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Sand Lizards *Lacerta agilis* Linaeus, 1758 (Lacertidae) as Hosts for Tick-borne Pathogens in the Wielkopolska National Park, Poland

***Jolanta Behnke-Borowczyk*[1], *Rafał Kurczewski*[2] *& Dariusz J. Gwiazdowicz*[1]**

[1] Faculty of Forestry, Poznan University of Life Sciences, Wojska Polskiego 71C, 60–625; Poznań, Poland; E-mail: jolanta.behnke@up.poznan.pl (orcid.org/0000-0003-2085-038X); dariusz.gwiazdowicz@up.poznan.pl (orcid.org/0000-0002-0064-2316)
[2] Wielkopolska National Park, Jeziory, 62-050 Mosina, Poland; E-mail: r.kurczewski@wielkopolskipn.pl

Abstract: The incidence of tick-borne diseases has increased in recent years. Studies on sand lizards have demonstrated that they carry a number of acarid species. The purpose of this study was to identify the transmissible pathogenic organisms in *Ixodes ricinus* removed from *Lacerta agilis* in the Wielkopolska National Park in 2015. The ticks were identified using a stereomicroscope and by comparing with taxonomical descriptions. Isolation of DNA was performed using a Modified Genomic Maxi AX Direct. The primers used were those used for the detection of haemoparasites transmitted by ticks. In total, 56 nymphs and 34 larvae were removed from 15 of 47 examined lizards (overall tick prevalence 31.9%). Larvae and nymphs of *Ixodes ricinus* were found on 12 lizards. The highest tick burden was 14 (nymphs). Successful PCR amplicons were from *Rickettsia* spp. These mono-specific infections did not allow analysis of interactions between co-occurring pathogens. It is clearly necessary to extend this work targeting a much larger sample size of sand lizards and to combine this with concurrent sampling of blood from lizards.

Key words: *Ixodes ricinus*, haemoparasites, *Rickettsia*, parasitic, natural reserve

Introduction

Parasitic acarid arthropods, such as ticks and mites, play an important role as vectors of a range of infectious diseases, including those caused by parasitic protozoans, viruses and bacteria (Cholewiński et al. 2017). The incidence of tick-borne diseases in humans around the world has increased in recent years and, therefore, there is a need for a better understanding of the tick-host relationship and the epidemiology of infectious agents that they carry (Deryło 2012, Brochocka et al. 2014). Previous studies on ticks have focused primarily on mammalian and avian hosts; however, ticks also infest other vertebrate hosts, including reptiles (Gut & Prokopowicz 2002, Gern 2008).

Relationships between ectoparasites such as mites and ticks and reptilian hosts have been the subject of several studies (Fain 1962, Bauwens et al. 1983, Haitlinger 1987, Gwiazdowicz & Filip 2009a). Such studies on sand lizards *Lacerta agilis* L., 1758 have demonstrated that they carry a number of different acarid species: *Dermacentor reticulatus* (Fabricius, 1794), *Haemaphysalis concinna* (Koch, 1844), *Ixodes trianguliceps* (Birula, 1895) and *Ophionyssus saurarum* (Oudemans, 1901) (Siuda 1993, Gwiazdowicz & Filip 2009b). To-date, attention has focused largely on the castor bean tick (*Ixodes ricinus* L., 1758), which is a common ectoparasite of reptiles in the western Palaearctic (Barnard & Durden 2000). This species has the widest distribution range among European ticks and has been observed often on sand lizards (Bauwens et al. 1983, Matuschka et al. 1991, Gryczyńska–Siemiątkowska et al. 2007, Földvári et al. 2009). Infestations with this species have pathological consequences for infected hosts and therefore represent a significant handicap (cost of infestation) for lizards, e.g. European green lizards (Václáv et al. 2007).

Ixodes ricinus is known to be a vector of *Borrelia burgdorferi* s. l., *B. lusitaniae* and bacteria of the family Anaplasmataceae. Until recently, it has been considered that *Borrelia* spp. do not occur in reptilian hosts but reside only in mammals and birds (Ekner et al. 2011). However, a relatively low prevalence of *Borrelia* spp. has been observed in *Lacerta agilis,* which were heavily infested with ticks, an infection level contrasting with that in rodents, in which prevalence is generally higher (av. 4.9%) (Dudek et al. 2016, Bajer et al. 2014). In this context, it is pertinent that Lane & Quistada (1998) have reported a bactericidal effect on *Borrelia* spirochetes of serum complement from the lizard *Sceloporus occidentalis* (Baird & Girard, 1852).

