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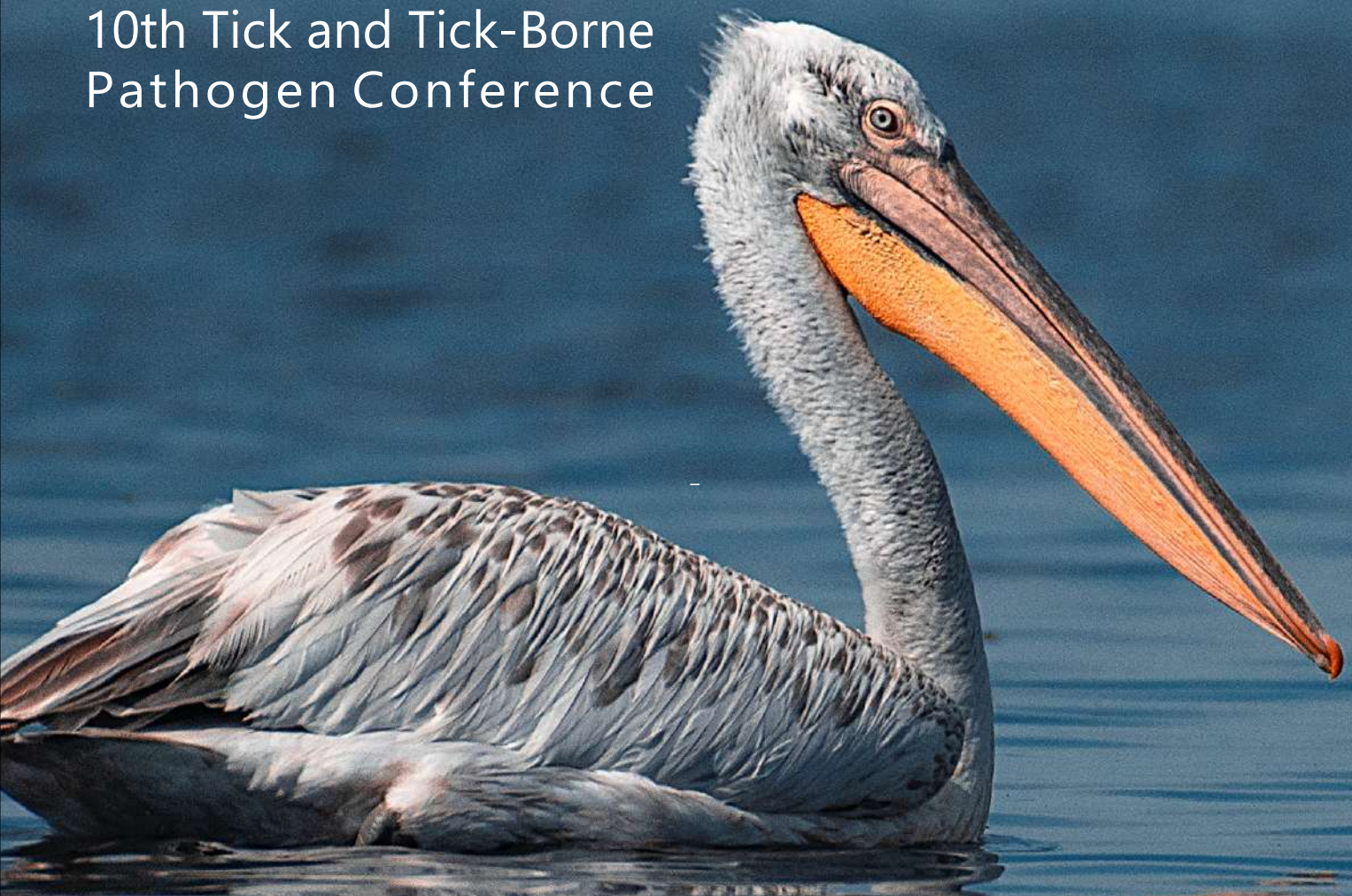

29 August-2 September 2022

Murighiol, Danube Delta, Romania

Abstracts

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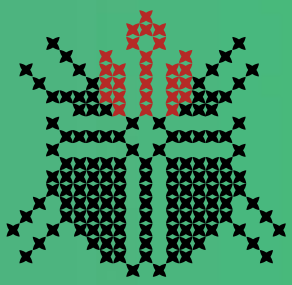

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PLENARY TALKS (PL01-PL05)

PL01 *Ixodes ricinus*-borne disease risk: disentangling tick and pathogen life cycles

Sprong H

Centre for Infectious Disease Control, National Institute of Public Health and Environment, The Netherlands

The density of questing *Ixodes ricinus* nymphs infected with tick-borne pathogens is one of the parameters that determines tick-borne disease risk. Vertebrate species contribute differently to the feeding and propagating ticks, and differ in pathogen competence: the ability to transmit a pathogen to ticks. Therefore, we investigated how wildlife communities contribute to tick-borne disease risk. The density of *I. ricinus* nymphs infected with *Borrelia burgdorferi* sensu lato, *Neoehrlichia mikurensis* and *Anaplasma phagocytophilum* among forest sites were correlated to the density of mammals and birds, determined by (camera) trapping and mathematical modelling. We found that the density of *I. ricinus* nymphs was positively associated with ungulates, and negatively associated with leporids and foxes. The density of nymphs infected with a pathogen increased with the density of competent hosts: rodents for *B. afzelii* and *N. mikurensis*, ungulates for *A. phagocytophilum* and birds for *B. garinii* and *B. valaisiana*. The density of nymphs infected with *B. miyamotoi* increased with the density of questing nymphs and with rodent density. Remarkably, increasing densities of rodents were associated with decreasing densities of nymphs infected with bird-associated *Borrelia*. An increasing diversity of the vertebrate community was not associated with decreasing densities of questing *Ixodes ricinus* nymphs. In a separate field study, rodent densities were manipulated in plots of 2500 m^2 by (mock-)supplementing acorns or by rodent trapping during two years. The density of nymphs infected with pathogens were correlated with the (fluctuations in) rodent densities. Strong positive associations between rodent density and the density of nymphs infected with *B. afzelii* or *N. mikurensis* were found. Pathogens that predominantly rely on vertical transmission, for example *Borrelia miyamotoi*, only showed moderate associations between rodent density and the density of infected nymphs. Remarkably, the infection prevalence of nymphs infected with *B. garinii*, decreased with increasing rodent density, but the density of *B. garinii*-infected nymphs remained stable. Furthermore, temporal fluctuations in rodent densities did not result in detectable fluctuations in tick and pathogen densities. Our results draw attention to the importance of considering spatial and temporal scales as well as transmission modes of tick-borne pathogen in the generation of *Ixodes ricinus*-borne disease risk.

