



Occurrence of *Dermacentor reticulatus* in central-southern Poland, and potential threats to human and animal health

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Abstract

Introduction and Objective. *Dermacentor reticulatus* is one of the tick species of the greatest epidemiological importance in Europe. To date, the Eastern European and Western European populations of this tick species have been separated by an area located in Poland where the species has never been found. In this study, newly discovered *D. reticulatus* localities in areas transformed by human activities in central-southern Poland are described.

Materials and method. The specimens of the ornate dog tick were identified among ticks collected from companion animals in 2010, 2012, 2013, and 2014. They were examined using PCR methods to detect *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Anaplasma phagocytophilum*, *Bartonella* spp., *Babesia* spp., and *Toxoplasma gondii*. In the case of the positive results, the amplicons were sequenced and examined by a BLAST search.

Results. In total, 6 specimens of *D. reticulatus* were collected (3 females and 3 males). As declared by the owners, animal hosts stayed in the same area throughout the study period and had never travelled outside their place of residence. As many as 3/6 (50%) of *D. reticulatus* adults removed from dogs were infected with *Rickettsia raoultii*.

Conclusions. The results expand the available data on the spread of the ornate dog tick and indicate that, since 2010, this tick species and *Rickettsia raoultii* transmitted by this tick species have probably been present in this area, which has a strongly transformed agricultural structure and had previously been regarded as a *D. reticulatus*-free zone. The presence of the ornate dog tick in urban and suburban habitats in central-southern Poland poses new threats to the health of companion animals and humans associated with the transmission of pathogens by this species.

Key words

Poland, *Dermacentor reticulatus*, *Rickettsia raoultii*, companion animals, tick-borne pathogens, tick expansion

INTRODUCTION AND OBJECTIVE

The meadow or ornate dog tick *Dermacentor reticulatus* (Fabricius, 1794) was regarded as one of the most expansive tick species at the turn of the 21st century. Its geographical range has increased across Europe [1, 2], especially in its northern, central, and eastern parts. The boundary of the *D. reticulatus* occurrence in the west and east of the continent, historically referred to as Western European and Eastern European populations, has been regarded as a tick-free area in Poland [3]. The spread and increase in the abundance of the ornate dog tick have been caused by climate and environmental changes, which create favorable conditions for the species in various geographical regions [4–7]. The climate warming and weather phenomena recorded recently in Europe have contributed to prolonged seasonal activity of adults of this species, which may begin in early March and last until December in the northern and central parts of the continent. The first activity peak is recorded from April

to May in spring, and the next peak occurs in late summer and autumn, usually persisting from mid-August/early September to November [5, 7–10]. Currently, host seeking *D. reticulatus* specimens can be found even in winter months at an ambient temperature of approximately 0°C in the absence of snow cover [8, 9, 11]. Juvenile stages of this tick species are active in summer and autumn, with the highest activity of larvae observed in June or July and nymphs in July or August, depending on the region [12–15].

In the European part of Eurasia, the entire life cycle of *D. reticulatus* from oviposition to adult stages usually lasts from 1–2 years [16]. Unengorged adults can live even 3–4 years [16]. Their longest survival of 617.8 days was recorded at 5°C and 100% relative humidity RH, and the shortest lifespan (33.6 days) was noted at 27°C and 15% RH [17]. In the temperature range 5°C–27°C and at RH from 15%–100%, the mortality rate of unengorged adults was reported to increase, regardless of their gender, with an increase in relative humidity and a temperature decline.

D. reticulatus is mainly transported to new habitats by migrating mammals, mainly representatives of Cervidae and carnivores [18, 19]. Companion animals travelling with their keepers may also play a role in the spread of adult ticks [20,

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21]. Additionally, birds may be involved in the transmission of *D. reticulatus*, as its larvae have been collected from these animals [22]. Further research is necessary to determine the role of these hosts in the spread of *D. reticulatus* in nature.

