mazovia endemic for *D. repens* was evaluated.

Universal primers were designed for simultaneous detection of *D. repens* and *D. immitis* cytochrome oxidase subunit one gene fragments using Real Time PCR. Mosquitoes collected in July 2012 were examined for the presence of *Dirofilaria* spp. Species determination was conducted by High Resolution Melting and PCR product sequencing.

The results show that the designed xenomonitoring assay allows *D. repens* to be detected in mosquito populations. Furthermore, it was revealed that *Setaria tundra*, a parasite that has not yet been described in any of the vertebrate hosts inhabiting Poland, was present in the local ecosystem.

To conclude, Real Time PCR xenomonitoring of filaria in mosquitoes can be used for detection of the filarial parasites in ecosystems. Using the method, filarial species whose local vertebrate hosts are not known can be detected.

The research was supported by NCN grant no. N N404 256840.

## Ticks species (Ixodida) on dogs in Olsztyn city area

Mirosław M. Michalski, Rajmund Sokół

Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland

Corresponding author: Mirosław Michalski; e-mail: michmm@uwm.edu.pl

Ticks parasitizing on dogs belong to mites of order Ixodida, class Arachnida. In Poland the invasion of four species has been recorded most frequently: *Ixodes ricinus*, *I. hexagonus*, *I. crenulatus*, and *Dermacentor reticulatus*. Their particular importance is due to the fact that they are vectors of many dangerous diseases of humans and animals. There is also a phenomenon of diapause, describing two peaks of tick's activity – spring and autumn. In the veterinary practice the tick's species affiliation is hardly ever determined, hence the lack of full knowledge about the species and its percentage in the city area.

The object of the examination were ticks from dogs, patients of several veterinary clinics from the city of Olsztyn. Ticks were collected from May to June in four consecutive years. Mites removed from dogs skin were placed in 70% ethanol and then their species affiliation was determined according to their morphology, using the key to the species. Obtained results are shown in the Table.

The predominant species was *Ixodes ricinus* that accounted for 67.11% of all the collected ticks. In the years 2009 and 2010 adult ticks were predominant (58.06% and 40.61%, respectively) and in the years 2011 and 2012 larvae (nymphs) (51.18% and 48.23%, respectively). In case of *Dermacentor reticulatus* adult ticks were predominant in years 2009 and 2012 (20.5% and 17.26%, respectively).

	Total	<i>Ixodes ricinus</i>		<i>Dermacentor reticulatus</i>	
		nymphs	adults	nymphs	adults
2009	434	78 (17.97%)	252 (58.06%)	17 (3.92%)	87 (20.05%)
2010	394	48 (12.18%)	160 (40.61%)	141 (35.79%)	45 (11.42%)
2011	424	217 (51.18%)	81 (19.10%)	83 (19.58%)	43 (10.14%)
2012	481	232 (48.23%)	95 (19.75%)	71 (14.76%)	83 (17.26%)
	1733	575 (33.18%)	588 (33.93%)	312 (18%)	258 (14.89%)
		1163 (67.11%)		570 (32.89%)	

## Comparison of *Babesia canis* infection between eastern and western populations of *Dermacentor reticulatus* ticks

Ewa J. Mierzejewska, Maciej Kowalec, Anna Bajer

Department of Parasitology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland

Corresponding author: Ewa Mierzejewska; e-mail: e.mierzejewska@biol.uw.edu.pl

*Dermacentor reticulatus* ticks are the main vector of *Babesia canis* parasites in Poland. The distribution range in Europe covers the temperate climate zone and is divided in two populations. The western population spreads to the south of Great Britain, while the eastern population reaches the Yenisei River in Siberia. The territory between the Vistula River and western Poland is known as 'the gap' separating those populations. In recent years the range of marsh tick has expanded rapidly. The first cases of canine babesiosis in non-endemic regions are associated with the appearance of *D. reticulatus* in new areas. Although new foci of *D. reticulatus* have been recently recognized in different parts of Poland, there is still lack of data on the prevalence of infection with *B. canis*.

