Detection of selected pathogens in ticks collected from cats and dogs in the Wrocław Agglomeration, South-West Poland

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Abstract

Background: Tick-borne infections are no longer confined to rural areas, they are documented with increasing frequency in urban settlements across the world. They are known to cause diseases in humans as well as in their companion animals.

Methods: During a period of 2 years, from January 2013 until December 2014, ticks were collected from dogs and cats in 18 veterinary clinics in the Wrocław Agglomeration, Poland. In total, 1455 ticks were found on 931 pets: 760 domestic dogs and 171 cats. For molecular examinations 127 I. ricinus ticks (115 females and 12 males) were randomly selected, all collected I. hexagonus (n = 137, 32 females, 98 nymphs, 7 larvae) and all collected D. reticulatus (n = 46, 31 females, 15 males) were taken. Ixodes ricinus and I. hexagonus ticks were tested for Rickettsia spp., Anaplasma phagocytophilum, Candidatus Neoehrlichia mikurensis and Babesia spp., while D. reticulatus ticks were investigated for Rickettsia spp. and Babesia spp. only.

Results: In total, 65.4 % I. ricinus ticks were infected with at least one pathogen. Over 50 % of I. ricinus were positive for Rickettsia spp. (R. helvetica and R. monacensis). The infection level with A. phagocytophilum was 21.3 %. DNA of Cand. N. mikurensis was detected in 8.1 % I. ricinus ticks. Interestingly only female ticks were infected. The prevalence of Babesia spp. was confirmed in 9.0 % of I. ricinus involving the species B. microti and B. venatorum. A total of nineteen double, one triple and two quadruple infections were found in I. ricinus ticks only. Almost 11 % of I. hexagonus ticks were positive for at least one of the tested pathogens. Rickettsia spp. infection was found in 2.2 %, while A. phagocytophilum was detected in 8.1 % of I. hexagonus ticks. Only one nymph was positive for Cand. N. mikurensis and none of I. hexagonus ticks harbored a Babesia spp. Over 60 % of D. reticulatus ticks were positive for rickettsial DNA, exclusively belonging to the species R. raoultii.

Conclusion: The high tick infestation rates and the prevalence of pathogens found in these ticks demonstrate a serious level of encounter to tick-borne diseases in urban dogs in the Wrocław area, and provide evidence that dogs and cats themselves may substantially contribute to the circulation of the ticks and pathogens in the urban area.

Keywords: Rickettsia spp., Anaplasma phagocytophilum, Candidatus Neoehrlichia mikurensis, Babesia spp., Ixodes ricinus, Ixodes hexagonus, Dermacentor reticulatus, Ticks, Dogs, Cats
Background
Tick-borne pathogens, such as Anaplasma phagocytophilum, Candidatus Neoehrlichia mikurensis and Rickettsia spp., belong to the order Rickettsiales, while such as Babesia spp. are parasitic protozoans. All these pathogens, are known to cause diseases in humans as well as in their companion animals, and are considered to be emerging across Europe and other parts of the world [1–6].

Rickettsia spp. are divided into four groups: the spotted fever group (SFG), the typhus group, the Rickettsia bellii group, and the Rickettsia canadensis group [7]. Rickettsiae of the SFG are known to be transmitted by ticks and cause DEBONEL (Dermacentor-borne necrosis erythema lymphadenopathy), also known as TIBOLA (tick-borne lymphadenopathy) in humans [8]. In Poland, rickettsioses caused by rickettsiae of the SFG were described in forest workers, dogs and ticks [9–11].

Anaplasma phagocytophilum and Candidatus Neoehrlichia mikurensis are gram-negative obligate intracellular bacteria. Anaplasma phagocytophilum may cause granulocytic anaplasmosis in humans, dogs, horses and ruminants [4]. Anaplasmosis has been reported in dogs, cats and humans from Poland with evidence of autochthonous human cases [12–15]. Neoehrlichiosis was described in immunodeficient and previously healthy humans but also in immunodeficient dogs [1, 3, 16]. The presence of Cand. Neoehrlichia mikurensis has been proven in Ixodes ricinus ticks and in asymptomatic humans from Poland [17, 18].