The colonization of various habitats by the ornate dog tick is supported by its special adaptation abilities and biological and physiological features, e.g. high tolerance to humidity and temperature conditions [5, 7, 14, 17, 22, 23]. Moreover, this tick species is characterized by a wide range of hosts of juvenile [14, 22] and adult stages [1, 18, 21, 24], which facilitates finding sources of food (blood) necessary for further development in new habitats.

Across its entire geographical range, *D. reticulatus* adults infest dogs (*Canis lupus familiaris*) [1], which may play an important role in the maintenance of the population of this tick in urban environments. Companion animals, especially dogs, may be involved in the circulation of tick-borne pathogens (TBPs) transmitted by the ornate dog tick in urban areas [25].

The expansion of the *D. reticulatus* occurrence range and changes in their phenology have increased the exposure of tick hosts to infection by various pathogens transmitted during feeding, e.g. tick-borne encephalitis virus (TBEV), spotted-fever group rickettsiae (SFGR), *Babesia* spp., *Theileria equi*, and *Francisella tularensis* [1].

In Europe, *D. reticulatus* is one of the most important vectors of *Babesia canis*, the causative agent of canine babesiosis which poses a high risk of death in dogs [26], and spotted fever group rickettsiae [1], e.g. *Rickettsia slovaca* and *Rickettsia raoultii* bacteria from the SF group responsible for a syndrome characterized by scalp eschars and neck lymphadenopathy following tick bites (SENLAT).

This article describes the new localities in central-southern Poland of *D. reticulatus* and the pathogens transmitted by this tick species in a zone that has so far been free of this tick species. Attention is drawn to the consequences of the presence of the tick to animal and human health.

MATERIALS AND METHOD

Study area. The study was carried out in central Poland, in the Radomsko and Żytno Communes in Łódź Province and the Kłobuck Commune in the Silesian Province. The climate in this area is transitional, which is associated with the influences of air masses characteristic of the continental and oceanic zones. The climate in this region is also determined by differences in terrain elevation and soil moisture. In recent years, an increase in the mean air temperature has been observed in the study area.

The field and forest landscape of this area with relics of natural forest and meadow-peatland ecosystems are favourable habitats for numerous animal species that can be potential tick hosts, e.g. moose, red deer, fallow deer, roe deer, wild boar, hare, fox, raccoon dog, marten, badger, beaver, weasel, and stoat.

Radomsko Commune (51°04'01"N, 19°26'41"E, 223 m a.s.l.) is situated in the valley of the Warta River and on the Radomsko Hills. Its southern part is located in a nationally important ecological corridor covering the valley of the Warta River, and in the area of the main underground water reservoir in Upper Cretaceous formations. Its area covers numerous wet meadows, swamps, and peat bogs, where many northern

plants can be found (marsh horsetail, swamp sedge, several species of mosses, long-leaved chickweed, and wild rosemary). Species characteristic of Central Europe, e.g. European beech, common hornbeam, English oak, broad-leaved linden, and Norway maple, are the most numerous plants. Herbaceous plants are represented by finger sedge, wood anemone, and dog's mercury. Riparian forests and oak-hornbeam forests are the dominant habitats in the valley of Warta tributaries. The plant cover in the valley bottom is closely related to the habitat conditions and land use. The mid-field tree stands and forests in this area are characterized by a high degree of habitat degradation caused by changes (intensive drainage) or neglect of water relations. The areas of former mid-forest meadows provide a favourable living habitat for mammals and birds. The wetlands and forest coppices located near agricultural fields are frequent habitats for roe deer and boars. The area comprises large forest complexes, mainly the communities of fresh mixed coniferous forest, wet mixed coniferous forest, and alder forest. The tree stand is dominated by pine, alder, birch, and oak.

Żytno Commune (50°55'38"N 19°37'39"E, 202 m a.s.l.) covers a largely swampy area between the Pilica and Warta River valleys. Marsh and peat wetlands are a frequent element of its landscape. Nearly 50% of the area is covered by forests dominated by pine trees. Dębowiec Nature Reserve was established in the commune to protect a natural oak-hornbeam forest with broad-leaved linden at the margins of its occurrence range and an elm-ash riparian forest with rare herbaceous plants. Dębowiec Reserve and the neighbouring forest complexes are located in wet valleys covered with natural alder and ash forests. The proglacial valley and the old riverbeds of the Pilica River are covered by riparian forests, rushes, herbaceous plants, and wet meadows in addition to fertile oak-hornbeam forests.

