OTKA K 115854 (NKFI 130216) zárójelentés

The following results were achieved in the course of four years:

(1) We demonstrated for the first time, that bats may act as reservoirs of one of the most important tick-borne protozoan pathogens of the dog, i.e. Babesia canis. The results were published in a journal paper.

(2) We organized the first phylogeographical survey of bat ticks encompassing Eurasia, which demonstrated that (a) bat ticks show high degree of genetic heterogeneity over large geographical distances, and (b) all three ixodid bat ticks that occur in Europe appear to be also widespread in Asia. The results were published in a journal paper.

(3) We screened large numbers of Haemaphysalis sp. ticks for protozoan pathogens, and detected certain piroplasms for the first time in Hungary/Europe. The results were published in a journal paper.

(4) We assessed the phylogeography of bird ticks arriving in Hungary from various directions. We discovered for the first time that a widespread tick species of birds (Ixodes frontalis) has two well separated, distinct genetic lineages. We also found a tick species (Ixodes festai) for the first time in Hungary. The results were published in a journal paper.

(5) We demonstrated for the first time in a worldwide context, that (a) insectivorous bird species not only consume large amounts of insect moulting hormones (ecdysteroids) with their food, but these hormones also appear in high concentrations in their blood. (b) These ecdysteroids can be taken up by ixodid ticks sucking blood from insectivorous birds, and will damage these ticks by inducing earlier moulting (i.e. detachment from their avian host). The results were published in a journal paper.

(6) We screened large numbers of Dermacentor reticulatus ticks (collected in urban habitats) for the protozoan pathogen Babesia canis. The two genotypes of Babesia canis were demonstrated to have a shift in their seasonality. Two very rare malformed ticks were also found, and illustrated the potential consequences of environmental contamination in cities. The results were published in a journal paper.

(7) We described a new ticks species (ixodes collaris sp. nov.) from two bat species that also roost in buildings. The results were published in a journal paper.

(8) We finished the description of the life cycle stages of an ixodid tick species, Ixodes ariadnae. This species has recently been discovered and described (for the first time in the world) in Hungary. Ixodes ariadnae almost exclusively infests bats of the genus Myotis, several species of which prefer buildings as summer roosts, thus having potentially high epidemiological significance. The results were published in a journal paper.

(9) We found the westernmost occurrence of the bat tick species *Ixodes ariadnae* (discovered and described by S. Hornok) in Europe (in Belgium). This result is relevant to point B1 of the original research work plan.

(10) In this study we screened 308 ixodid bat ticks (collected from 17 bat species) for piroplasms. The results indicated the presence of DNA from various *Babesia* and *Theileria* species of ruminants, humans or dogs. Bat ticks are not known to infest the latter hosts, therefore the most likely origin of these piroplasms is the blood of bats. These results are relevant to point B1 (and because of the zoonotic pathogen slightly to point ABC2) of the original research work plan.

(11) We collected 321 *Haemaphysalis concinna* ticks from 121 passerine birds, and screened them for piroplasms. Central and East Asian *Babesia* genotypes were identified with sequencing, suggesting a connection of relevant tick populations via migratory birds. These results are relevant to points A2 and B1 of the original research work plan.

(12) We collected the second most important and emerging tick species of Central Europe, *Dermacentor reticulatus* in urban habitats and analysed them for rickettsiae. The results showed that single habitat or seasonally biased sampling is inconsistent with the actual prevalence of these zoonotic bacteria. These results are relevant to point ABC2 of the original research work plan.

(13) In this nearly pan-Mediterranean study we demonstrated for the first time that two genetic lineages of the world-wide most important tick species, *Rhipicephalus sanguineus* are geographically separated in the Mediterranean Basin. In addition, these data mean the first sequence-verified, autochthonous and not imported case of the occurrence of this tick species in Hungary. These results are relevant to point ABC1 of the original research work plan.

(14) We detected a newly discovered *Neoehrlichia* species/genotype for the first time in a badger and in a less common tick species, *Ixodes canisuga*. In addition, we reported the occurrence of badger babesiae (known to occur in Western, Southern Europe) for the first time in Central Europe. These results are relevant to point C1 of the original research work plan.

(15) We found the DNA of free-living bodonids, *Bodo saltans* and neobodonids (which are important in the evolution of pathogenic trypanosomes) in bat ticks. We suppose that the source of this PCR positivity was the blood of bats, which may have had access to these bodonids (or at least their DNA) from natural water surfaces. These results are relevant to point B1 of the original research work plan.

(16) In this nearly pan-European study we clarified the morphological differences of four tick species of (primarily) carnivores. We also reported here the occurrence of *Ixodes kaiseri* for the first time in Hungary, Germany and Serbia. These results are relevant to point ABC1 of the original research work plan.

(17) We discovered two new, highly divergent genotypes of the economically important pest, the common bedbug (*Cimex lectularius*) associated with bats. These results are in part relevant to points C2 and ABC2 of the original research work plan.

(18) We demonstrated that specimens of the bat soft tick species, *Argas vespertilionis* collected in Central Asia are genetically much more closely related to geographically distant conspecific populations in Central Europe, that to others in Southeast Asia. This can be interpreted with geographical barriers in the latter direction, whereas confluent bat populations and/or migration between Central Europe and Central Asia. This was confirmed by sequences of *Babesia vesperuginis* detected in these soft ticks. These results are relevant to point B1 of the original research work plan.

(19) We also examined mitochondrial genetic markers of *Argas vespertilionis* in the Old Word. Samples from Europe were different from those collected in Kenya and Vietnam, in the latter context both morphologically and genetically. These results are also relevant to point B1 of the original research work plan.