The purpose of our study was to compare the prevalence of *B. canis* infection among ticks from new foci in western and eastern areas of Poland depending on different time of colonization by *D. reticulatus*. Ticks from western population were collected with flagging method in spring 2012. *D. reticulatus* ticks from eastern population were collected in the autumn 2011 and 2012 in the oldest focus in the Masurian Lake District, and in spring 2009, autumn 2011 and 2012 in Mazovia near Tłuszcz, where marsh ticks have been found for several years. Ticks from the sites where the expansion of *D. reticulatus* has been observed in the last few years were collected on the western side of the Vistula River in Warsaw (Siekierki) in spring 2012, and in the vicinity of Żyrardów in spring 2011 and 2012. Prevalence of *B. canis* infections was determined using PCR. The highest prevalence of *B. canis* infection was found in ticks collected in the focus which has existed since year 2005 in the area of Tłuszcz (3.77–4.5%). In Urwiągł the prevalence is relatively lower (0.94–1.6%), and the lowest prevalence among ticks from the eastern population was recorded in Żyrardów (0.84%). No ticks from western Poland were positive for *B. canis* DNA.

Our study has shown significant differences in the prevalence of infections with *B. canis* between ticks from eastern and western populations. In addition, in the eastern populations there are noticeable differences depending on the time of ticks occurrence in the area. This indicates that new tick foci detected in the area may constitute a risk factor for canine babesiosis in the future.

This study was supported by the National Science Centre grant OPUS 2011/03/B/NZ802212.

## Co-infections involving TBE virus, *Babesia* and *Rickettsia* spp. in *Dermacentor reticulatus* ticks

Ewa J. Mierzejewska<sup>1</sup>, Agnieszka Pawelczyk<sup>2</sup>, Renata Welc-Falęciak<sup>1</sup>,  
Marek Radkowski<sup>2</sup>, Anna Bajer<sup>1</sup>

<sup>1</sup>Department of Parasitology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland

<sup>2</sup>Department of Immunopathology of Infectious and Parasitic Diseases, Medical University of Warsaw, Pawińskiego 3c, 02-106 Warsaw, Poland

Corresponding author: Ewa Mierzejewska; e-mail: e.mierzejewska@biol.uw.edu.pl

*Dermacentor reticulatus* is the second most abundant species of ticks in Poland. Although it does not feed on humans, it may take part in circulation and maintaining of pathogens dangerous to people in the environment. Marsh ticks may be involved in the transmission of tick-borne encephalitis virus (TBEV) and intracellular bacteria of the genus *Rickettsia* among variety of wildlife species. *Dermacentor reticulatus* is the main vector for *Babesia canis*, the protozoan causing canine babesiosis. The province of Mazovia is an endemic area either for TBE or canine babesiosis. Molecular detection of TBEV infections depends on RNA detection, as opposed to the detection of *Babesia* or *Rickettsia* based on DNA amplification. Therefore, not much research comprise the concurrent extraction and amplification of these different nucleic acids.

The purpose of our study was to determine the prevalence of concurrent infections with TBEV, *B. canis* and *Rickettsia* spp. in *D. reticulatus* ticks from TBE endemic region.

Ticks (n=163) were collected from fallow lands with flagging method in autumn 2012 in the area of Tłuszcz in Mazovia. RNA and DNA were extracted from the collected ticks using AllPrep DNA/RNA Mini Kit Qiagen, and stored at -80°C for further use. DNA of *B. canis* and *Rickettsia* spp. was detected using PCR. RNA of TBE virus was detected using RT-PCR. DNA and RNA amplicons from positive samples were purified and sequenced for further analysis.

High prevalence of *Rickettsia* infection was observed (40%), the prevalence of *B. canis* infection was 3.6%. The co-infections with these pathogens and TBEV were analyzed.

