CASE REPORT

Annals of Agricultural and Environmental Medicine
www.aaem.pl

A seven-legged tick – anomalous *Ixodes ricinus* female (Acari: Ixodidae) from Poland

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Koczwarska J, Nowakowska J, Polaczyk J, Welc-Falęciak R. A seven-legged tick – anomalous *Ixodes ricinus* female (Acari: Ixodidae) from Poland. Ann Agric Environ Med. doi: 10.26444/aaem/172533

Abstract

Morphological anomalies are considered a rare phenomenon among natural tick populations. New cases of abnormalities in ticks are being described, such as body assymetries, nanism, gynandromorphism and limb malformations. The tick removed from a cat was morphologically identified to species and developmental stage. The time of feeding on the host was determined. The specimen was tested using PCR and Real-Time PCR methods for the presence of the common tick-borne pathogens: *Anaplasma phagocytophilum*, *Babesia* spp, *Borrelia* spp., *Neoehrlichia mikurensis*, *Rickettsia* spp. For visualisation of the anomalous structures, scanning electron microscopy (SEM) was performed. The tick was identified as a slightly engorged adult female of *I. ricinus* exhibiting ectromely of leg I on the left side of the idiosoma. The specimen was tested positive for two medically important pathogens: *A. phagocytophilum* and *N. mikurensis*. The case report describes a rare case of a morphological anomaly in an *I. ricinus* tick from Poland.

Key words

Europe, tick, deformities, *Ixodes ricinus*, teratology, abnormalities

INTRODUCTION AND OBJECTIVE

Since the first three cases of morphological anomalies in ticks were described by Neumann in 1899 [1], there have been numerous subsequent reports of teratological ticks [2–8]. The abnormalities are found both in Argasid (genera: *Argas* and *Ornithodoros*) and much more frequently in Ixodid ticks, including genera: *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus*, [9–13]. Morphological anomalies, which can appear at every developmental stage [14], are considered a rare phenomenon in natural tick populations [11] and noted in less than 2% of ticks [14–18]. In contrast to the majority of studies that show very low rates of morphologically changed ticks, there are single reports documenting higher percentages of anomalous ticks in areas contaminated with heavy metals [19].

Classification systems have been developed to categorize anomalies, the most widely used of which is the one formulated by Campana-Rouget [20]. It provides a division based on the location and magnitude of the anomalies on the tick's body: 1) general anomalies, including changes in an overall body shape, gynandromorphism, gigantism and nanism; 2) local anomalies affecting the various body parts, such as deformations of scutum, capitulum, and femur, changes in shape of anal plates and grooves, missing or extra spiracles and porous areas, abnormal chitinous formations, and malformations of limbs. The latter are further subdivided into: polymely, symely, schizomely, heteromorphoses, ectopy, atrophy and ectromely [14, 16, 18, 20].

In addition to the above, Buczek [21] modified classification of limb anomalies by introducing the terms oligomely (absence of limbs), heterosymely (fusion of limbs on the same side of idiosoma), symely (fusion of limbs on the opposite side of idiosoma) and ectomely (change of attachment side of the limb to the idiosoma). Anomalies of internal structures are also described, as well as complex abnormalities with simultaneous general and local anomalies in one specimen [10, 11 20, 22].

The case report presents an example of a local anomaly – ectromely, in an adult female *Ixodes ricinus* tick in Poland.

MATERIALS AND METHOD

The tick was submitted for research conducted under grant OPUS 2020/37/B/NZ6/01587 from the National Science Centre (NCN) in Crakow, Poland. The tick was removed from the host, a domestic cat in the Masovian Province in north-eastern Poland. The specimen was delivered in a tightly-sealed, ethanol-filled container to the Faculty of Biology at the University of Warsaw, where it was subsequently morphologically identified to species and developmental stage level using a standard taxonomic key [23]. The tick feeding time was estimated from the scutal and coxal indexes according to Gray et al. [24]. For detailed visualisation of teratologically changed structures, scanning electron microscopy (SEM) was used. The procedure for preparation of the material for Scanning Electron Microscopy (SEM) has been described previously [25].