Babesiosis is a zoonotic disease occurring worldwide which is caused by intraerythrocytic parasites of the genus Babesia [2]. In Europe, Babesia divergens-like organisms are mainly responsible for the disease in humans. Babesia spp. are reported in Ixodes ricinus and Dermacentor reticulatus from Poland [19]. Nowadays, cases caused by tick-borne pathogens are emerging in urban regions in Europe [20, 21]. Dogs and cats should be taken into account as important hosts of ticks in urban areas [21, 22].

In Poland, 5 of the 19 detected tick species (I. ricinus Linnaeus, 1758, I. hexagonus Leach, 1815, I. crenulatus Koch, 1844, I. rugicollis Schulze et Schottke, 1929, and D. reticulatus Fabricius, 1794) parasitize on cats and dogs [23]. Most commonly, however, I. ricinus and D. reticulatus, the important vectors of Rickettsia spp., Anaplasma phagocytophilum and Babesia spp., are detected on pets [21, 24–28]. In a previous study, ticks collected from cats and dogs from the Wroclaw Agglomeration, SW Poland, were tested for the presence of Borrelia spp. [29].

The aim of this follow-up study was to evaluate the prevalence of Babesia spp., A. phagocytophilum, Cand. Neoehrlichia mikurensis and Rickettsia spp. in ticks collected from cats and dogs in the Wroclaw Agglomeration, Poland.

Methods
Tick collection
During a period of 2 years, from January 2013 till December 2014, ticks were collected from dogs and cats in the veterinary clinics in the Wroclaw Agglomeration, Poland. Wroclaw city (c292.8 km²) is located in the south-west of Poland (51°07’N, 17°02’E). Tick collection from 2013 [29] was extended with specimens collected in the next year. In total, 18 veterinary clinics submitted 1455 ticks found on 931 pets: 760 domestic dogs and 171 cats (Table 1). Tick specimens were determined by life stage, sex and species [30]. Tick collection consisted of: 46 D. reticulatus ticks (31 females, 15 males), 137 I. hexagonus (32 females, 98 nymphs, 7 larvae), and 1272 I. ricinus (1160 females, 103 males, 9 nymphs).

DNA isolation and biological material
All collected ticks were kept in 70 % ethanol until isolation of DNA was performed. Before DNA extraction, ticks were washed in sterile water. All ticks were individually homogenized using sterile polystyrene pistils and then genomic DNA was extracted by using a Tissue Genomic Extraction GPB Mini Kit with proteinase K (Genoplast Biochemicals, Poland) according to the manufacturer’s instructions. All of the obtained lysates were stored at -20 °C until examined.

For further examinations 310 ticks were selected: 127 randomly chosen I. ricinus ticks (115 females and 12 males), all collected I. hexagonus ticks (n = 137; 32 females, 98 nymphs, 7 larvae) and D. reticulatus ticks (n = 46; 31 females, 15 males) (Table 2). Ixodes ricinus and I. hexagonus ticks were tested for Rickettsia spp., Anaplasma phagocytophilum, Candidatus Neoehrlichia mikurensis and Babesia spp., while D. reticulatus ticks were only investigated for Rickettsia spp. and Babesia spp.

Molecular detection of Rickettsia spp., Anaplasma phagocytophilum, Candidatus Neoehrlichia mikurensis and Babesia spp.

For detection of Rickettsia spp., a real-time PCR targeting the gltA genome region (70 bp) was used [31]. A real-time PCR targeting msp2 gene fragment (77 bp) was performed to detect A. phagocytophilum [32]. In order

Table 1 Ticks collected from pets in the Wroclaw Agglomeration (Poland), 2013-2014

| Species               | Number of ticks / number of hosts |
|-----------------------|-----------------------------------|
|                       | Cats     | Dogs     | Total    |
| Dermacentor reticulatus | 2 / 2   | 44 / 34  | 46 / 36  |
| Ixodes hexagonus       | 53 / 7   | 84 / 37  | 137 / 44 |
| Ixodes ricinus         | 267 / 162 | 1005 / 689 | 1272 / 851 |
| Total                 | 322 / 171 | 1133 / 760 | 1455 / 931 |
to detect Candidatus Neoehrlichia mikurensis, a real-
time PCR targeting the partial groEL gene (99 bp) was
used [33, 34]. All PCR methods were carried out using
the Mx3000P real-time cycler (Stratagene).