Kłobuck Commune (50°54'02"N, 18°56'12"E, 239 m a.s.l.) is located in the catchment area of the Warta River. Numerous smaller rivers flow from the wet valleys into its largest tributary, the Liswarta River. The vegetation of the area is represented by coniferous (56%), forest (27%), mountain (16%), and alder (1%) communities. Pine and larch are the main forest-forming species (66.4%), with a lower percentage of spruce, oak, maple, sycamore, elm, ash, birch, beech, and others [27].

Tick collection. Ticks were collected from companion animals (six dogs and a cat) during routine health checks carried out by veterinarians in the study area. The ectoparasites were removed from the companion animals as part of hygiene practices. Ticks removed from the skin of the animal hosts were stored in sterile plastic test tubes with 70% ethanol pending identification in the laboratory at the Department of Biology and Parasitology, Medical University of Lublin. The species, developmental stage, and gender of the tick specimens were identified based on the guide to tick identification by Nowak-Chmura [28].

Molecular analysis. Ticks collected from the companion animals were examined for the presence of TBPs, e.g. *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Anaplasma phagocytophilum*, *Bartonella* spp., *Babesia* spp., and *Toxoplasma gondii* at the Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice.

The DNA was isolated from single tick specimens with the ammonia method [29]. Next, the concentration was measured spectrophotometrically in the NanoPhotometer PEARL (Implen, Munich, Germany) at the 260/280 nm wavelength. The samples were then frozen and stored at -20°C until further molecular analyses. *B. burgdorferi* sensu lato was detected in the ticks with the real time polymerase chain reaction (qPCR) method with the use of a ready Borrelia qPCR detection Kit (EURx, Gdańsk, Poland), according to the manufacturer's protocol. In turn, two pairs of primers specific to the 16S rRNA and B1 gene were used for detection of *A. phagocytophilum* and *T. gondii*, respectively [30, 31]. *Bartonella* spp., *Babesia* spp., and *Rickettsia* spp. were detected in the ticks by single PCR. To detect these pathogens in *D. reticulatus*, primers specific to the *rpoB*, 18S rRNA, and *gltA* genes were used [32–34]. The polymerase chain reaction (PCR) and nested PCR amplification products were separated electrophoretically in 2% ethidium bromide-stained agarose gels and visualized under ultraviolet light in a device for the agarose gels analysis (Vilber Lourmat, Collegien, France). Next, the amplicones were isolated from the gels, cleaned with the use of an Agarose-Out DNA Purification Kit (EURx, Gdańsk, Poland), according to the manufacturer's protocol, and sequenced by Genomed (Warsaw, Poland).

Statistical and phylogenetic analyses. The sequencing results were revised and aligned manually using BioEdit v.7.0.5.3 [35] and compared to the GenBank references by BLAST (<http://www.ncbi.nlm.nih.gov/>, accessed on 30 October 2023) to determine *Rickettsia* spp. The three sequences of the citrate synthase (*gltA*) gene obtained in this study were identical. To test the phylogenetic relationships among the *gltA* haplotype derived in this study and sequences downloaded from GenBank, a phylogenetic tree was constructed using a maximum-likelihood (ML) algorithm in Mega v.6.06 [36] with 1,000 bootstrap replicates used to assess the support for the tree nodes. In the phylogenetic analyses, the nucleotide substitution model TIM3+I was determined under the Akaike information criterion [37] implemented in jModelTest v. 0.1.1 [38]. Additional *gltA* gene sequences of *Rickettsia bellii* downloaded from GenBank (Accession No. DQ146481) were used as an outgroup.