Although several publications have reported aspects of the host-parasite relationships of ticks on lizards and of the pathogens that the ticks may carry (Eisen et al. 2001, Dsouli et al. 2006, Majláthová et al. 2006, Scali et al. 2001, Tälleklint-Eisen & Eisen 1999), there is still a lack of fundamental knowledge on the epidemiology and epizootiology of these relationships, especially regarding the most common lizard species in Central Europe, *L. agilis*. Therefore, the purpose of this study was to identify the transmissible pathogenic organisms in *Ixodes ricinus* removed from *L. agilis* that were caught in the Wielkopolska National Park.

Materials and Methods

The survey was carried out in April–August 2015 in the Wielkopolska National Park (52˚13'39.4"–52˚19'08.0"N, 16˚32.0"–16˚46'08.0"E), which is a protected natural reserve. The collected ticks were preserved in 90% ethanol, counted and identified with the use of a stereomicroscope and by comparison with taxonomical key (Siuda 1993, Nowak-Chmura 2013).

Isolation of DNA was performed using a Modified Genomic Maxi AX Direct (A&A Biotechnology, Gdynia, Poland) kit. An elongated lysis was used, which was carried out at 50°C for 72 hours with continuous shaking. Quantitative and qualitative evaluation of DNA extraction was carried out spectrophotometrically with the NanoDrop® ND-1000 system (PeqLab Erlangen, Germany). The primers applied were those used for the detection of tick haemoparasites. The primers and cycling conditions used in this study are describe by Armstrong et al. (1998) and Bonnet et al. (2007), for *Babesia* spp., Norman et al. (1995), Maggi et al. (2006) and Paziewska et al. (2011) for *Bartonella* spp., Regnery et al. (1991) and Roux & Raoult (2000) for *Rickettsia* spp., Inokuma et al. (2002) for *Hepatozoon*, Noyes et al. (2002, 1999) for *Trypanosoma*. As a positive control for the DNA, haemoparasites were taken from the blood of the bank vole (*Myodes glareolus*), the eastern spiny mouse *Acomys dimidiatus* (Cretzschmar)*,* the golden spiny mouse *A. russatus* (Wagner) and Wagner's dipodil *Dipodillus dasyurus* (Wagner) (Bajer et al. 2014, Alsarraf et al. 2016). PCR products were separated using electrophoresis on a 1.5% agarose gel and visualised with Midori Green stain (Nippon Genetics, Dueren, Germany). Marker Dramix (A&A Biotechnology, Gdynia, Poland) DNA was used as a molecular-weight size marker. The PCR products were sequencing by Genomed S.A. (Warszawa, Poland).

The prevalence of infection (percentage of animals infected) was calculated separately for total tick larvae and nymphs recovered; values were reported with 95 % confidence limits, calculated by bespoke software based on the tables of Sokal & Rohlf (1995). Values for mean abundance of infection (mean tick burden among all sampled lizards including those that were not infected) are given with standard errors of the mean (S.E.M.). Mean intensity of infection (mean tick burden per infected tick) is also provided.

Results

In total, 90 ticks (56 nymphs and 34 larvae) were removed from 15 of 47 examined lizards giving an overall tick prevalence of 31.9 % (CL_{95}=17.57–49.59%), an abundance of 1.9 ± 0.52 and intensity of 6.0 ± 0.995. For nymphs, the values were: prevalence 31.9% (CL_{95}=17.57–49.59%), abundance 1.2 ± 0.41, intensity 3.7 ± 1.02. For larvae, these values were: prevalence 25.5% (CL_{95}=12.84–43.14%), abundance 0.7 ± 0.25 and intensity 2.8 ± 0.72. Both larvae and nymphs of *I. ricinus* were found on 12 lizards. Three individuals harboured only nymphs. Larval tick burdens ranged from 0 to 8 larvae, those of nymphs from 0 to 14 ticks, and the highest tick burden was 14 (all nymphs).