For detection of Babesia spp., a conventional PCR
amplification of the small 18S subunit of the rRNA gene
(411–452 bp) with primers BJ1 and BN2 was performed
[35]. Samples positive for Rickettsia spp. DNA by real-
time PCR were further investigated using a conventional
PCR in which a 811-bp fragment of the ompB gene was
amplified [36]. The PCR products were visualized by electrophoresis on 1.5 % agarose gels stained with Mid-
ori Green (NIPPON Genetics, Düren, Germany). Ran-
donally selected positive PCR products (n = 22) were
purified using the NucleoSpin® and PCR Clean-up Kit
(MACHEREY-NAGEL, Düren, Germany) according to
the manufacturer’s instructions. Purified PCR products
were sequenced (Interdisziplinäres Zentrum für Kli-
nische Forschung, Leipzig, Germany) with forward and
reverse primers, and analyzed with Chromas Lite (Tech-
neiyum Pty Ltd, Australia). Nucleotide sequences were
obtained by RT-PCR (CT > 35). The prevalence of A.
phagocytophilum was detected in 14.4 % (n = 37) of
Ixodes species. Further, 21.3 % of I. ricinus, and 8.1 %
of I. hexagonus were positive for this pathogen. The infec-
tion level was statistically higher in I. ricinus ticks than I.
hexagonus (χ² = 8.599, df = 1, P = 0.003). Candidatus N.
mikurensis was found in 4.2 % of Ixodes samples. It was
detected in 8.1 % of I. ricinus and 0.7 % of I. hexagonus
(the difference being statistically significant, χ² = 8.599,
df = 1, P = 0.003). The prevalence of Babesia spp. was
the lowest among the tested pathogens, 3.6 % (n = 11)
for all tick species, but only I. ricinus ticks (9.0 %) were
infected (χ² = 16.934, df = 2, P < 0.001). Babesia microti
was detected in 83.3 % (all samples with identity over
96 % to acc. no. JQ711225.1), and B. venatorum in 16.7 %
(identical with 99 % to acc. no. KR493907.1 and
98 % to KF500410.1) of sequenced I. ricinus samples.

Ixodes ricinus ticks were more often infected, with
minimum one pathogen, than I. hexagonus or D. reticu-
latus (χ² = 90.019, df = 2, P < 0.001). In total, 65.4 % (n =
83) I. ricinus ticks were positive for at least a single
infection. The most often detected pathogen was Rickettsia
spp. (χ² = 84.505, df = 3, P < 0.0001), in 50.4 % of I. rici-
nus (n = 64; Table 3). There were no significant differ-
ces in infection levels between females and males (χ²
= 0.404, df = 1, P = 0.525) nor ticks collected from cats or
dogs (χ² = 0.694, df = 1, P = 0.405). Anaplasma phagocy-
tophilum was verified in 21.3 % specimens (n = 26, no
statistically significant differences were observed be-
tween females and males, χ² = 0.259, df = 1, P = 0.611, or
between ticks parasitizing cats or dogs, χ² = 0.002, df = 1,
P = 0.964). Candidatus N. mikurensis was detected in
81 % I. ricinus (n = 10), only females were infected (χ²
= 0.202, df = 1, P = 0.653); infection level of ticks collected
from pets was not statistically significant (χ² = 0.097, df
= 1, P = 0.755). The prevalence of Babesia spp. was con-
firmed in 9.0 % of specimens (n = 11); there were neither
significant differences between females and males (χ²
= 0.214, df = 1, P = 0.644) nor ticks infesting cats or dogs
(χ² = 3.086, df = 1, P = 0.079).

In total, 10.9 % (n = 15) I. hexagonus ticks were posi-
tive for at least one of the tested pathogens. Anaplasma
phagocytophilum was the most common infection in

### Table 2 Ticks investigated for pathogens, the Wroclaw Agglomeration (Poland), 2013–2014

| Pathogens     | Number of tick stages | Females | Males | Nymphs | Larvae | Total |
|---------------|-----------------------|---------|-------|--------|--------|-------|
|               |                       | Total   | Cats   | Dogs   | Total  | Cats  | Dogs  | Total  |
| D. reticulatus|                       | 31      | 2      | 29     | 15     | 15    |       | 46     |
| I. hexagonus  |                       | 32      | 5      | 27     | 98     | 43    | 55    | 137    |
| I. ricinus    |                       | 115     | 34     | 81     | 12     | 4     | 8     | 127    |
| Total         |                       | 178     | 41     | 137    | 27     | 4     | 23    | 310    |
### Table 3