Rickettsia bellii is an α -proteobacterium that together with *Rickettsia canadensis* and *Rickettsia kingi* belongs to the phylogenetic ancestral group [39]. Four different groups of *Rickettsia* species were recognized based on the molecular analysis [39], and all their members were included in the phylogenetic analysis.

RESULTS

In this study, *D. reticulatus* ticks were found for the first time in central-southern Poland in three localities (Radomsko, Żytno, and Kłobuck) situated at a distance of approx. 25–60 km from each other (Fig. 1). To date, this area has been regarded as a zone separating the eastern and western ornate dog tick populations in Poland [3].

In total, six *D. reticulatus* were collected from six dogs and one specimen was removed from a single cat (Fig. 2, Table 1). There were three fully engorged females, one partially engorged female, and three males. As declared by the owners of the dogs and the cat, their animals stayed in the same area throughout the study period and had never travelled outside their place of residence.

In the present study, as many as 3/6 (50%) of *D. reticulatus* adults removed from dogs were infected with *Rickettsia* spp. The sequence analysis of *Rickettsia* spp., showed that the obtained sequences were 100% compatible with the sequence of the *Rickettsia raoultii* clone HL01 citrate synthase (*gltA*) gene (GenBank Accession No. KM386690.1). None of other pathogens, i.e. *B. burgdorferi* s.l., *Babesia* spp., *A. phagocytophilum*, *Bartonella* spp., and *T. gondii*, were detected in the studied ticks.

The obtained sequence of the *gltA* gene was identical to the sequences of *Rickettsia* (GenBank Accession No. EF030949) and *R. raoultii* (GenBank Accession Nos. KF003007, KF003009, KM279354, KM386690). The maximum-likelihood phylogenetic reconstructions revealed that the *gltA* haplotype identified in this study formed a phylogenetic branch together with haplotypes belonging to *R. massiliae* and *R. aeschlimannii* (Fig. 3).

A limitation in the presented phylogenetic analysis was the length of the analyzed fragment of the *gltA* gene. It was insufficient for a more precise arrangement of the particular groups of *Rickettsia* on the phylogenetic tree. The insufficient sequence length did not allow inclusion of other species of *Rickettsia* in the analysis, as it was impossible to distinguish some *Rickettsia* species based on the analyzed *gltA* gene fragment.

DISCUSSION

The incidents of attachment of *D. reticulatus* adult ticks to companion animals in the different study years indicate that this species has been present in central-eastern Poland since 2010. The collection of the *D. reticulatus* specimens in central-southern Poland confirms the findings of the

Table 1. *Dermacentor reticulatus* ticks collected from companion animals in central-southern Poland (2010–2014)

Date of collection	Collection site	Host species	Anatomical location on host	Life stage of removed tick	<i>Rickettsia raoultii</i>
22.10.2010	Radomsko *	<i>Canis lupus familiaris</i>	head	almost fully engorged female	+
11.05.2012	Radomsko	<i>Canis lupus familiaris</i>	abdomen	partially engorged female	+
10.04.2012	Radomsko	<i>Felis catus</i>	head	male	n.d.
03.05.2013	Kłobuck **	<i>Canis lupus familiaris</i>	head	male	-
27.05.2014	Radomsko	<i>Canis lupus familiaris</i>	head	male	-
20.06.2014	Kłobuck	<i>Canis lupus familiaris</i>	neck	almost fully engorged female	+
10.05.2014	Żytno ***	<i>Canis lupus familiaris</i>	leg	almost fully engorged female	-

Geographical coordinates of collection sites * 51°04'01"N, 19°26'41"E, 223 a.s.l.; ** 50°54'02"N, 18°56'12"E, 239 a.s.l.; *** 50°55'38"N 19°37'39"E, 202 a.s.l.; n.d. – no data



Figure 1. Geographical locations in Poland where *Dermacentor reticulatus* ticks were found on companion animals in 2010–2014.

O- 51°04'01"N, 19°26'41"E, 223 m a.s.l.; - 50°54'02"N, 18°56'12"E, 239 m a.s.l.; Δ- 50°55'38"N 19°37'39"E, 202 m a.s.l.