For DNA extraction, the tick was sterilised in 70% ethanol and then homogenised with Qiagen TissueLyser II. Genomic DNA was isolated using the DNeasy Blood & Tissue Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). Genomic DNA was used

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Received: 20.04.2023; accepted: 18.09.2023; first published: 13.10.2023

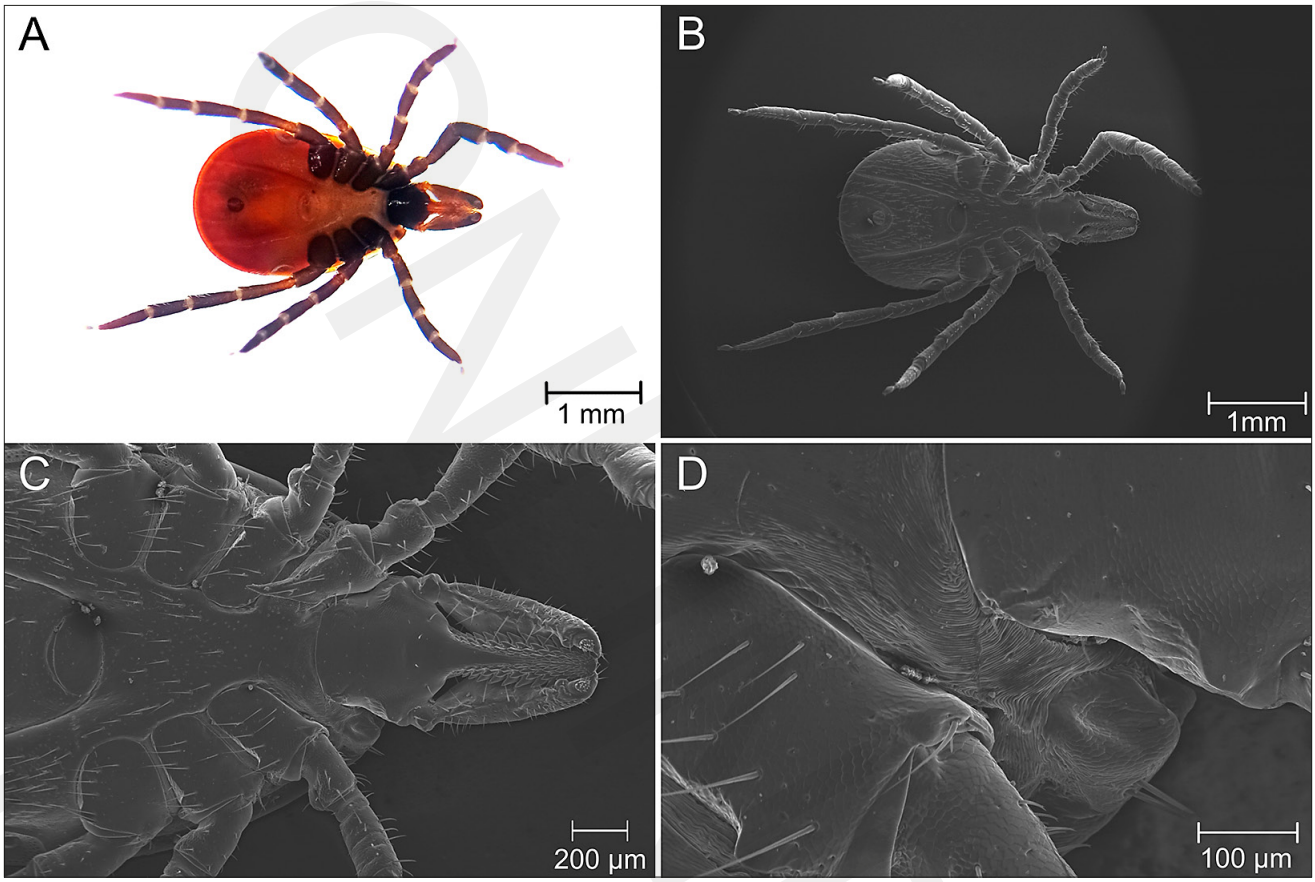

Figure 1. Ectromely on an *Ixodes ricinus* female. Microphotographs with light (A) and scanning electron microscopy (B, C, D). Ventral view of the whole tick (A, B), podosoma (C) and spot with missing limb (D)

for molecular identification of tick to species level and detection of the following tick-borne pathogens: *Anaplasma phagocytophilum*, *Babesia* spp., *Borreliella* spp., *Neoehrlichia mikurensis*, *Rickettsia* spp., the causative agents for human granulocytic anaplasmosis, human babesiosis, Lyme disease, neoehrlichiosis, and rickettsioses, respectively.

In order to confirm tick species, a conventional PCR method was used with two sets of primers targeting 519 bp *nad5* mitochondrial gene fragment and 653 bp *18S rRNA* gene fragment. The primers sequences and PCR conditions have been described earlier [26, 27]. Genomic DNA was used for molecular identification of tick-borne pathogens by amplification of different loci: *16S rRNA* fragment for *A. phagocytophilum*, [28] *18S rRNA* fragment for *Babesia* spp., [29] *groEL* heat shock operon for *N. mikurensis* [30] and *gtlA* gene fragment for *Rickettsia* spp. [31]. Negative controls were performed in the absence of template DNA. *Babesia microti* King's College strain DNA isolated from infected BALB/c mice blood and sequenced *A. phagocytophilum*, *N. mikurensis* and *Rickettsia helvetica* DNA obtained from infected ticks [32–33] were used as positive controls. The PCR products were separated by electrophoresis on 1.2% agarose gel stained with Midori Green Stain (Nippon Genetics Europe, Düren, Germany) and visualised on Gel Doc XR+ (Bio-Rad, Hercules, California, USA). In order to detect *Borreliella* DNA ready to use *Borrelia* qPCR Detection Kit (EurX, Gdańsk, Poland) was used according to the manufacturer's instructions. Amplicons were sequenced in