Ticks collected from dogs and cats infected with pathogens, the Wroclaw Agglomeration (Poland), 2013-2014

| Pathogens          | Number of infected ticks/number of investigated ticks (%) |
|--------------------|----------------------------------------------------------|
|                    | L. ricinus | L. hexagonus | D. reticulatus | TOTAL |
|                    | F   | M   | T   | F   | N   | L   | T   | F   | M   | T   | F   | M   | T   |
| Rickettsia spp.    | 59/115 (51.3) | 5/12 (41.7) | 64/127 (50.4) | 2/32 (6.3) | 1/98 (1.0) | 0/7 (0.0) | 3/137 (2.2) | 19/31 (61.3) | 9/15 (60.0) | 28/46 (60.9) | 95/310 (30.6) |
| A. phagocytophilum  | 25/112 (22.3) | 1/10 (10.0) | 26/122 (21.3) | 2/31 (6.4) | 8/97 (8.2) | 1/7 (14.3) | 11/135 (8.1) | – | – | – | 37/257 (14.4) |
| Cand. N. mikurensis| 10/113 (8.8) | 0/11 (0.0) | 10/124 (8.1) | 0/32 (0.0) | 1/97 (1.0) | 0/7 (0.0) | 1/136 (0.7) | – | – | – | 11/260 (4.2) |
| Babesia spp.       | 10/112 (8.9) | 1/10 (10.0) | 11/122 (9.0) | 0/31 (0.0) | 0/97 (0.0) | 0/7 (0.0) | 0/135 (0.0) | 0/31 (0.0) | 0/15 (0.0) | 0/46 (0.0) | 11/303 (3.6) |

*Abbreviations: F females, M males, N nymphs, L larvae, T total*
these ticks ($\chi^2 = 20.661, df = 3, P < 0.001$), it was detected in 8.1% of ticks ($n = 11$); no statistically significant differences were observed between life stages ($\chi^2 = 0.473, df = 2, P = 0.789$) or for ticks parasitizing cats or dogs ($\chi^2 = 1.373, df = 1, P = 0.241$). *Rickettsia* spp. infection was found in 2.2% ticks ($n = 3$), there were no statistically significant differences between life stages, ($\chi^2 = 3.245, df = 2, P = 0.197$) or for ticks from different hosts ($\chi^2 = 0.166, df = 1, P = 0.684$). Only one of the *I. hexagonus* ticks was found to be infected by *Babesia* spp.

Among *D. reticulatus* only *Rickettsia* spp. was detected; 60.9% of ticks were positive ($n = 28$). Statistically significant differences were not detected in infection levels between males and females ($\chi^2 = 0.007, df = 1, P = 0.933$) or ticks from cats or dogs ($\chi^2 = 0.175, df = 1, P = 0.676$). *Babesia* spp. DNA was not amplified in any of the *D. reticulatus* sample.

Co-infections were detected only in *I. ricinus* ticks, mainly females. Only one male tick had a double-infection with *Rickettsia* spp. and *Anaplasma phagocytophilum*. The most common pathogen combination was *Rickettsia* spp. *+ A. phagocytophilum*, followed by *Rickettsia* spp. *+ Candidatus Neoehrlichia mikurensis*, *Rickettsia* spp. *+ Babesia* spp. and one of *Candidatus Neoehrlichia mikurensis* *+ Babesia* spp. (Table 4). Apart from these, two quadruple-infections and one triple-infection (*Rickettsia* spp., *Candidatus Neoehrlichia mikurensis*, *A. phagocytophilum*).

**Discussion**

From three ticks species identified as parasites of dogs and cats in the Wroclaw Agglomeration, *Ixodes hexagonus* was predominant, followed by *I. hexagonus* and *D. reticulatus*. Similar findings were obtained in Belgium [37], Switzerland [38], Germany [39] and Great Britain [40], as well as in Bosnia and Herzegovina [41].