Source: Marcin Wasilewski, marcinwasilewski.eu on the basis of the OpenStreetMap; Copyright © authors OpenStreetMap



Figure 2. Engorged female of *Dermacentor reticulatus* (arrow) collected from a dog's head in Radomsko in October 2010 (scale in cm) (Photo by Alicja Buczek)

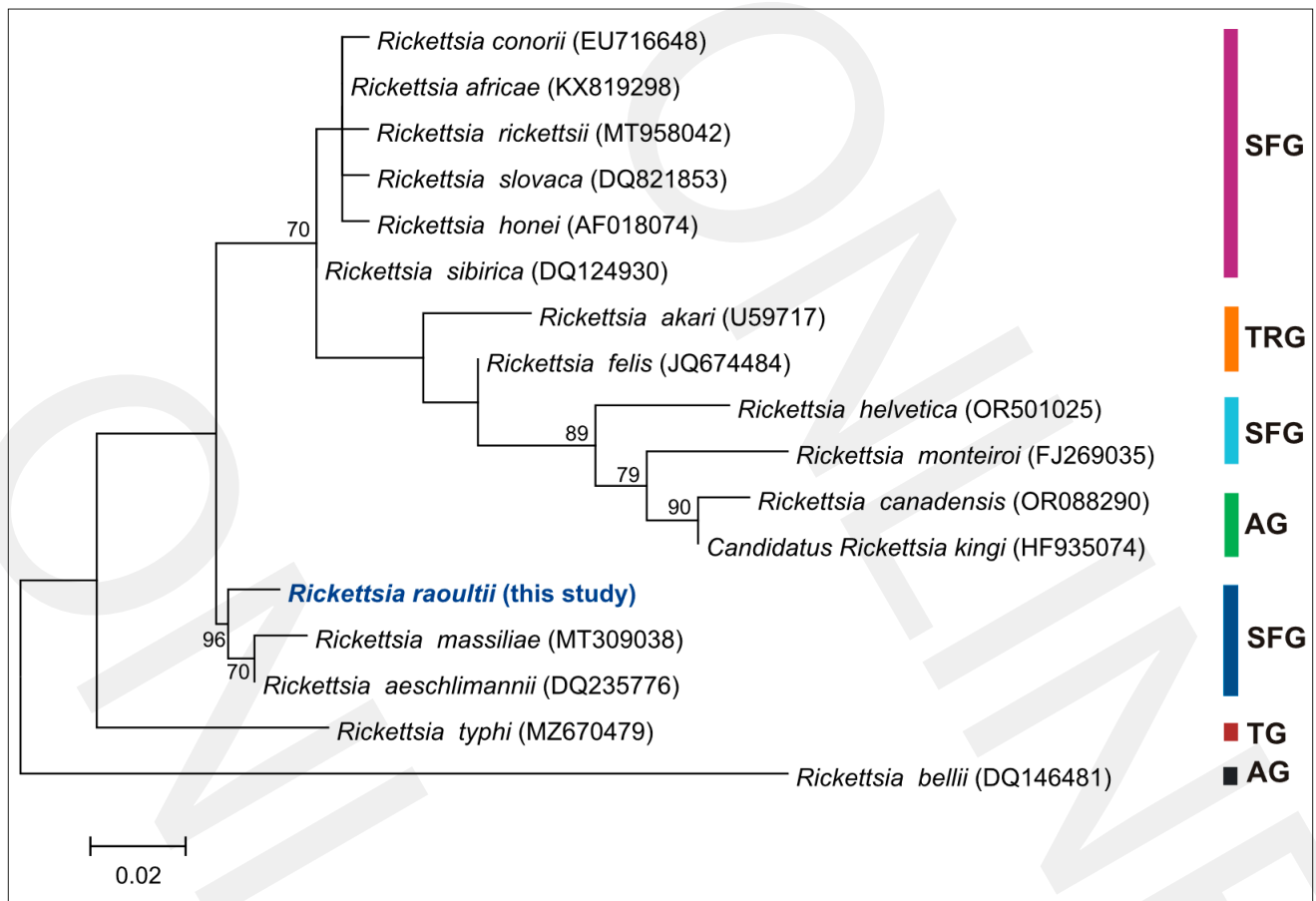


Figure 3. Maximum-likelihood topology computed with the TIM3+I model of substitution evolution, representing the phylogenetic relationships among the sequences of the *gltA* gene in *Rickettsia* species. Numbers listed at the nodes represent the percent support for the node from 1,000 bootstrap replicates. Bootstrap values are indicated at the nodes if found in more than 70% of 1,000 bootstrap trees. The haplotype obtained in this study is marked in dark blue. SFG, TRG, TG, and AG refer to the spotted fever group, transitional group, typhus group, and ancestral group of *Rickettsia*, respectively.

constant migration of this tick in Poland reported by other researchers [3, 40, 41].

At the end of the 20th century and the beginning of the 21st century, the eastern and western occurrence range in Europe of *D. reticulatus* was separated in Poland by an area between the Vistula and Oder Rivers [3]. The first localities of *D. reticulatus* west of the Vistula River were recorded in Pomeranian Lake District in the early 2000s [42, 43] and in subsequent years, as reported by Dwużnik-Szarek et al. [3]. In western Poland, the tick species was first found near the border with Germany in Lower Silesian and Lubuskie Provinces [44–47]. In 2022, *D. reticulatus* specimens were collected near Poznań (west-central Poland) [48]. Two specimens (one female and one male), probably from a local population, were removed from a dog in Racibórz in south-western Poland [49].

Dermacentor reticulatus ticks were probably brought to the area analyzed in this study by animals migrating from the western regions of Poland and/or eastern Germany, where stable populations of this species have been identified [4, 6]. The new *D. reticulatus* localities in central Poland are situated in or near urbanized areas. The shift in the range of these ticks towards urban areas has been observed for two decades in other parts of Poland [e.g., 48, 50–54] and Europe [e.g., 9, 55, 56].