both directions by a commercial company (Genomed S.A., Warsaw, Poland). Obtained nucleotide sequences were analysed using BLAST NCBI and MEGA v. 11.0 software [34] for sequence alignment and species typing using sequences deposited in GenBank NCBI. The new nucleotide sequences have been deposited in the GenBank database – Accession Nos. OQ457017 (*18S rRNA* gene fragment) and OQ413242 (*nad5* gene fragment) – for sequences of *Ixodes ricinus*, OR544014 for sequence of *N. mikurensis* (*groEL* heat shock operon), and OR532503 for sequence of *A. phagocytophilum* (*16S rRNA* fragment).

The Internal Review Board of the Medical University in Warsaw was informed about the study protocol (No. AKBE/73/2021), which followed the ethical guidelines of the 2013 Declaration of Helsinki. Informed consent was obtained from participants included in the study.

RESULTS AND DISCUSSION

The tick was removed from a domestic cat in the Masovian Province in north-east Poland, but the exact place in which the specimen was collected is not known. The tick was identified as an *Ixodes ricinus* female, based on the morphological key and molecular analysis of *18S rRNA* and *nad5* gene fragments. The *18S rRNA* gene sequence demonstrated 100% similarity with two *I. ricinus* isolates obtained from Spain (GenBank: Z74479.1) and Slovakia (GU074648.1). *The nad5* gene sequence showed >99% similarity with *I. ricinus* questing female found in Italy (GenBank: FN394357.1), but differed from it by four nucleotide substitutions at positions: 21 (A→T), 30 (A→G), 42 (C→A) and 204 (A→G). The tick was slightly engorged, and according to the scutal and coxal

indexes, attached to the host for an average 23 hours [24]. The tested tick showed ectromely of leg I on the left side of the idiosoma with associated slight asymmetry of appendages, compared to the right side of the body (Fig. 1). The tick was positive for two medically-important pathogens. The amplicon of *groEL* gene fragment showed 100% similarity with *N. mikurensis* isolate obtained from *I. ricinus* in the Czech Republic (GenBank: MN151365). The sequence of *16S rRNA* gene fragment was identical with *A. phagocytophilum* originally isolated from *I. ricinus* in Poland (GenBank: MH122891).