PL02 The many scales of the tick-borne pathogen's relationships: an epidemiological tale

Estrada-Peña A

Department of Animal Health, University of Zaragoza, Spain

In the several years that happen since the concept of "One Health" was built, few studies have been devoted to the integral application of the concept to the tick-borne pathogens affecting human health. In the last decades, we assisted to the development of powerful sequencing methods that are raising a deeper knowledge on these pathogens. We also improved our knowledge about tick-host relationships (i.e., main hosts of each species of tick) in orders of magnitude. Information about the geographical distribution of wild hosts is massively available, as are databases containing details on morphological and physiological traits of vertebrates. Finally, weather data for large regions are routinely captured by a cloud of earth-orbiting satellites, at a resolution unexpected only a few years ago. A change of paradigm seems to be necessary if we aim to integrate all these data into a unifying framework. The main purpose is the proposal for implementing an agenda for research. That agenda is specifically focused on the preparation of our society for the impact, adaptation, and resilience. It must to be supported by the concepts of the One Health approach, with a background of climate change and considering the contributions of both livestock and wildlife in the circulation of tick-borne pathogens. In this presentation I would like to introduce different methods of integration of these data into an approach implementing different perspectives. Even if we continue capturing the intricate processes of these pathogens at the local or regional scales (the foundations over which that agenda for research must be erected), it seems unnecessary to continue promoting alternatives of control at that scale: a large area approach is an essential part of a new paradigm. The relationships between climate, human habits, livestock management and niche overlap of ticks with the wildlife could be understood and translated to diverse strategies of control at large scales. This is the scale at which major webs of interacting organisms are studied: the proposed agenda must take our current knowledge one step forward. This is also the purpose of the One health concept: the integration of approaches from different fields into a unique frame of reference.

PL03 Paradigms in tick evolution

Mans BJ[1, 2, 3]

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[2]Department of Life and Consumer Sciences, University of South Africa, South Africa
[3]Department of Veterinary Tropical Diseases, University of Pretoria, South Africa

Ticks adapted to a blood-feeding lifestyle by evolving various mechanisms unique to tick biology. This includes mechanisms to detect the host, modulate the host immune and hemostatic systems, and process and digest the blood meal and eliminate excess water derived from the blood meal. Evolution of these mechanisms entailed novel structural and morphological innovations and/or exaptation of existing structures for blood feeding. This included genetic innovations that allowed evolution of new functions involved at the feeding interface. Not only did these innovations need to be efficient and specifically suited to its functional purpose to allow effective feeding, but had to evolve within existing biological frameworks that shaped and influenced the evolutionary trajectories followed by different tick lineages. The unique biology of different tick lineages may be explored and interpreted from this perspective to allow a synthesis of shared and novel characters that define ticks as monophyletic, obligate blood-feeding arachnids that nonetheless display remarkable lineage specific diversity. The reconstruction of ancestral characters enables contextualization of these paradigms, while technological advances illuminate the complexities involved in the mechanisms behind blood-feeding. For such reconstruction accurate systematic frameworks is essential that require the use of extensive systematic markers that may include mitochondrial genomic and nuclear phylogenomic approaches. A robust understanding of tick biology requires integration of knowledge from different disciplines that will allow definition and description of a fundamental and holistic model of tick evolution. Essential to this endeavor is the construction of well annotated sequence databases and empirical confirmation of predicted functions encoded by proteins involved in tick-host interactions.

PL04 *Ixodes ricinus* – a personal perspective

Gray JS

UCD School of Biology and Environmental Science, University College Dublin, Ireland

Ixodes ricinus, the most abundant tick in northern and eastern Europe, has been the subject of scientific research for approximately 90 years, and more papers have been published on this tick species than on any other. This presentation will consider past, present and potential future studies. Early research, in the 1930s and 1940s, concerned the tick's role as a disease vector and parasite of farm animals in northern Britain, with attention subsequently shifting to the transmission of zoonotic tick-borne encephalitis in central and eastern Europe. Another surge of interest in *I. ricinus* resulted from its incrimination as a vector of Lyme borreliosis in the 1980s, and since then there has been a marked increase in studies on the detection of pathogens in questing stages, mainly driven by the advent of molecular methods. There are now many ongoing and developing studies on *I. ricinus*, but since it is impossible to pay appropriate attention here to every topic, three in particular will be briefly considered. The methods involved in the identification of vertebrate hosts by analysis of blood-meal remnants, a potentially a powerful tool for the study of tick-borne disease ecology, will be reviewed together with recent progress in its use in the field. Genetic variation between vector populations, particularly in relation to ecology and pathogen transmission, has received little attention so far, but there are now indications that important differences may occur between discrete populations of *I. ricinus*. The evidence will be reviewed and suggestions made for further research. Lastly, recent observations on the apparent effects of climate change on the distribution of *I. ricinus* will be described and possible future scenarios discussed.

PL05 The genus *Anaplasma*: comparative to functional genomics

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Although tick transmitted pathogens of the genus *Anaplasma* have been known for over a century, the obligate intracellular nature of these organisms has impeded progress in elucidating their biology and developing vaccines. Undoubtedly a genome sequence is a necessity to begin to understand the biology of these pathogens. Although rickettsial pathogens have reduced genomes, the technical challenges of working with these organisms has resulted in relatively few genomes being available for organisms in the genus *Anaplasma*, with only two species, *A. marginale* and *A. phagocytophilum*, having more than a single strain that have been sequenced, while *A. centrale*, *A. ovis* and *A. platys* are represented by a single strain each. Even for *E. coli*, approximately 35% of the genes lack a known function, and for these rickettsial organisms the percentage of the genome encoding genes of unknown function is a bit higher. Ascribing function to previously uncharacterized genes is painstaking work; we have employed both comparative and functional genomic approaches in attempts to reveal the biology of these pathogens. Comparative genomics analyses have provided some insight into mechanisms of persistence, have highlighted issues with species definition, and generated candidate lists of genes involved in tick transmission, however, many comparative analyses result in candidate gene lists, and without robust genetic systems in place we are unable to test these candidate genes for phenotype. *Anaplasma marginale* has been transformed only twice, however a breakthrough transposon mutant library was recently developed for *A. phagocytophilum* which has provided information on essential genes and provides a tool for characterizing genes of interest. Recent studies to elucidate the effectome – the set of proteins translocated by the type IV secretion system - of *Anaplasma* will be presented.