The only successful PCR amplicons were for *Rickettsia* spp. Four nymphs (removed from three lizards) yielded PCR products with the *Rickettsia* specific primers, giving a prevalence of *Rickettsia* of 4. 4% (CL_{95}=1.07–13.59%) among the 90 isolated ticks, 20.0% (CL_{95}=6.7–46.57 %) among the 15 tick-infested lizards, and 6.4% (CL_{95}=1.23–20.44%) among all the sampled lizards. The sequence was compared with GenBank entries by Blast N2.2.13 and revealed 100% homology with 100% similarity to *R. helvetica* (Genbank AM418450, DQ821857, DQ910785 and EF392725).

Discussion

Rickettsia spp. are the most widespread parasites found in lizards and ticks, with generally a higher infestation among nymphs compared with larvae (Sousa et al. 2012). Our results confirm the occurrence of *Rickettsia helvetica* in *Ixodes ricinus* ticks in Poland as well as the observed infection rate (prevalence of 4.4% among sampled ticks) is comparable to those registered in Slovenia (4.6%) (Prosenc et al. 2003) and Austria (4.8%) (Rehacek et al. 1997). However, it is much lower than that in similar studies in Spain (16.7%) (Fernandez-Soto et al. 2004) and Germany (12%) (Wölfel et al. 2006). Our results are similar to those reported in earlier studies in Poland (from 0.6% to 16.3%, depending on the region studied). Regional differences in prevalence values of this bacterium in ticks (Madej & Śliwa 2014) emphasise the need for long-term monitoring and further systematic analysis of tick samples to determine whether infection is stable or spreading, and if it is spreading, the directions of the spread of *R. helvetica*. Our current state of knowledge regarding this pathogen among ticks and wild animals is still insufficient, and our study contributes to complement current knowledge.

In Poland, cases of *Borrelia burgdorferi* s. l. have been reported in ticks derived from sand lizards (Cieniuch 2016). Only larvae and nymphs, but no adult stages of ticks, have been observed on reptiles. Similar results have been obtained in studies on the viviparous lizard *Zootoca vivipara* (Jacquin, 1787) carried out in Germany (Matuschka et al. 1992) and our results concur with these findings. In Poland, approximately 1.2% of sand lizards have been reported to be infected with *Borellia lusitaniae,* which is the only species detected in all the studied lizard species as well as in the majority of ticks infecting them. These observations underline the association of this pathogen with particular species of reptiles and suggest tight host-specificity (Dsouli et al. 2006, Amore et al. 2007, Földvári et al. 2009).

Reports of tick infestations of reptiles carrying *Anaplasma* spp. include hosts such as the western fence lizard *Sceloporus ocidentalis* (Baird & Girard, 1852), the sagebrush lizard *S. graciosus* (Baird & Girard, 1852) and the northern alligator lizard *Elgaria coeruleus* (Crother, 1828) in North America, and sand lizards in Europe (Nieto et al. 2009, Nowak at al. 2010, Tijsse-Klasen et al. 2010, Václav et al. 2010). Prevalence with *Anaplasma* spp. in ticks (detection based on DNA identification) removed from lizards can be up to 28.3% (Nieto et al. 2009, Václav et al. 2010) but, nevertheless, despite these records of relatively high prevalence of *Anaplasma* spp., our tests failed to reveal the presence of this pathogen in samples of ticks from sand lizards.

The probability of host exposure to infestation by tick-borne pathogens is correlated with tick abundance (Ginsberg 1993, 2008). The more ticks that are acquired by a lizard, the greater the probability of contact with infected ticks, and thus the greater the likelihood of acquiring a tick-borne infection. Ticks can be infected by two or more microorganisms simultaneously (Ginsberg 2008, Schouls et al. 2009, Dietrich et al. 2010, Václav et al. 2010) but the relationships between these co-infecting microorganisms are variable (Ekner et al. 2011). Some infections exhibit antagonistic interactions with one another, while others show synergistic interactions and many clearly do not interact (Ginsberg 2008). When they do occur, such interactions between microorganisms can be subject to the influence of many factors, including the number of co-infections, microclimate, vegetation and density of ticks (Hildebrandt et al. 2003). Future studies, based on larger sample sizes should also reveal whether the lizards and ticks in our study sites carry other micro-organismal pathogens and if so, in due course should allow the relationships between the identified pathogens to be understood.

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