Our study confirmed the importance of *D. reticulatus* in circulation and maintaining of pathogens constituting health risk either to humans or animals in the environment.

This study was supported by the National Science Centre grant OPUS 2011/03/B/NZ802212.

## Ticks (Acari: Ixodida) and the threat of tick-borne diseases in the Bieszczady National Park and its buffer zone

Magdalena Nowak-Chmura<sup>1</sup>, Krzysztof Siuda<sup>1</sup>, Marek Asman<sup>2</sup>,  
Krzysztof Solarz<sup>2</sup>

<sup>1</sup>Department of Invertebrate Zoology and Parasitology, Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Cracow, Poland

<sup>2</sup>Department of Parasitology, Medical University of Silesia in Katowice, Jedności 8, 41-218 Sosnowiec, Poland

Corresponding author: Magdalena Nowak-Chmura; e-mail: mnowak@up.krakow.pl

The Bieszczady National Park is the third largest national park in Poland covering an area of 29.200 hectares, of which 63% is under strict protection. Together with the surrounding buffer zone it is perhaps the most attractive part of the Polish Bieszczady mountains. The Park covers a part of the Western Bieszczady Mountains, the only area of the Eastern Carpathians within Poland.

Until now, three tick species have been found in the Bieszczady National Park. Two of them are from the Ixodidae family: *Ixodes ricinus*, *Ixodes trianguliceps*, and one from the Amblyommidae family: *Dermacentor reticulatus*. Within the Bieszczady National Park and neighbouring areas the best studied distribution is that of the *I. ricinus* species. The studies were conducted using the flagging method by which active ticks waiting for hosts in grassy vegetation were collected. The occurrence of *I. ricinus* was found in large forested spaces throughout the Bieszczady National Park and its buffer zone. Thirty three sites were identified in places frequently used by tourists, such as mountain paths, nature trails, forest roads, peatlands, camp sites, and parking lots. The largest groupings of active ticks were noted in the Nasiczniański stream valley. Bukowska Pass was the highest place (1107 m a. s. l.) where ticks were found. *I. ricinus* was recorded many times on humans and livestock in these areas.

*I. trianguliceps* was found on the host *Myodes glareolus* on the south-eastern side of the foothill of Połonina Caryńska mountain. *D. reticulatus* was recorded on hosts *Lepus capensis* (location: Ustrzyki Górne) and *Canis familiaris* (location: Stare Sioło).

Epidemiological studies have demonstrated that *I. ricinus* ticks in the Bieszczady National Park are vectors of transmissible diseases pathogens such as: *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *Babesia* sp. Wide spectrum of hosts occurring in the Bieszczady National Park and numerous cases of ticks attacking humans indicate that it is an area of high risk of exposure to ticks and contracting tick-borne diseases. All this supports the necessity to carry out broadened faunistic studies on the occurrence of natural habitats of ticks in order to identify the hot spots from which transmissible diseases spread, with particular attention paid to *I. ricinus* as a potential vector.

## Molecular characteristic of *Babesia* isolated from dogs in Mazovia province

**Wioletta Rożej-Bielicka<sup>1</sup>, Danuta Cielecka<sup>1</sup>, Rafał Stachurski<sup>2</sup>,  
Katarzyna Stachurska<sup>2</sup>, Mariusz Piechocki<sup>3</sup>, Elżbieta Gołąb<sup>1</sup>**

<sup>1</sup>Department of Medical Parasitology, National Institute of Public Health–National Institute of Hygiene, Warsaw, Poland

<sup>2</sup>Antecedo Veterinary Practice, Książenice, Poland

<sup>3</sup>Veterinary Diagnostic Laboratory Haema-lab Express, Milanówek, Poland

Corresponding author: Wioletta Rożej-Bielicka; email: wrozej@pzh.gov.pl

Infections with *Babesia* in dogs are common in Mazovia. Babesiosis may cause anaemia, haemoglobinuria, hepato- and splenomegaly, and if untreated, can lead to death of the animal. The disease is diagnosed based on microscopic examination of blood smears stained by May-Grünwald or Giemsa.