The specific reason for ectromely in the studied tick could not be determined. The causes of morphological abnormalities in natural populations of ticks are diverse and may be related to both internal (germinal mutations, somatic mutations, abnormalities during embryonic development, e.g. fusion of eggs, incorrect egg divisions) [18, 35], and environmental factors (antropopression, heavy metal pollution, mechanical injuries, adverse moulting conditions) [19, 35, 36]. The influence of the host-related factors and conditions during blood meal are also discussed, such as host resistance [37], feeding of multiple ticks close together, feeding on unusual hosts, and lack of food for a long period [22]. Anomalies have also been successfully induced under laboratory conditions by exposing ticks to chemical agents (among others: colchicine, ivermectin, acaricides) [38, 39], high temperature and humidity [21, 40]. The causes of limb malformations in ticks include abnormalities at the embryogenesis stage, adverse moulting conditions, body injuries, and defective regeneration, as well as genetic factors and breeding in high humidity conditions [16].

Ixodes ricinus is the most numerous tick in Europe and a major vector of various pathogenic microorganisms [41]. Whether or not morphological anomalies affect the ability of ticks to acquire and transmit pathogens is not known. Only a few studies have confirmed the presence of tick-borne pathogens and co-infections in abnormal ticks. Molaei et al. [42] reported adult *I. scapularis* female showing ectromely, and tested positive for *Borreliella*, *A. phagocytophilum* and rickettsial endosymbiont.

It is believed that co-infections in ticks with morphological anomalies may occur with higher frequency than in normal ticks. Žygutienė et al. [43] during their research conducted in 2005 in the parks of Vilinius, Lithuania, have noted that the abnormal ticks were much more often infected with *Borreliella* and had a higher rate of co-infections than ticks without anomalies. Molalei et al. [44] noted that if the tick's morphological anomalies were associated with environmental stress, this factor could also affect the tick's immune response and thus increase susceptibility of ticks to pathogens. However, further research is needed to verify this hypothesis. Alekseev et al. [45] documented higher locomotor activity of *Borreliella*-infected *I. persulcatus* females with exoskeleton anomalies, compared to those without abnormalities. The ticks examined came from areas with higher concentrations of cadmium in the soil, which may suggest the emergence of a new tick population characterized not only by a higher prevalence of anomalies, but also having features enhancing adaptation to adverse environmental conditions [45].

Nevertheless it appears that developmental anomalies, especially associated with the crotch legs may adversely affect the locomotor abilities of ticks and, as a result, their effectiveness in finding and attaching to hosts [19, 21]. It is worth noting that the presence of anomalies, especially those associated with gnathosoma and deformities of the legs and certain leg segments, may hinder correct taxonomic identification of the tick, subsequently making epidemiological predictions more difficult [12, 9, 21].

CONCLUSIONS

The study presents a rare case of ectromely in a medically-significant *Ixodes ricinus* tick. Further studies on the prevalence of anomalies and pathogens in ticks are needed to determine the possible effects of anomalies on the vector competence and usefulness of ticks as an indicator of environmental pollution. Reporting anomalies among ticks can also help in reducing taxonomic errors that may affect epidemiological predictions of tick-borne disease risk.

Acknowledgement
The study received a grant (Grant No. OPUS 2020/37/B/NZ6/01587) from the National Science Centre (NCN), Kraków, Poland

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