The prevalence of pathogens differed between the tick species. *Ixodes ricinus* individuals were the most often infected species. The lowest infection levels were observed in *I. hexagonus* ticks. From all tested pathogens (*Rickettsia* spp., *Babesia* spp., *Candidatus Neoehrlichia mikurensis* and *Anaplasma phagocytophilum*), rickettsial infections were the most common. The infection levels of *A. phagocytophilum*, *Candidatus Neoehrlichia mikurensis* and *Babesia* spp. (*B. microti, B. venatorum*) were the highest in *I. ricinus* ticks and co-infections were detected only in this species. Interestingly, *Babesia* spp. DNA was only found in *I. ricinus* ticks, none of the *D. reticulatus* ticks and *I. hexagonus* ticks were infected by *Babesia* spp.

| Co-infections                                      | No. of ticks (collected from cats/dogs) |
|---------------------------------------------------|-----------------------------------------|
| *Rickettsia* spp. + *Babesia* spp.                | 3 (1/2)                                 |
| *Rickettsia* spp. + *Candidatus Neoehrlichia mikurensis* | 4 (1/3)                                |
| *Rickettsia* spp. + *A. phagocytophilum*          | 11 (3/8)                                |
| *Candidatus Neoehrlichia mikurensis + Babesia spp.* | 2 (2/0)                                 |
| *Rickettsia* spp. + *Candidatus Neoehrlichia mikurensis + A. phagocytophilum* | 1 (0/1)                                |
| *Rickettsia* spp. + *Candidatus Neoehrlichia mikurensis + A. phagocytophilum + Babesia spp.* | 2 (1/1)                                |

The prevalence of *Babesia* spp. (*B. microti and B. venatorum*) in *I. ricinus* was higher than in Belgium [54] and Germany [45], where the infection was also detected in *I. hexagonus* ticks. In Poland, also in Lower Silesia, only 1–3% of questing *I. ricinus* ticks were infected [52,
Similar to our results, all tested *D. reticulatus* in Germany were free of babesial parasites [45]. However, 11% of *D. reticulatus* ticks infesting dogs in central Poland were infected [51]; in Austria 2 of 6 specimens and in Hungary almost 30% of *D. reticulatus* (only female ticks) were positive for *B. canis* [56, 57].

As results of this study show, the risk of tick-borne diseases (TBD) is high in the Wrocław Agglomeration. However, due to the limitation of this study (no blood samples of dogs and cats were investigated for the pathogens), the tick-borne situation among pets in this area is not fully estimated. In Poland, canine tick-borne diseases pose an emerging veterinary problem. The most common TBD among dogs are *B. canis* and *A. phagocytophilum* reaching levels of 28 and 12%, respectively [58, 59]. Apart from the above, dogs were infected with *Borrelia burgdorferi* (s.l.), and *Ehrlichia canis*.

**Conclusion**

The high infection levels were detected for *Rickettsia* spp. (*R. raoelli*, *R. helvetica* and *R. monacensis*), *Anaplasma phagocytophilum*, *Candidatus Neoehrlichia mikurensis* and *Babesia* spp. (*B. microti* and *B. venatorum*) in ticks infesting dogs and cats in the Wrocław Agglomeration, Poland. These findings, as well as the high tick infestation rates, demonstrate a serious level of encounter to tick-borne diseases in urban dogs and cats in the Wrocław area, and provide evidence that dogs and cats themselves may substantially contribute to the circulation of the ticks and the pathogens in the urban area.

**Abbreviations**

DEBONEL, dermacentor-borne necrosis erythema lymphadenopathy; SFG, spotted fever group; TBD, tick-borne disease; TIBOLA, tick-borne lymphadenopathy

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**Authors’ contributions**

NK organized the collection of samples, carried out the morphologic determination of ticks, and prepared the samples in the laboratory. MP, AO and NK designed, planned and organized the study. NK and AO tested the samples for pathogens, performed the sequence analysis. NK conducted data analysis. NK, AO, MP, DK and EL drafted the manuscript and wrote the final version. MP, DK and EL contributed to acquire funding. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