ORAL PRESENTATIONS

Ecology and epidemiology (EE01-EE43)

EE01 Tick-borne relapsing fever caused by *Borrelia persica*, epidemiology, transmission pathways and animal reservoirs

Baneth G

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Relapsing fever is an acute infectious disease caused by spirochetes of the genus *Borrelia* in the family of Borreliaceae. The disease in humans is characterized by spirochetemia with episodes of fever, separated by afebrile intervals. Tick-borne relapsing fever caused by *Borrelia persica* and transmitted by the tick *Ornithodoros tholozani* is common in Israel and other countries in the Near East extending from India and Central Asia to Egypt. Human infection is Israel is frequently associated with entrance to caves or ruins where the host tick is abundant. *Borrelia persica* has been found to cause infection and disease in domestic cats and dogs. We studied the life cycle and transmission of *B. persica* under natural conditions and in an experimental model using membrane feeding and maintaining the life cycle of the tick in all stages by artificial feeding. We also determined tick blood meal origins and detected *B. persica* infection in wildlife animals for the characterization of transovarial and transstadial transmission patters. The results of these studies indicated that *B. persica* is mainly transmitted transstadially while transovarial transmission is minimal. *Borrelia persica* infects a variety of wildlife mammals including wildlife canids such as golden jackals (*Canis aureus*) and red foxes (*Vulpes vulpes*), rock hyraxes (*Procavia capensis*) and also rodents. Infection in *O. tholozani* ticks was significantly associated with the presence of blood meals in general and specifically with blood meals from golden jackals and red foxes. This strongly suggests that wildlife canids are natural reservoirs for *B. persica* infection and are potentially able to transfer infection between remote populations of *O. tholozani* ticks in remote caves and shady environments.

EE02 Maternal behaviour in soft ticks

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[3]Department of Veterinary Tropical Diseases, University of Pretoria, South Africa

Female spiders, whip spiders and scorpions are known to display maternal behaviour by carrying the eggs and/or newly hatched offspring on the opisthosoma until they are able to support themselves independently. Certain whip scorpions and whip spiders enclose their eggs in a protective sac to their ventral opisthosoma. This ancestral arachnid phenomenon evolved in response to high predation or parasitism risk. Maternal behaviour was limited to *Antricola* (*Parantricola*) *marginatus* and was linked to its unique lifecycle where only the larvae are hematophagous. However, maternal behavior was also observed for *Argas* (*Argas*) *striatus* Bedford, 1932 and *Argas* (*Secretargas*) *transgariepinus* White, 1846. In addition, brooding behavior over egg batches were observed for *A.* (*S.*) *transgariepinus* adding to previous observations of the same phenomenon for *Argas* (*Argas*) *arboreus* Kaiser, Hoogstraal and Kohls, 1964, *Argas* (*Chiropterargas*) *boueti* Roubaud and Colas-Belcour, 1933, *Argas* (*Ogadenus*) *brumpti* Neumann, 1907, *Ornithodoros* (*Ornithodoros*) *moubata* Murray, 1877 and *Ornithodoros* (*Reticulinasus*) *salahi* Hoogstraal, 1953. Selection for maternal behavior may be due to evolutionary adaption to the harsh ecological habitats these ticks are living in that is unfavorable to larval survival. To circumvent this and ensure species survival, female ticks adapted special setae or a heavily corrugated posterio-ventral surfaces to aid the attachment of the larvae via their pulvilli which are reduced or absent in most argasid nymphs and adults. This biological adaption contributes to the uncommon sexual dimorphism between males and females of the Argasidae. The possibility that maternal behavior as unique adaption for argasids is an ancestral trait seem plausible since evolution of attachment pads and hooks has occurred more than once independently in insects and arachnid attachment structures and are considered analogous characters. Phylogenetic analysis would also support the inference that maternal behaviour is an ancestral trait and ancient adaptation in the Argasinae.

EE03 Ticks and tick-borne pathogens in Australia

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Australia is an island continent in south-east Asia, with unique fauna, including many native (endemic) tick species. There are also several imported (exotic) tick species that have become well established in Australia. Many of these are important reservoirs and vectors of animal diseases. Overall however there are only a few bacterial infections known to be tick-transmitted in Australia and, surprisingly, no viral tick-transmitted human or animal pathogens are yet recognised. The imported tick species include *Rhipicephalus sanguineus* (now *R. linnaei*, the former "tropical" clade of the brown dog tick), that arrived with the first human Aboriginal immigrants' dogs about 50,000 years ago; *Rhipicephalus australis* (Australian cattle tick) that arrived with European immigrants' cattle about 200 years ago; and *Haemaphysalis longicornis*, a more recent arrival. A. Tick transmission of human pathogens: (1) *Ixodes holocyclus* (+ *I. tasmani* + *I. cornuatus*) transmit *Rickettsia australis* (Queensland Tick Typhus) and *Coxiella burnetii* (Q fever); (2) *Bothriocroton hydrosauri* transmits *R. honei* (Flinders Island Spotted Fever); (3) *Amblyomma triguttatum* transmits *C. burnetii*. A study is currently underway to determine the aetiology of the "Lyme-like syndrome" that is said to follow tick-bite in Australia, and called "Debilitating symptom complex attributed to ticks (DSCATT)". B. Tick transmission of animal pathogens: (4) *R. linnaei* has recently become colonised with the exotic bacterium *Ehrlichia canis* and is causing widespread infection and death in dogs in northern Australia. This tick also transmits *Anaplasma platys*, *Babesia* spp. and *Mycoplasma* spp. to dogs; (5) *R. australis* transmits *A. marginale*, *Babesia* spp. and *Theileria* spp. to cattle; (6) *H. longicornis* transmits *Theileria* spp. to cattle. There remains much to be discovered about tick-transmitted infections in Australia.