The aim of the study was molecular characteristics of *Babesia* isolates derived from blood samples of dogs from the Mazovia province. Samples, including 53 obtained from dogs with clinically diagnosed babesiosis and 130 previously tested for *Dirofilaria* infection, were collected between February 2012 and May 2013. Average age of the examined dogs was 5.4 years (2 months to 16 years). Most blood samples were obtained from animals kept at home (84.6%). Tests were performed using PCR. DNA fragment, 539 bp long, of the 18S rRNA gene sequence encoding the small ribosomal subunit of *Babesia* spp. was amplified using BAB-GF2/BAB-GR2 primer pair. The results of amplification were recorded after electrophoresis in 1.5% agarose gel stained with ethidium bromide and visualization in UV light. The PCR products were sequenced and the obtained sequences were compared with those deposited in GenBank database.

*Babesia* DNA was detected in blood samples of 79 dogs. Co-invasion of *Babesia* and *Dirofilaria* was found in two dogs. The examined PCR products were identified as *Babesia canis canis* gene fragments. The analysis of DNA sequence from the obtained isolates revealed presence of polymorphisms at positions 559 and 560 of the 18S rRNA gene encoding the small ribosomal subunit of *Babesia*. The study confirmed the presence of *B. canis canis* infection in dogs in the Mazovia province and showed genetic diversity of isolates within the *B. canis canis* species.

## Molecular screening for asymptomatic *Babesia* infection among people at risk of tick-borne diseases in Poland

Wioletta Rozej-Bielicka<sup>1</sup>, Aleksander Masny<sup>1</sup>, Małgorzata Sadkowska-Todys<sup>2</sup>, Anna Bauman-Popczyk<sup>2</sup>, Elżbieta Gołąb<sup>1</sup>

<sup>1</sup>Department of Medical Parasitology, <sup>2</sup>Department of Epidemiology; National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland

Corresponding author: Wioletta Rozej-Bielicka; email: wrozej@pzh.gov.pl

Protozoan *Babesia* are parasites of red blood cells of humans and other vertebrates. Human infection is the result of a bite by an infected tick or a transfusion of infected blood. Babesiosis can be life-threatening for immunosuppressed persons. Since diagnostics of the disease is usually based on microscopic examinations, low intensity infections frequently remain undetected. Little is known about human *Babesia* infection in Poland. The aim of the study was to evaluate, using molecular techniques, the frequency of asymptomatic *Babesia* infection cases among people at-risk of tick-borne diseases.

We examined blood samples of 487 hunters coming from different parts of Poland, aged 21–81 years, mainly men (97% of the examined persons). A 539 bp length fragment of 18 S rDNA *Babesia* spp. gene was amplified using PCR method.

*Babesia* DNA was found in the blood sample of a 22 year old man from the Warmia-Masuria province. The sequence of the isolate matched the sequences of *Babesia* EU1 (new species *B. venatorum*) deposited in GenBank. Among the examined hunters 0.21% were *Babesia*-positive.

The discovery of an asymptomatic *Babesia* infection points to the necessity of an epidemiological survey for babesiosis in the Warmia-Masuria province since the disease constitutes a serious threat to haemotherapy.

## Seasonal abundance of mosquito larvae (Diptera: Culicidae) in stormwater catch basins in the center of Wrocław (Poland)

Katarzyna Rydzanicz<sup>1</sup>, Piotr Jawień<sup>1</sup>, Monika Kaliwoda<sup>2</sup>, Elżbieta Lonc<sup>1</sup>

<sup>1</sup>Department of Microbial Ecology and Environmental Protection, Institute of Genetics and Microbiology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław

<sup>2</sup>Environmental Protection and Agriculture Division, Municipality of Wrocław, Bogusławskiego 8/10, 50-032 Wrocław; Poland