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**References**

1. Dzin PP, Schulz BS, Hartmann K, Breitschwerdt EB. “Candidatus Neoehrlichia mikurensis” infection in a dog from Germany. J Clin Microbiol. 2011;49: 2059–62.
2. Homer MJ, Aguilar-Delfin I, Telford 3rd SR, Krause PJ, Persing DH. Babesiosis. Clin Microbiol Rev. 2000;13(3):451–69.
3. Li H, Jiang JF, Liu W, Zheng YC, Huo QB, Tang K, Zuo SY, Liu K, Jiang BG, Yang H, Cao WC. Human infection with *Candidatus Neoehrlichia mikurensis*. China Emerg Infect Dis. 2012;18:1636–9.
4. Woldehivzet Z. *The natural history of Anaplasma phagocytophilum*. Vet Parasitol. 2010;167(2-4):108–22.
5. Oteo JA, Portillo A. Tick-borne rickettsioses in Europe. Ticks Tick Borne Dis. 2012;3:271–8.
6. Sollano-Gallego L, Kidd L, Trotta A, Gellin B, Maltais M, Furlanello T, Breitschwerdt E. *Ehrlichia* illness associated with *Rickettsia conorii* infection in dogs from Sicily. Emerg Infect Dis. 2006;12:1985–8.
7. Parola P, Paddock CD, Socolovschi C, Labruna MB, Medrannikov O, Kernif T, Abdal AM, Stenos J, Bitam I, Fournier P, Raoult D. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. 2013;26(4):657–702.
8. Ibarra V, Oteo JA, Portillo A, Sanz-Bianes S, Blanco JR, Motela L, et al. *Rickettsia slovaca* infection: DEBONEL/TIBOLA. Ann N Y Acad Sci. 2006;1078:206–14.
9. Podsiadly E, Chmielewski T, Kaborwii G, Kręda E, Tylewska-Wierzbanowska S. The occurrence of spotted fever rickettsioses and other tick-borne infections in forest workers in Poland. Vector Borne Zoonotic Dis. 2011;11:985–9.
10. Rymaszewska A, Adamska M. Molecular evidence of vector-borne pathogens coinfecting dogs from Poland. Acta Vet Hung. 2011;59:215–23.
11. Starczak J. Detection of spotted fever group (SFG) rickettsiae in *Dermacentor reticulatus* (Acari: Ixodidae) in Poland. Int J Med Microbiol. 2006;296 Suppl 40:144–8.
12. Grzeszczuk A, Stanczak J, Kubica-Biernat B, Racewicz M, Kruminis-Lozowska Bejnokova J, K, Cikol, G: *Ehrlichia canis* in dogs from Northwestern Poland. Acta Vet Brno. 2004;73:347–353.
13. Welc-Falciak R, Kowalec M, Zajkowska J, Pancewicz SA, Sirlski E. Clinical and molecular features of one case of human infection with *Anaplasma phagocytophilum* from Podlaskie Province in eastern Poland. Ann Agric Environ Med. 2015;22(3):414–7.
14. Skotarczak B, Adamska M, Supron M. Blood DNA analysis for *Ehrlichia* (Anaplasma) phagocytophilum and *Babesia* spp. in dogs from Northern Poland. Acta Vet Bmo. 2004;73:347–51.
15. Gorna A, Adaszek M, Kowalec M, Wierczyk A. Detection of *Anaplasma phagocytophilum* in a cat. Vet Med. 2013;58:39–43.
16. Wellinder-Olsson C, Kjellin E, Vaht K, Jacobsson S, Wennners C, E. T. a. C. *Candidatus Neoehrlichia mikurensis* infection in a febrile patient with chronic lymphocytic leukemia. J Clin Microbiol. 2010;48(5):1956–9.
17. Welc-Falciak R, Sirlski E, Kowalec M, Zajkowska J, Pancewicz SA. Asymptomatic "*Candidatus Neoehrlichia mikurensis*" infections in immunocompetent humans. J Clin Microbiol. 2014a;52(3):3072–4.
18. Welc-Falciak R, Kowalec M, Kaborwii G, Bajer A, Behnke JM, Sirlski E. Rickettsiaceae and Anaplasmataceae infections in *Ixodes ricinus* ticks from urban and natural forested areas of Poland. Parasit Vectors. 2014b;7:119–31.
19. Wójcik-Falla A, Bartosik K, Buczek A, Dutkiewicz J. Babesia microti in adult *Dermacentor reticulatus* ticks from eastern Poland. Vector Borne Zoonotic Dis. 2012;12(10):841–3.
20. Kiewra D, Starczak J, Richter M. *Ixodes ricinus* ticks (Acari, Ixodidae) as a vector of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* in Lower Silesia. Poland—preliminary study. Ticks Tick Borne Dis. 2014;5(6):892–7.
Ixodes ricinus and other ticks (Acari, Ixodidae) collected in urban areas.