(20) We investigated the presence of *Babesia* and *Theileria* species in cervids (red, fallow, and roe deer), as well as in water buffaloes, mouflons, and wild boars. The latter were PCR negative, and in the former only *Theileria capreoli* was detected. The genotypes of these showed significant association with their host species (genotype "capreoli-CE1 with roe deer, genotype "elaphi-CE1" with red and fallow deer. These results are also relevant to point A2 of the original research work plan.

(21) We demonstrated for the first time that building freeways may result in habitat fragmentation of ticks, and therefore in biased occurrence of different tick species and tick-borne pathogens on the two sides of a fenced freeway. We also reported the high prevalence of the Lyme disease agent, *Borrelia burgdorferi* sensu lato in forested resting places, rendering these into high risk areas (Lyme disease hotspots). These results are also relevant to point ABC3 of the original research work plan.

(22) We collected bat ticks (Ixodes simplex) in a man-made artificial habitat (mine). We provided and illustrated morphological characters of the males in a publication, because of its poor original description. These results are relevant to points A3, A4 of the original research work plan.

(23) As part of the subproject of monitoring tick species that may emerge in Hungary, in the previous year we published the first, molecularly verified and autochthonous occurrence of the brown dog tick (Rhipicephalus sanguineus) in Hungary. In this second study we evaluated if cats can also be important hosts of this important tick species. These results are relevant to point ABC1 of the original research work plan.

(24) For this study we chose a non-invasive sampling method, because of the highly protected nature of bat and bird species evaluated as tick-borne pathogen reservoirs. Relevant to bats, we identified

several Rickettsia spp./genotypes and Neorickettsia risticii (the latter for the first time in Europe). Samples from two highly urbanized bird species contained rickettsia DNA and Anaplasma phagocytophilum. These results are relevant to point B1 of the original research work plan.

(25) We analysed ticks and blood samples from rural dogs and badgers for the DNA of an important group of tick-borne pathogens, the family Anaplasmataceae. Four new species/genotypes were found, which are new in canine hosts in a world-wide context. These included Anaplasma marginale, a badger-associated Ehrlichia sp., a Candidatus Neoehrlichia lotoris-like genotype and arthropod-associated wolbachiae. These results are relevant to points A2, C1 and ABC1 of the original research work plan.

(26) Bats are known to be carriers of one species of piroplasms (tick-borne protozoan parasites), i.e. Babesia vesperuginis. In this study, we amplified its 18S rRNA sequence from bat soft ticks collected in Hungary and evaluated it in a broad phylogenetic context. Our results demonstrated for the first time that B. vesperuginis does not belong to Babesia sensu stricto, but is more closely related to theilerids. These results are relevant to points A4, B1 of the original research work plan.

(27) We evaluated molecularly blood samples from rural dogs for the presence of piroplasms. For the first time in a world-wide context, we identified Babesia sp. badger type-A in dogs, in particular in a higher number of animals than those in which Babesia canis was detected. This badger-associated piroplasm was most frequently found in dogs often taken to forests and used for hunting; and among ticks only in Ixodes canisuga (not in I. hexagonus). These results are relevant to points A2, C1 and ABC1 of the original research work plan.

(28) During a large scale survey of game animals and buffaloes for vector-borne bacteria in Hungary, we found that forest-dwelling game animal species (i.e., cervids, wild boars) are significantly more important carriers of an Ixodes ricinus-borne bacterium, Anaplasma phagocytophilum, than animals grazing grassland (i.e. buffaloes). Examined species rarely carried other tick-borne bacteria, i.e. rickettsiae. These results are relevant to point A2 of the original research work plan.

(29) Blood samples of 259 long distance migratory birds (warblers: Acrocephalus spp.) were analysed for tick-borne bacteria (Anaplasmataceae). Unexpectedly, we found arthropod-associated Wolbachia DNA (most likely of ectoparasite origin) in avian blood. This result is relevant to point B1 of the original research work plan.

(30)-(32) Three further studies did not target ticks or tick-borne pathogens, but alternative blood-sucking arthropod vectors of the latter. Some of these were analysed first taxonomically (and not for tick-borne pathogens), because of the low number of specimens available for each species. We discovered one new bat bug species and several new flea genotypes (two of which are potentially new spp./ssp.). In addition, we screened tick- and flea-borne rickettsiae in cat fleas, and found two new pathogens in two countries of the Mediterranean Basin. These results are in part relevant to point C2 of the original research work plan.

(33) We collected and described the male and larva of the new bat tick species, Ixodes collaris Hornok, 2016. These results are relevant to points B1 and C1 of the original research work plan.

(34) We screened all bat-associated soft tick DNA samples for the presence of a broad range of bacterial pathogens. Among the others, we identified several Bartonella and Rickettsia species, among the latter also R. africae for the first time in Europe. These results are relevant to point B1 of the original research work plan.

(35) We collected specimens of a tick species, Hyalomma anatolicum, which is indigenous in the Mediterranean region and Asia (but not yet reported from Hungary), to evaluate their mitochondrial markers and to screen them for tick-borne pathogens. We detected a Trypanosoma sp. and we obtained the sequence of Babesia occultans for the first time from this tick species. These results are partly relevant to point C1 of the original research work plan.

(36) We examined bat ticks collected in Pakistan for mitochondrial markers and tick-borne pathogens, and we discovered a hitherto unknown Argas vespertilionis haplotype, a potentially new bat-associated soft tick species and a novel Rickettsia honei genotype. These results are relevant to point B1 of the original research work plan.

Preparation of the map (summary) of tick and tick-borne pathogen species in Hungary is in progress.