EE04 Masting by beech trees predicts the abundance of *Ixodes ricinus* nymphs two years later

Bregnard C[1], Voordouw MJ[2]

[1]University of Neuchatel, Switzerland
[2]Department of Veterinary Microbiology, University of Saskatchewan, Canada

Understanding the ecological factors that determine the abundance of ticks is important for predicting the risk of tick-borne disease. In Europe, the sheep tick (*Ixodes ricinus*) is the most important vector of Lyme disease and other tick-borne diseases. We conducted a long-term study (15 years) at a location in Switzerland, in order to determine the ecological factors that drive inter-annual variation in abundance of *I. ricinus* ticks. Over a 15-year period (2004 to 2018), we monitored the abundance of *I. ricinus* ticks on a monthly basis at four different elevations on a mountain near Neuchâtel, Switzerland. We collected climate variables and obtained data on beech seed production from the literature. We used AIC-based model selection to determine which ecological variables best explain inter-annual variation in tick density. The top model explained 73.2% of the variation in our annual estimates of nymph density and contained the explanatory variables of elevation, year, beech tree masting index two years prior, and relative humidity. Increasing the beech tree masting index from very poor to full mast increased the abundance of nymphs by 86.2% two years later. The hypothesized underlying mechanism is that masting by deciduous trees in year 0 increases rodent density and the feeding success of larval ticks in year 1, which in turn increases the density of nymphal ticks in year 2. Public health officials in Europe should be aware that seed production by deciduous trees is a critical driver of the abundance of *I. ricinus*, and hence the risk of tick-borne disease.

EE05 Seasonality of host-seeking *Ixodes ricinus* nymph abundance according to different climates

Hoch T, Madouasse A, Jacquot M, Beugnet F, Bournez L, Cosson JF, Huard F, Moutailler S, Plantard O, Poux V, René-Martellet M, Vayssier-Taussat M, Verheyden H, Vourc'h G, Chalvet-Monfray K, Agoulon A

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There is a growing concern about climate change and its impact on human health. Specifically, global warming could increase the probability of emerging infectious diseases, notably because of changes in the geographical and seasonal distributions of disease vectors such as mosquitos and ticks. For the tick *Ixodes ricinus*, which is widespread in Europe, there is evidence of settlement further north and at higher altitudes. However, little is known about the seasonal variation in tick activity in different climates. Studies on the seasonality of *I. ricinus* abundance are rare and often limited in time, which impedes their generalizability. Our objective was to describe seasonal variations in *I. ricinus* abundance under different climates. Therefore, a longitudinal study was carried out in France, in six locations corresponding to different climates. Questing nymphs of *I. ricinus* were collected monthly during six years, using the drag sampling method. Meteorological variables (temperature, relative humidity) were recorded hourly and summarized daily. Seasonal patterns of *I. ricinus* nymphal abundance were investigated for the 6 different sites using linear regression incorporating sine and cosine functions of time as covariates (harmonic regression). The model parameters were estimated separately for each location. The seasonal patterns of evolution were different depending on the climate considered. "Temperate oceanic" sites showed an early spring peak, a summer minimum and a moderate autumn and winter activity. "Continental" sites show a later peak in spring, and a minimum in winter. The pick occurred in summer for the "mountainous" site, with no winter activity. In most cases, the timing of the spring peak could be related to the sum of degree days since the beginning of the year. Winter activity was positively correlated to the corresponding temperature. While until now mostly based on expert opinions reported in the literature, the seasonality of tick abundance was estimated from field data in the present study. We were able to clearly describe different patterns in different climates thanks to long term monthly samplings at several sites.

EE06 Can *Ixodes ricinus* be neglected any longer in Greece? A case study reveals its dominance in forest habitats of mountain Vermio in northern Greece

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Ixodes ricinus is considered to be one of the most important vectors of human and zoonotic pathogens, however data on its distribution in Greece are scarce and outdated, not to mention the lack of density data and their relation to ecological factors. This might lead to an underestimation of its public and animal health burden. As a case study and by using a dragging approach we herewith demonstrate that *Ixodes ricinus* is the dominant questing tick species in forested areas of mountain Vermio in northern Greece. From April 2021 until April 2022 dragging was applied at 87 locations. An effort was made to resample locations visited during the spring/summer period 2021 also during autumn/winter 2021/2022 resulting in a total of 148 samplings. Dragging took place at deciduous (dominated by *Carpinus orientalis*, *Ostrya carpinifolia*, *Buxus sempervirens*, *Quercus frainetto*, *Q. pubescens*, *Q. trojana*, *Castanea sativa*, *Tilia tomentosa*, *Fagus sylvatica* either alone or in combination) as well as coniferous/evergreen (dominated by *Pinus nigra*) forested areas and typical maquis vegetation dominated by the shrub species *Quercus coccifera*. This included forested sites adjacent to agricultural and/or grazing areas, local recreation sites, hiking trails, hunting and NATURA 2000 sites of the mountain. Sampling altitude ranged between 65 and 1900 masl. In total 489 ticks belonging to eight species were collected: *Ixodes ricinus* (90.4%, with an adult: nymph: larvae ratio of 6.2:1:0.03), *Haemaphysalis inermis* (2.9%), *Dermacentor marginatus* (2.1%), *Ixodes frontalis* (1.6%), *Haemaphysalis parva* (1.2%), *Rhipicephalus turanicus* (1.0%), *Haemaphysalis punctata* (0.6%) and *Rhipicephalus sanguineus* s.l. (0.2%). *Ixodes ricinus* was collected in 47.1% (41/87) of the sampled locations at altitudes ranging from 300 to 1530 masl, in deciduous forests (all tick stages), but also in typical maquis vegetation (only adults). Adult mean density for all *Ixodes ricinus* positive sites (defined through the presence of either adults/nymphs or both at a particular site) did not significantly differ between the spring/summer (1 adult/100m^2 ±1; range: 0-4.1 adults/100m^2) and the autumn/winter (1.4 adults/100m^2 ±1.2; range: 0.2-5.5 adults/100m^2) period (p=0.128), whereas *Ixodes ricinus* nymph mean density did (p=0.039) significantly differ (spring/summer: 0.3 ticks/100m^2 ±0.7; range: 0-3.8 ticks/100m^2 vs autumn/winter: 0.04 ticks/100m^2 ±0.1; range: 0-0.7 ticks/100m^2). Owing to the lack of both distribution as well as time series data of this important species at its southern distribution limit we posit that systematic data collection needs to be initiated also in Greece as part of a comprehensive public and animal health policy.