22. Rizzoli A, Silaghi C, Obiegala A, Rudolf I, Hubálek Z, Foldvári G, et al. Ixodes ricinus and its transmitted pathogens in urban and peri-urban areas in Europe: New hazards and relevance for public health. Front Public Health. 2014;2:511.

23. Hamer SA, Tsai JL, Walker ED, Mansfield LS, Foster ES, Hickling GJ. Use of tick surveys and serosurveys to evaluate pet dogs as a sentinel species for emerging Lyme disease. Ann J Vet Res. 2009;70(1):49–56.

24. Nowak-Chmura M, Siuda K. Ticks of Poland review of contemporary issues and latest research. Ann Parasitol. 2012;58:125–55.

25. Siuda K, Bliska M, Nowak M. Kleszcze (Acari: Ixodidae) atakujace psy w okolicach Wadowic. Zoonozy: problem nadal aktualny. Conference Materials. Warszawa, 2002; 68. [in Polish].

26. Zygner W, Wedrychowicz H. Occurrence of hard ticks from Warsaw area. Ann Agric Environ Med. 2006;13(2):355–9.

27. Michalski M, Sokół R. Ticks species (Ixodida) on dogs on Olsztyn city area. Med. 2013;159:180.

28. Martinod S, Brossard M, Moreau Y. Immunity of dogs against Babesia canis, its vector tick Dermacentor reticulatus, and Ixodes ricinus in endemic area. J Parasitol. 1985;71(3):269–73.

29. Medranykov O, Matsumoto K, Samoylenko I, Drancourt M, Roux V, Rydkina E, Davout B, Tarasevich I, Brouqui P, Fournier PE, Rickertsia rattiui sp. nov., a spotted fever group rickettsia associated with Dermacentor ticks in Europe and Russia. Int J Syst Evol Microbiol. 2008;58:1635–9.

30. Křol N, Kewka D, Szymański M, Lonc E. The role of domestic dogs and cats in the zoonotic cycles of ticks and pathogens. Preliminary studies in Vlaamse Agglomeratie (SW Poland). Vet Parasitol. 2015;214:208–12.

31. Nowak-Chmura M, Fauna kleszczy (Ixodida) Europy Środkowej. Kraków: WNPJ; 2013. [in Polish].

32. Wölfel R, Essbauer S, Dobler G. Diagnostics of tick-borne rickettsioses in Germany: A modern concept for a neglected disease. Int J Med Microbiol. 2008;298:968–74.

33. Courtney JW, Kostelnik LM, Zeidner NS, Massung RF. Multiplex real-time PCR for detection of Anaplasma phyocytophilum and Borelia burgdorfi. J Clin Microbiol. 2004;42:3164–8.

34. Jahfari S, Forville M, Henggerveld P, Reusken C, Scholte E, Takken W, Heyman P, Medlock J, Heylen D, Kleve J, Spong H. Prevalence of Neoehrlichia mikurensis in ticks and rodents from north-west Europe. Parasit Vectors. 2012;5:74.

35. Silaghi C, Woll D, Mahling M, Pfister K, Pfeffer M. Candidatus Neoehrlichia mikurensis in rodents in an area with sympatric existence of the hard ticks Ixodes ricinus and Dermacentor reticulatus. Parasites Vectors. 2012;5:285.

36. Casati S, Sager H, Gern L, Piffaretti JC. Presence of potentially pathogenic Neoehrlichia microbi in human in Ixodes ricinus in Switzerland. Ann Agric Environ Med. 2006;13:65–70.

37. Roux V, Raoult D. Phylogenetic analysis of members of the genus Rickettsia using the gene encoding the outer-membrane protein OmpB (ompB). Int J Syst Evol Microbiol. 2000;50:1449–55.

38. Claerbout E, Lesson B, Cochez C, Casaert S, Dalemans AD, De Cat A, Maddon M, Segerman C, Heyman P, Lemperere L. Ticks and associated pathogens collected from dogs and cats in Belgium. Parasit Vectors. 2013; doi: 10.1186/1756-3305-6:303-6.

39. Eichinger RM, Deplazes P, Mathis A. Ticks on dogs and cats: A pet owner-based survey in a rural town in northeastern Switzerland. Ticks Tick-Borne Dis. 2015;6:267–71.

40. Dietrich MM, Schreiber C, Krücken J, Beck J, Maaz D, Pachnicke S, Krieger K, Bohn M, von Samson-Himmelstjerna G. Pathogens in ticks collected from dogs in Berlin/Brandenburg. Germany. Parasit Vectors. 2014;7:515.