The environmental conditions in central Poland are highly favourable for this tick species. Within its distribution range,

the species is most often found in riparian forests, river and lake banks, meadows, mid-forest clearings, and wastelands with a high groundwater level [1, 7, 22, 23, 40, 41]. The *D. reticulatus* colonization of the study area may have been facilitated by human activities changing the hydrographic network (conversion of smaller tributaries of the main rivers in the region into drainage ditches and construction of drainage ditches, regulation of rivers, construction of artificial water reservoirs) and landscape transformation (mid-field afforestation, fallow areas, urbanization, conversion of forests into agricultural land). The mosaic landscape creates not only open habitats preferred by *D. reticulatus* ticks, but also various habitats suitable for their potential hosts in central Poland. The relationships between the reduction of forested areas and the presence of *D. reticulatus* have been confirmed by Mierzejewska et al. [57].

In Poland, juvenile stages of *D. reticulatus* (larvae and/or nymphs) have been collected from various species of rodents from the families Murinae, Microtinae, and Soricidae [12, 13, 15, 58–60]. Adult ticks parasitize large wild-living mammals, mainly representatives of the families Cervidae: moose (*Alces alces*), fallow deer (*Dama dama*), and red deer (*Cervus elaphus*) [43, 47], Canidae: wolf (*Canis lupus*) and fox (*Vulpes vulpes*) [61], Bovidae: European bison (*Bison bonasus*) [62], and Suidae – wild boar (*Sus scrofa*) [47, 63]. In the *D. reticulatus* occurrence range, adult specimens are collected from dogs and other domestic animals [52, 54, 64]. In some

habitats of central-eastern Poland, it is the dominant tick species infesting dogs [64].

The presence of *D. reticulatus* ticks in the study area poses a threat to the health of not only companion animals, but also humans, who may be accidental hosts of adult stages [21, 24, 65, 66]. An interesting case of attachment of a female *D. reticulatus* tick to human skin was recorded in eastern Poland where the tick was transferred to the apartment by a pet dog [66]. As demonstrated by the research, after ingestion of dog's blood, female *D. reticulatus* ticks can lay eggs that hatch into larvae in household conditions.