EE07 Tick-borne pathogens in *Rhipicephalus sanguineus* sensu stricto collected from dogs in the steppe and high plateau regions of Algeria

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[2]Institute for Parasitology, University of Veterinary Medicine Hannover, Germany
[3]Gertrud Theiler Tick Museum, Onderstepoort Veterinary Research, Pretoria, South Africa
[4]Bundeswehr Institute of Microbiology, Munich, Germany

In Algeria, the epidemiology of canine tick-borne diseases and both their veterinary and zoonotic significance are poorly known. The present study describes a molecular investigation of important vector-borne pathogens in *Rhipicephalus sanguineus* sensu stricto collected from domestic dogs in steppe and high plateau areas of central and eastern Algeria. In total, 1,043 ticks were collected from 147 dogs, including 756 ticks from 124 dogs in the steppe region of Djelfa and 287 ticks from 23 dogs in the high plateau region of Bordj Bou Arreridj. Ticks were divided into 384 pools (309 pools from Djelfa and 75 pools from Bordj Bou Arreridj) and analysed for genomic material of Crimean-Congo hemorrhagic fever virus (CCHFV) as well as *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Rickettsia* spp., *Babesia* spp., and *Hepatozoon* spp. All 384 pools were found negative for CCHFV and *A. phagocytophilum*, while *A. platys* was detected in 92 samples (24.0%). DNA of *E. canis* was present in 15 samples (3.9%). *Rickettsia* spp. were detected in 24 (6.3%) samples, including eleven samples identified as *R. massiliae*, six samples identified as *R. conorii conorii*, and seven samples that could not be identified to species level. *Babesia* spp. were determined in 50 samples (13.0%), and sequencing of a subset of eleven samples identified Babesia vogeli in all cases. *Hepatozoon* spp. were found most frequently, with 160 positive samples (41.70%). Of these, 12 were sequenced and identified as *H. canis*. The present study provides first molecular data on the occurrence of *A. platys*, *B. vogeli* and *H. canis* in *Rh. sanguineus* s.s. infesting Algerian dogs. Further studies in other regions are desirable to complete the picture of tick-borne pathogens in Algeria.

EE08 Mediterranean spotted fever: the role of reptiles and arthropod vectors

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Spotted fever group *Rickettsia* spp. are transmitted by arthropod vectors (e.g., ticks, lice, fleas and mites) to a wide range of vertebrates, including reptiles and humans. Several pathogenic rickettsial species may be associated to reptile reservoirs, such as *Rickettsia africae*, the agent of the African tick bite fever and *Rickettsia honei*, the causative agent of the Flinder island spotted fever. Other *Rickettsia* spp. are well known as causative agents of the Mediterranean Spotted Fever (MSF). To investigate the role of reptiles and arthropod vectors in the epidemiology of the MSF, from March to August 2018 (i.e., spring and summer) 172 reptiles were captured in a woody area of southern Italy and ticks and mites on them were removed and identified. In addition, *Ixodes ricinus* (n=219) ticks were collected by dragging and flagging in the same area under the frame of a previous study. Rickettsia spp. were detected by genomic extraction and PCR amplification targetting a fragment encoding for the gene citrate synthase (gltA; 401bp in length) of *Rickettsia* spp. Positive samples for this gene were tested by a second PCR using a pair of primers that amplify a fragment (632 bp) of the ompA gene, present only in Spotted fever group (SFG) Rickettsiae. Amplicons were purified and sequenced, and homologies verified with corresponding sequences available from GenBank. A high parasitic load of ticks and mites was observed on all the captured reptiles (i.e., 162/172; 94.18%). Overall, 3/168 (1.78%) lizards' tails (i.e., 1 *Podarcis muralis* and 2 *Podarcis siculus*), 98/145 (67.58%) I. ricinus ticks, and 8/36 (22.22%) *Neotrombicula autumnalis* mites collected on reptiles scored positive for *Rickettsia* spp. Also 71/219 (32.42%) of I. ricinus ticks collected from the environment yielded positive results for *Rickettsia* spp. and *Rickettsia monacensis* was the most prevalent species (n=89; 83.17%) followed by *Rickettsia helvetica* (n=18; 16.82%). Data presented suggest that Squamata reptiles may play a role in the maintenance of the MSF infection in southern Europe, as well as *I. ricinus* ticks and *N. autumnalis* mites feeding on them. Both Acari species are able to parasitize humans, representing a potential public health threat.

EE09 Tick-borne pathogens in *Hyalomma marginatum* (Acari: Ixodidae) collected from animal hosts in the South of France

Bernard C[1, 2], Vial L[1], Moutailler S[3], Galon C[3], Sabardeil J[1], Falchi A[4, 5], Cicculli V[4, 5], Poli PE[4, 5], Pollet T[1], Holzmuller P[1]