41. Stensvold CR, Al Marai D, O’Brien Andersen K, Krogfelt KA, Jensen JS, Saholt Larsen K, Nielsen HV. Babesia spp. and other pathogens in ticks recovered from domestic dogs in Denmark. Parasit Vectors. 2015;8:262.

42. Chmielewski T, Podsiadly E, Karbowiak G, Tylowska-Wierzbawowska S. Rickettsia spp. in ticks. Parasit. 2015;2:223–7.

43. Starczak J, Racewicz M, Michalik J, Buszek A. Distribution of Rickettsia helvetica in Ixodes ricinus tick populations in Poland. Int J Med Microbiol. 2008;298:231–4.

44. Mierzejewska E, Welc-Fałecki R, Kowalew M, Alsarif M, Bajer A. Comparison of prevalence of vector-borne pathogens in Dermacentor reticulatus from eastern and western Poland. The 16th International Symposium ‘Parasitic and Allergic Arthropods – Medical and Sanitary Significance’. Kazimierz Dolny. 2014:76.

45. Krücken J, Schreiber C, Maaz D, Kohn M, Demeler J, Beck S, Schein E, Olias P, Richter D, Matuschka FR, Pachnicke S, Krieger K, Bohn M, von Samson-Himmelstjerna G. A novel high-resolution melt PCR assay discriminates Anaplasma phagocytophilum and ‘Candidatus Neoehrlichia mikurensis’. J Clin Microbiol. 2013;51(6):1998–61.

46. Zygner W, Jaros S, Wedrychowicz H. Prevalence of Babesia canis, Borrelia afzelii, and Anaplasma phagocytophilum infection in hard ticks removed from dogs in Warsaw (central Poland). Vet Parasitol. 2008;153:139–42.

47. Sytwiński K, Karbowiak G, Hapunik J, Szepczynski T, Supergan-Marwicz M, Gołąbka S, Sprawka I, Czermiecz P. Molecular evidence of Anaplasma phagocytophilum and Babesia microti co-infections in Ixodes ricinus ticks in central-eastern region of Poland. Ann Agric Environ Med. 2012;19:499–509.

48. Kiewra D, Zaźleń G, Czulowska A. The risk of infection with Anaplasma phagocytophilum and Babesia microti in Lower Silesia, SW Poland. In: Buszek A, Blaszcik C, editors. Stawonogi: Zagrożenie zdrowia człowieka i zwierząt. Lublin: Kolarz; 2014. 103–110.

49. Lemperere L, Decat A, Caron Y, Madder M, Claerbout E, Segerman C, Lesson B. First molecular evidence of potentially zoonotic Babesia microti and Babesia sp. EU1 in Ixodes ricinus ticks in Belgium. Vector Borne Zoonotic Dis. 2011;11:125–30.

50. Cieniuch S, Starczak J, Ruczaj A. The first detection of Babesia EU1 and Babesia canis canis in Ixodes ricinus ticks (Acari, Ixodidae) collected in urban and rural areas in northern Poland. Pol J Microbiol. 2009;58(3):231–6.

51. Leschnik MW, Kanaahak D, Duscher G, Wille-Piazzai W, Hońcęg C, Joachim A, Stanek G. Species, developmental stage and infection with microbial pathogens of engorged ticks removed from dogs and questing ticks. Med Vet Entomol. 2012;26(4):440–6.

52. Földvári G, Márialigeti M, Solymosi N, Lukács Z, Majorgó K, Göd AI, et al. Hard ticks infesting dogs in Hungary and their infection with Babesia and Borrelia species. Parasitol Res. 2010;107:25–34.

53. Welc-Fałęcki R, Rodo A, Sirlski E, Bajer A. Babesia canis and other tick-borne infections in dogs in Central Poland. Vet Parasitol. 2009;166:191–8.

54. Krámer F, Schaper R, Schautack B, Połozowski A, Piekar ska J, Szvedko D, Jodes R, Kowalska D, Schüpbach D, Pantchev N. Serological detection of Anaplasma phagocytophilum, Borrelia burgdorferi sensu lato and Ehrlichia canis antibodies and Dirofilaria immitis antigen in a countrywide survey in dogs. Parasitol Res. 2014;113(6):3229–39.