The expansion of *D. reticulatus* ticks into central-southern Poland may lead to the spread of *R. raoultii* and other pathogens transmitted by this species in the environment, which should be taken into account in the clinical diagnosis of tick-borne diseases in domestic animals and humans. In Poland and its western and southern neighbors, numerous pathogens with medical and veterinary importance have been detected in this tick species, e.g. *R. raoultii* and other SFG rickettsiae, *Babesia* spp., TBEV, *A. phagocytophilum*, and *F. tularensis* [52, 67–70]. Spotted fever group rickettsiae, mainly *R. raoultii*, exhibit the highest prevalence in *D. reticulatus*. In western Poland, they were detected in 38.3% of specimens collected from vegetation [52] and in 60% of ticks removed from dogs [70]. In the endemic regions in eastern and northern Poland, the percentage of host seeking *D. reticulatus* infected with these bacteria ranged from 34.18%–56.7% [67–69]. A high prevalence of *R. raoultii* and host seeking *D. reticulatus* specimens was also recorded in Poland's western and southern neighbouring countries. The mean rates of infection with this bacterium were 64%–70.5% in Germany [9, 71] and 47.9% in the Czech Republic and Slovakia [72]. Depending on the habitat, TBE viruses causing tick-borne encephalitis in humans were detected in 0.33%–10.8% of *D. reticulatus* specimens from the tick population in eastern Poland [51, 69, 73, 74]. The presence of TBE viruses in this tick species has been reported from the district of northern Saxony near the Polish border [75].

The greatest threat to the health of dogs attacked by ticks is associated with the transmission of *Babesia* protozoa. In Poland, the prevalence of *B. canis* in ornate dog ticks is usually in the range of 1–4%, depending on the region [68, 69]; however, it can reach even 21.3% in swampy habitats in eastern Poland [76]. The incidence of clinical babesiosis in the Polish population of dogs is higher in eastern and central Poland (Eastern tick population) than in western Poland (new endemic region for the ornate dog tick) and in the tick-free area [3].

CONCLUSIONS

In conclusion, the observations presented in this study expand the data on the spread of *D. reticulatus* in Poland and confirm its progressive expansion in Central Europe. The presented results suggest that since at least 2010, *D. reticulatus* ticks have been present in a zone in Poland that had so far been considered to be free of this species. The field studies (collection of ticks from vegetation) may indicate the distribution of these ticks and the size of their population in central-southern Poland. The need to continue this type of research is supported by the fact that no field studies on the prevalence of *D. reticulatus* and/or other tick species have been conducted in this region to date.

The occurrence of *D. reticulatus* in central-southern Poland and the detection of *Rickettsia raoultii* in these ticks enlarges the areas of potential foci of rickettsiosis and possibly other diseases transmitted by this tick species, e.g. babesiosis in dogs and rickettsiosis in humans, both of which have been diagnosed in other parts of Poland. The detection of *R. raoultii* in the *D. reticulatus* specimens collected from the animals, previously also identified in three (6.4%) of 47 *I. ricinus* ticks collected from vegetation in this region [67], indicates the necessity to continue research on the prevalence of this bacterium in ticks, and the need for clinical diagnostics to take into account the possibility of the development of rickettsial diseases in humans in central-southern Poland. Given the high vector competence and the wide spectrum of *D. reticulatus* hosts, as well as its co-occurrence with *Ixodes ricinus* in the same habitats, the analyzed tick species can significantly contribute to the circulation and maintenance of other TBPs in the environment, which are one of the most important biological agents posing a threat to the health of companion animals and humans. However, a large scale molecular and epidemiological study should be conducted to reveal the real picture of the tick distribution and transmission dynamics of pathogens carried by *Dermacentor reticulatus* in the area.

Monitoring the occurrence of *D. reticulatus* and pathogens transmitted by this tick species can help develop strategies for reduction of the incidence of tick-borne diseases in humans and animals.

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