Corresponding author: Katarzyna Rydzanicz; e-mail: katarzyna.rydzanicz@microb.uni.wroc.pl

Stormwater catch basins and underground storm drain systems in urban areas are identified as key oviposition and larval development sites for mosquitoes. Of special interest group is the *Culex pipiens* s. l. complex that consists of several species, subspecies, forms, races, physiological variants or biotypes according to various authors. The polycyclic females of this species complex diapause in wintertime and produce non desiccation-resistant eggs laid directly on water with high organic content, while the larvae thrive in brackish and polluted habitats. The ornitophilic members of *Cx. pipiens* s. l. are the most common vector species for West Nile virus both in Europe and the USA. The newly established in European countries Asian species such as *Ochlerotatus japonicus japonicus* (Theobald) also develops in catch basins.

This study examines the species composition as well as the effects of abiotic factors on catch basin larval production in the centre of Wrocław (the Market Square and the Cathedral Island), and thus on mosquito nuisance for tourists and residents. The basins were sampled for larvae from 18 May to 30 September 2012. Larvae and pupae were counted and collected in each of 3 dips. All existing and potential breeding habitats in the study area were recorded using a GPS receiver (Magellan Mobile Mapper CX) and transferred to the database. A sufficient number of larvae were taken to the laboratory for rearing to the 4<sup>th</sup> instar or adult stages and identified to species or genus (*Culex* spp., mainly *Cx. pipiens* and *Cx. torrentium*).

In total, 969 larval samples were collected from geocoded 1160 catch basins throughout the season. The results show that mosquitoes breeding in the catch basins in Wrocław during this study consisted principally of the species complex *Culex pipiens* s.l.. The abundance of mosquito larvae in catch basins varied between months. The highest mosquito abundance was observed in July (n=478) and the lowest in May (n=4). Environmental factors that may support the development of mosquitoes within catch basins in the city centre of Wrocław are low precipitation, the presence of organic pollutants and high mean daily temperature.

In conclusion, our data suggest that storm water catch basins are an important source of *Culex* spp. and should be strongly considered in the local mosquito control strategy.

This work was supported by Municipality of Wrocław as part of the Wrocław's Mosquito Control Program.



## Detection of *Setaria tundra* microfilariae in mosquito populations from irrigated fields in Wrocław (Poland)

Katarzyna Rydzanicz<sup>1</sup>, Elżbieta Lonc<sup>1</sup>, Elżbieta Gołąb<sup>2</sup>, Aleksander Masny<sup>2</sup>

<sup>1</sup>Department of Microbial Ecology and Environmental Protection, Institute of Genetics and Microbiology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

<sup>2</sup>National Institute of Public Health–National Institute of Hygiene, Chocimska 24, 00-791 Warsaw, Poland

Corresponding author: Katarzyna Rydzanicz; e-mail: katarzyna.rydzanicz@microb.uni.wroc.pl

In general, filarioses are vector-borne infections transmitted by haematophagous arthropods, such as mosquitoes (Culicidae) or arachnids, such as mites (Acari). It can be assumed from studies in other European countries that several *Onchocerca* spp. are transmitted by blackflies and biting midges, the *Dirofilaria* species by various mosquitoes, and *Eufilaria* spp. by biting midges. *Aedes* spp. mosquitoes possibly serve as the most important and competent vectors for *S. tundra* in Finland (*Ae. communis*, *Ae. punctor*, *Ae. hexodontus* and *Ae. excrucians*) and in Germany (*Ae. vexans* and *Oc. sticticus*). The veterinary importance of *S. tundra* is due to a reported outbreak of peritonitis with significant economic losses in Finnish semi-domestic reindeer in the years 2003–2005. Similar outbreaks may occur in any other wild or semi-domesticated cervid population outside Finland, e.g. in zoos. Warm summers apparently improve transmission and genesis of disease outbreaks by favouring the development of *S. tundra* in its mosquito vectors.