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Ticks, vectors of several zoonotic pathogens, represent an important and increasing threat for human and veterinary health. *Hyalomma marginatum*, one of the main tick vectors of the Crimean-Congo Haemorrhagic Fever (CCHF) virus, has been present in Corsica for decades. Its establishment in continental France is apparently much more recent. This species is known to be a vector of different microbial pathogens agents like bacteria, viruses or parasites. In order to decipher the potential infectious risks of its installation in France (introduction of a vector of pathogens not yet present on the territory or introduction of an additional vector for pathogens already present) we build up an analytical strategy to find out which pathogens are carried by H. marginatum ticks collected in the field from the Italian to the Spanish borders. To do this, we used two complementary approaches, one biased to specifically target known tick-borne pathogens and the other one unbiased to screen the microbiota of the tick for pathogens. The biased approach is using a powerful new high-throughput technology based on microfluidic real-time PCR amplification using 48.48 Dynamic Array™ IFC chips and the BioMark™ real-time PCR system (Fluidigm, CA, United States). This advanced methodology permitted the simultaneous detection of 14 bacterial, 8 virus and 3 parasitic species, belonging to the genera *Anaplasma*, *Bartonella Borrelia*, *Coxiella*, *Ehrlichia*, *Francisella*, *Rickettsia* for the bacteria, Bandavirus, Flavivirus, Orthonairovirus, Thogotovirus for the viruses, *Babesia* and *Theileria* for the parasites). CCHF virus was investigated individually. The unbiased approach is using proteogenomics technology with trypsin-digested proteins from ticks and corresponding microbiota analyzed in a liquid chromatography-coupled quantitative quadrupole tandem mass spectrometer Orbitrap Exploris™ (Thermo Scientific, MA, United States), with high-resolution precision mass (HRAM). To date, among the pathogenic species identified in a concordant way by the 2 approaches, we found *Rickettsia aeschlimannii*, *Anaplasma phagocytophilum*, *Theileria equi* and West Nile virus. This confirms the need for close monitoring of *H. marginatum* in areas where the tick has established itself, but also requires further investigations into its probability of geographic expansion and its precise role as a vector of pathogens in France.

EE10 Ticks and tick-borne pathogens in ticks from northern Sweden: data from a frontier area

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The environmental climate changes in Northern regions have shaped a potentially geographical area where new tick populations and new species could become established and introduce along with them new tick-borne pathogens. The present study was aimed at describing the tick species composition and the tick-borne pathogens present in northern Sweden, from Norrland (above latitude 60○N) in 2018 and from the three northernmost counties (Norrbotten, Västerbotten, Jämtland) in 2019. Ticks collected from domestic animals or people were morphologically identified at the species level. Species identification was confirmed with a PCR analysis in the case of morphologically similar species (i.e. *Ixodes ricinus* and *I. persulcatus*). Around 4200 ticks were collected in 2018. A subset of them (n= 1398) were identified as follows: 1357 *I. ricinus*, 27 *I. persulcatus*, 13 *I. trianguliceps* and 1 *Hyalomma marginatum*. Seven ticks resulted to be positive at PCR both for *I. ricinus* and *I. persulcatus*. Identification of 507 ticks from 2019 (total collection=942) were identified as follows: 239 *I. ricinus*, 268 *I. persulcatus*. Regarding tick-borne pathogens, the same subset of ticks from 2018 and 2019 were examined by a microfluidic analysis (FLUIDIGM®) with following results: *Rickettsia helvetica* 25% (2018)/5% (2019); *Borrelia* spp. 20/12%; *Anaplasma phagocytophilum* 9/0,4% and *Babesia* spp. 6/1%. No ticks were positive for tick-borne encephalitis virus. To our knowledge this is the first time that such a large collection has been performed in this geographical area. *I. ricinus* is still the dominant tick species, but I*. persulcatus* is expanding its geographical distribution. Also, this is the first report of *H. marginatum* at such a northern location. Ticks were found in all considered counties and not only on the coastal regions. These results confirm that tick-borne pathogens are present in northern Sweden ticks, but – even if a direct comparison from different sampling area is not possible - less ticks carrying tick-borne pathogens were detected 2019 in the far north compared to 2018. Updated knowledge on tick distribution is fundamental to design risk maps and to give proper advice to the community by suggesting measures to minimize diseases. The present work was partially funded by NordForsk, project CLINF, grant no. 76413 and the Swedish Research Council, project TICKBIOCON, grant no. 2018-03830.

EE11 Tick-borne pathogens, *Babesia canis* and *Borrelia burgdorferi* s.l., in sled and companion dogs from Central and North-Eastern Europe

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Ticks are important vectors of numerous pathogens of medical and veterinary significance. Among tick-borne pathogens (TBP) with the greatest impact on their hosts are spirochaetes of the *Borrelia burgdorferi* sensu lato (s. l.) complex, responsible for development of the multisystemic disease, borreliosis (Lyme disease). *Babesia* spp. are protozoan parasites of red blood cells responsible for the development of babesiosis, a potentially life-threatening disease of humans and animals. Babesiosis due to *Babesia canis* infection is an emerging tick-borne disease in dogs in Central Europe. The aims of the current study were to (1) determine the prevalence of *Babesia* spp. and *Borrelia burgdorferi* s.l. in sled dogs; (2) compare prevalence in sled and pet dogs from Poland and Ukraine; (3) determine species/genotypes of tick-borne pathogens occurring in dogs, and (4) assess the occurrence of co-infections. Neither *Babesia* spp. nor *B. burgdorferi* s.l. infections were detected in sled dogs from seven countries (Poland, Lithuania, Latvia, Estonia, Belarus, Russia and Finland). The DNA of *B. canis* was detected in 100% of symptomatic and 5.4% of asymptomatic pet dogs from Poland. Similarly, the DNA of *B. canis* was identified in 82% of symptomatic and 3.8% of asymptomatic pet dogs from Ukraine. The DNA of *Borrelia burgdorferi* s.l. was detected in 4.4% of pet dogs. Molecular typing confirmed the presence of *B. burgdorferi* sensu stricto (s.s.). Three dogs were co-infected by *B. canis*, *B. burgdorferi* s.l. and *Dirofilaria repens*. In conclusion, tick-borne pathogens constitute a serious health threat to pet dogs in Central and South-eastern Europe, but were not observed among sled dogs from the same region of Europe nor in the Baltic countries.

