

Preliminary studies on the prevalence of selected pathogens threatening human health in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from wild animals of Warmia and Mazury, part I

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As a result of human activity, ecosystems are a mix of natural habitats and farmland. Such transformed ecosystems affects the population abundance, its composition and integrity, additionally enable contact between wildlife, farm animals and humans. This, in turn, promotes the circulation of pathogens among many different hosts. Wild animals, even those with very low population densities, are important hosts having impact on the increase in tick abundance and affecting ticks reproduction. Wild ungulates passively spread ticks which are potentially infected with viruses, bacteria or protozoa. Transmitted microorganisms can be pathogenic to both humans and pets. Wild animals screening such as cervids and wild boar is essential to elucidate the linkages of pathogens transmitted by wild, livestock and domestic animals. These studies also provide basic information for assessing the risk to animal and human health in a given region. Among 22 genospecies belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex, five are considered pathogenic to humans. The most common diagnosed in Europe is *B. afzelii* and *B. garinii*. Rickettsiales including *Anaplasma phagocytophilum* and *Rickettsia* spp. from the spotted fever

group are potential risk to human health and animal welfare as well.

The aim of the research was to compare the frequency of *Borrelia* spp., *Rickettsia* spp. and *A. phagocytophilum* in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from wild ungulates (deer and wild boar) in north-eastern Poland during two hunting periods in 2018–2020. In total, 484 ticks were collected, 50% of which were *I. ricinus* (n = 242; collected from deer: 199 females, 21 males, n = 220, 91%; from wild boars: 16 females, 5 males, 1 nymph, n = 22, 9%) and 50% *D. reticulatus* (n = 242; harvested from deer: 46 females, 173 males, n = 219, 90%; from wild boars: 4 females, 19 males, n = 23, 10%).

Conducted preliminary studies, confirmed that almost 40% of the collected ticks (191 out of 484) were infected with the following pathogens: 3.3% *Borrelia* spp., 19.2% *A. phagocytophilum* and 26.9% *Rickettsia* spp. *Borrelia* spirochetes were only identified in ticks collected from deer. The restriction fragment length polymorphism (RFLP) method was used to identify the *Borrelia* species. RFLP was performed on the products of *fla* gene amplification. The presence of the following spirochete genospecies: *B. afzelii*, *B. garinii*, *B.*

lusitaniae and *B. miyamotoi* was confirmed. *Rickettsia* spp. was identified by *gltA* gene amplification and sequences analysis. GenBank data allowed to confirm the presence of *R. helvetica*, *R. raoultii* and *R. monacensis*. Identification of *A. phagocytophilum* in ticks was done targeting 16S rRNA gene fragment. Among the infections, monoinfections (151/191, 79.1%) prevailed over co-infections (40/191, 20.9%). The most frequently recorded co-infection was *A. phagocytophilum/Rickettsia* spp. (28/40, 70%). Coexistence of *B. afzelii/A. phagocytophilum* (4/40, 10%), *B. afzelii/Rickettsia* spp. (4/40, 10%), *B. afzelii/A. phagocytophilum/Rickettsia* spp. (2/40, 5%) *B. miyamotoi/A. phagocytophilum* (1/40, 2.5%) and one case of *B. afzelii/B. garinii/B. lusitaniae* co-infection (1/40, 2.5%) were recorded. Significant differences in the affinity of some pathogens for their vectors were observed. *Borrelia* spp. and *A. phagocytophilum* were more often identified in *I. ricinus* ticks (5.3% and 23.1%) than in *D. reticulatus* (1.2% and 15.3%, respectively). The incidence of *Rickettsia* spp. Infections was similar (approximately 25–29%) in both species of ticks. The prevalence of *A. phagocytophilum* and *Rickettsia* spp. In ticks obtained from deer animals was 19.8% and 27.1%, and in ticks isolated from wild boar – 13.3% and 24.4%, respectively.

Summing up, in ticks collected from wild ungulates in north-eastern Poland, spirochetes included in the *B. burgdorferi* sensu lato complex (*B. afzelii*, *B. garinii* and *B. lusitaniae*), but also *B. miyamotoi* spirochetes, *A. phagocytophilum* and *Rickettsia* spp. (*R. helvetica*, *R. raoultii* and *R. mo-*

nacensis) bacteria were identified. The low spirochetes prevalence among ticks collected from wild deer can be explained by the reduction of *Borrelia* spp. infection during tick feeding on large wild animals („deer have a zooprophylactic effect on *B. burgdorferi* s.s. in ticks”). The zooprophylactic effect of these hosts becomes evident in competition with smaller species of tick hosts, thereby diverting the vectors from feeding on the spirochete reservoir hosts. This may be due to the host’s natural immunity, including alternative complement activation in the blood of ungulates. This mechanism effectively eliminates *Borrelia* spp. in the body of ungulates, and in ticks feeding on them. On the other hand, the relatively high level of infestation by ticks infected with *A. phagocytophilum* and spotted fever rickettsiae may indicate that these animals are important reservoirs for both pathogens, contributing to their circulation in nature and a potential source of infection transmitted by ticks on people. The results of preliminary studies also indicate that in north-eastern Poland wild cloven-hoofed animals, strongly exposed to ticks, are infected with a single species of bacteria more often than with many pathogens with potential clinical significance. This can probably be associated with the large population dispersion of wild-living animals.

The authors of the study are currently starting the research of subsequent batches of ticks from the area of Warmia and Mazury and from the area of one northern Poland provinces and southern Poland, in order to conduct a comparative analysis of the research results.