In present study all trapping places were situated in irrigated fields located in the northeastern part of Wrocław. This area was constructed in 1890 in the Oder River Valley to provide wastewater treatment before disposal into river system. Floodwater mosquitoes, mainly *Ochlerotatus caspius* (Pallas) and *Ae. vexans* (Meigen), emerge from these fields in huge numbers every summer when there is intermittent flooding with wastewater entering infiltration fields, while canals/sewers are places of development for the larvae of species belonging to the *Culex pipiens* complex (mainly *Cx. pipiens* and *Cx. torrentium*). The total of 1950 mosquito females were collected in August and September 2012 with CO<sub>2</sub>-baited EVS (encephalitis vector survey) traps. The insects were divided and merged into pools for DNA isolation. The heads of 120 mosquitoes were analyzed separately from the rest of the body. The mitochondrial partial COI gene for cytochrome oxidase (subunit I) was used for simultaneous detection of *D. repens*, *D. immitis* and *S. tundra*. Positive results were obtained for DNA isolated from two pools of *Ae. vexans*. No filarial DNA was detected in the DNA isolated from the mosquito heads. The PCR product was sequenced and the NCBI BLAST analysis revealed that the obtained sequence was identical to a fragment of *S. tundra* COI gene. Our results confirm that *S. tundra* was present in the investigated mosquito populations from the genus *Aedes*.

## Molecular detection of *Rickettsia* spp. in *Ixodes ricinus* and *Dermacentor reticulatus* ticks from Poland

Renata Welc-Falęciak<sup>1</sup>, Anna Bajer<sup>1</sup>, Ewa Mierzejewska<sup>1</sup>, Maciej Kowalec<sup>1</sup>, Grzegorz Karbowski<sup>2</sup>, Edward Siński<sup>1</sup>

<sup>1</sup>Department of Parasitology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw,

<sup>2</sup>W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Warsaw; Poland

Corresponding author: Renata Welc-Falęciak; e-mail: rwelc@biol.uw.edu.pl

In Europe, *Ixodes ricinus* is the most prevalent and widely distributed tick species and serves as the most important vector for several microbial pathogens, i.e. *B. burgdorferi*, *Babesia* spp. and *Rickettsia* spp. *Dermacentor reticulatus* tick plays also an important role in the transmission of many animal tick-borne diseases (TBD), e.g. canine or cattle babesiosis, and may constitute an important reservoir and vector of human TBDs, including *Rickettsia* spp. Additionally, this tick species is not distributed throughout Poland and Europe. Tick-borne rickettsiosis is caused by intracellular bacteria belonging to the spotted fever group (SFG) of Rickettsiaceae family. At least half of about 30 known *Rickettsia* species have been identified so far as pathogenic for humans. Ticks are believed to act as vectors and reservoir of SFG group *Rickettsia*. These bacteria seem to be one of the most common pathogens found in *D. reticulatus* and *I. ricinus* ticks in Europe. Therefore, the impact of *Rickettsia* is likely to increase in the nearest future.

The aims of our study were: (i) to estimate the prevalence of *Rickettsia* spp. in two tick species collected from vegetation and the warm-blooded hosts, (ii) to evaluate the genetic diversity of *Rickettsia* isolates, (iii) to compare the prevalence and genetic diversity of *Rickettsia* spp. in two populations (Western and Eastern) of *D. reticulatus*, (iv) to compare the prevalence and genetic diversity of *Rickettsia* spp. in *I. ricinus* from two different habitats (natural and rural) in Central and Northeastern Poland.

The results of our study revealed a high prevalence of *Rickettsia* spp. in *D. reticulatus* ticks (40%) and relatively low rate of infections in *I. ricinus* ticks (5%). Our molecular studies confirmed the presence of pathogenic species/ strain of *Rickettsia* in tick populations in Poland.

This study was supported in part by the Ministry of Science and Higher Education, Grant NN404 795240 and the National Science Centre (NCN) grant OPUS 2011/03/B/NZ802212.