EE12 Imported *Hyalomma* ticks in the Netherlands 2018-2020

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Ticks of the genus *Hyalomma*, which are vectors for several tick-borne diseases, are occasionally found in areas outside their endemic range including northern parts of Europe. The objective of this study was to analyse adult *Hyalomma* ticks that were recently found in the Netherlands. *Hyalomma* ticks were morphologically identified. Cluster analysis, based upon sequence data (cox1 barcoding) for molecular identification, and pathogen detection were performed. Additionally, a cross-sectional survey of horses was conducted to actively search for *Hyalomma* ticks in summer 2019. Analysis of temperature was done to assess the possibility of (i) introduced engorged nymphs moulting to adults and (ii) establishment of populations in the Netherlands. Seventeen adult *Hyalomma* ticks (one in 2018, eleven in 2019, five in 2020) were found by citizens and reported. Fifteen ticks were detected on horses and two on humans. Twelve were identified as *H. marginatum*, one as *H. rufipes* and four, of which only photographic images were available, as *Hyalomma* sp. No Crimean-Congo haemorrhagic fever virus or *Babesia*/*Theileria* parasites were detected. One adult tick tested positive for *Rickettsia aeschlimannii*. In the cross-sectional horse survey, no *Hyalomma* ticks were found. Analysis of temperatures showed that engorged nymphs arriving on migratory birds in spring were able to moult to adults in 2019 and 2020, and that cumulative daily temperatures in the Netherlands were lower than in areas with established *H. marginatum* populations. Our results show that *Hyalomma* ticks are regularly introduced in the Netherlands as nymphs. Under the Dutch weather conditions, these nymphs are able to develop to the adult stage, which can be sighted by vigilant citizens. Only one human pathogen, *Rickettsia aeschlimannii*, was found in one of the ticks. The risk of introduction of tick-borne diseases via *Hyalomma* ticks on migratory birds is considered to be low. Establishment of permanent *Hyalomma* populations is considered unlikely under the current Dutch climatic conditions.

EE13 Eco-epidemiology of equine piroplasmosis in French draft horses in an enzootic area

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For vector-borne diseases, an 'eco-epidemiological' approach is essential to better understand the relationships between the pathogen, the hosts, the vectors and the environment, as well as to allow the determination of risk factors with limited bias, and therefore develop appropriate measures of surveillance, prevention or control. Thus, our objective was to study the eco-epidemiology of *Babesia caballi* and *Theileria equi* infections responsible for equine piroplasmosis in French draft horses, and for which very few multivariate studies, especially about the effect of environmental parameters on equid infections, were conducted up to now. From April to May 2021, we collected blood samples and ticks from 147 draft horses randomly selected from 38 farms, located in four departments in the Center and South-East of France. Questing ticks were also collected using the flagging method on the pastures of horses. Studied areas were chosen due to a high circulation of *B. caballi* and *T. equi*, a high density of draft horses, and heterogeneous environmental conditions. Collected ticks were identified by morphological characterisation, and identification was confirmed by RT-PCR. Pathogen detection was performed by PCR on both collected ticks and blood samples, the latter also being tested for the presence of antibodies against each parasite by ELISA tests. Data related to environment (altitude, vegetation type, and meteorological data), breeding practices (vaccination status, parasite control), and individual horse characteristics (breed, age, sex) were collected. Risk factors were determined through generalized linear models. Out of the 147 horses included in this study, 56.5% were infested by ticks, with a median of 1 (range 0-151) tick per horse. A total of 1048 ticks, belonging to the species *Dermacentor reticulatus* (58.2%), *Dermacentor marginatus* (23.0%) and *Ixodes ricinus* (18.8%), were collected on the horses (906 ticks) and on the pastures (142 ticks). Ticks infection by *B. caballi* and *T. equi* are under analysis. Regarding the horses infectious status, 14.4% and 50.7% of the sampled horses had a positive PCR result and 58.9% and 37.2% were seropositive against *B. caballi* and *T. equi*, respectively. Statistical analyses of risk factors related to individual and environmental parameters, and breeding practices are currently being conducted. The identification of potentially relevant risk factors will provide both recommendations for limiting tick infestation in horses, and the implementation of targeted methods against equine piroplasmosis infections.

EE14 *Dermacentor reticulatus* story in Poland: expanding range, wildlife hosts and vectored pathogens

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Dermacentor reticulatus is the second most common and abundant tick species in Poland with the expanding geographical range. It is the main vector of *Babesia canis*, causative agent of canine babesiosis and *Rickettsia raoultii* causing TIBOLA in humans. In comprehensive field studies we determined a seasonal and annual shift in the range of two *D. reticulatus* populations; we examined tick infestation on red fox (adult and pups) and small rodents; and finally we determined prevalence of *B. canis* and *Rickettsia* in adult and juvenile ticks from different populations. In 2016-2018, 5130 questing ticks from 330 sites were collected. The distance between two tick populations in each season and year was calculated. In total 366 adult hunted foxes and 25 live-trapped cubs were examined for ectoparasites (ticks, fleas and others). 345 rodents from three main regions defined by *D. reticulatus* presence/absence were examined for *D. reticulatus* larvae and nymphs. To confirm the vector role of D. reticulatus, 2497 adult questing ticks, 1096 larvae (150 pools) and 410 nymphs from rodents were subjected to molecular analyses for *Babesia* and *Rickettsia* DNA. Seasonal/annual shift in the range of two tick populations was documented, mainly along river basins. Adult *D. reticulatus* were found on adult foxes but not on fox cubs. Altogether 2866 *D. reticulatus* (2397L and 469N) were collected from rodents, mainly from *Microtus*/*Alexandromys* voles. Tick infestation was more than ten times higher in the Eastern and Central Poland compared to Western Poland. DNA of *B. canis* was detected only in ticks collected from the Eastern region of *D. reticulatus* occurrence (in every tick stage), all ticks from Western Poland tested negative. DNA of *Rickettsia* was found in ticks from all regions of *D. reticulatus* occurrence. Neither adult or juvenile *D. reticulatus* ticks were collected from rodents or foxes originating from the 'gap' area- region situated between two tick populations, historically free of ornate dog ticks. We noted significant decrease in the 'gap' area, with possible merge of two tick populations in Poland in next 10-13 years. Infestation of free-living hosts with *D. reticulatus* reflected ideally the range of adult questing ticks. Furthermore, prevalence of *B. canis* in ornate dog ticks reflected occurrence and incidence of canine babesiosis in the area of Poland. Acknowledgements: The study was financially supported by National Science Centre, Sonata Bis grant no. 2014/14/E/NZ7/00153 (AB).

EE15 *Babesia* "sensu lato" in South-Eastern, Central and North-Eastern Europe: emerging and re-emerging tick-borne disease of humans and animals

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Babesiosis is an important, life threatening disease for humans and animals. There is now considerable evidence that in Europe babesiosis is an emerging infectious disease, some of the causative species spreading as a consequence of the increasing range of their tick vector hosts. In this review, we summarize both historic records and recent findings on the occurrence and incidence of babesiosis in 20 European countries located in South-Eastern (Bosnia and Hercegovina, Croatia, Serbia), Central (Austria, Czechia, Germany, Hungary, Luxembourg, Poland, Slovakia, Slovenia, Switzerland) and Northern/North-Eastern (Lithuania, Latvia, Estonia, Iceland, Denmark, Finland, Sweden and Norway) parts of Europe, from humans and selected species of domesticated animals (cats, dogs, horses and cattle). This review covers the seven species of *Babesia* (*B. microti*, *B. divergens*, *B. venatorum*, *B. canis*, *B. gibsoni*. *B. vogeli* and *B. caballi*) and includes also the related piroplasm *Theileria equi* from horses. Recorded cases of human babesiosis are still rare but are expected to rise in the coming years because of the widespread and longer seasonal activity of *Ixodes ricinus* as a result of climate change and because of more extensive use of the better molecular diagnostic methods. Bovine babesiosis has re-emerging potential because of the likely loss of herd immunity and canine babesiosis is rapidly expanding in Central and NE Europe, its prevalence correlating with the rapid, successful expansion of the ornate dog tick (*Dermacentor reticulatus*) population in Europe. Taken together our analysis of the available reports shows clear evidence of an increasing annual incidence of babesiosis across Europe in both humans and animals that is changing in line with similar increases in the incidence of other tick-borne diseases. This situation is of major concern, and we recommend more extensive, standardised monitoring in future, in association with a One Health approach.

EE16 *Cytauxzoon* spp. and *Hepatozoon* spp. in questing ticks in north-eastern Italy

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Cytauxzoon spp. and *Hepatozoon* spp. are tick-borne pathogens infecting a wide range of felids and canids all over the world. Information about their transmission to wild and domestic felids in Europe is still scant. To date, no arthropod vectors were found positive for *Cytauxzoon* spp., whereas *Hepatozoon* DNA was already reported in engorged ticks, but their competence in transmission was not yet proved. A survey on *Cytauxzoon* and *Hepatozoon* detection in ticks was conducted in North-eastern Italy in areas known to be endemic for *Cytauxzoon* and *Hepatozoon* in domestic and wild cats. Ticks were collected from April to September 2021 by dragging and flagging, then morphologically identified. DNA from ticks was isolated and submitted to conventional PCR (16S- and 12S-rRNA) to confirm morphological identification. Then, a PCR targeting piroplasms 18S-rRNA was performed (Tabar et al. 2008). Amplicons were sequenced and sequences were compared to those in GenBank® dataset. A total of 582 questing ticks were collected and identified as follows: 547 *Ixodes ricinus* (42 adult males, 25 adult females, 480 nymphs) and 35 *Haemaphysalis punctata* (1 adult male, 4 adult females, 30 nymphs). Nymphs were grouped according to species and sampling date/site in pools (up to 10 individuals per pool), whereas adult ticks were examined individually. The infection rate for pools was obtained using generalised linear modelling to calculate maximum-likelihood estimates of prevalence. Among 54 *I. ricinus* nymph pools, *H. felis* was sequenced in 6, H. silvestris in 1, *Cytauxzoon* spp. in 5, and *Babesia venatorum* in 3, corresponding to an estimated pooled prevalence of 1.3%, 0.2%, 0.9%, and 0.6%, respectively. Besides, *H. felis* was detected in 2 males, *H. silvestris* in 4 males and 1 female, and *B. venatorum* in 1 adult male. Adults and nymph pools of *H. punctata* were found all negative to piroplasms. This study describes the molecular detection of *Cytauxzoon* and *Hepatozoon* feline species in questing ticks in an endemic area of North-eastern Italy. The obtained results suggest a potential role of *I. ricinus* in protozoa transmission, since the detection of *Cytauxzoon* and *Hepatozoon* in questing ticks supports the hypothesis that these parasites are maintained during the moult from larvae to nymphs and from nymphs to adult. However, further studies are needed to clarify the vectorial competence of I. ricinus. Worthy of note, the *B. venatorum* isolation in the study area for its zoonotic potential and consequently the risk exposure for humans.

EE17 Molecular detection of vector-borne agents in wild boars (*Sus scrofa*) and associated ticks from Brazil

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The present study aimed to investigate, by molecular techniques, the occurrence of Anaplasmataceae, Bartonellaceae, Rickettsiaceae, Mycoplasmataceae, Coxiellaceae e Babesiidae/Theileriidae in blood samples of free-living wild boars and associated ticks in southeastern Brazil. For this purpose, 67 blood samples and 265 ticks (264 *Amblyomma sculptum* and one *A. ovale*) were analyzed. In the screening for Anaplasmataceae agents by a PCR assay based on the 16S rRNA gene, 5.97% blood samples and 50.54% ticks were positive. In the PCR assay for *Ehrlichia* based on the dsb gene, 9.24% of ticks were positive. Despite the low occurrence, a possible new 16S rRNA genotype of *Anaplasma* was detected in a wild boar's blood sample. Based on phylogenetic analyses based on the groEL, gltA, sodB genes and ITS (23S- 5S) intergenic region, it was found that *A. sculptum* and *A. ovale* ticks collected from wild boars carry *Ehrlichia* genotypes phylogenetically associated with *E. ewingii*, *E. ruminantium*, and new *Ehrlichia* genotypes previously detected in horses, peccaries, and ticks collected from jaguars. In the screening for hemoplasmas by a qPCR based on the 16S rRNA gene, 88.06% of blood samples and 8.69% of ticks were positive. *Mycoplasma suis*, *M. parvum* and a possible new hemoplasma genotype were detected in wild boars in southeastern Brazil. In the screening for *Bartonella* using a nuoG-based qPCR, 3.8% of tick samples were positive. Phylogenetic inferences positioned four nuoG and one gltA *Bartonella* sequences into the same clade as *Bartonella machadoae*. No blood or tick samples from wild boars showed to be positive in the qPCR for *Coxiella burnetii* based on the IS111 gene. On the other hand, only 1.6% of ticks was positive in the nested PCR assay for piroplasmids based on the 18S rRNA gene. An 18S rRNA sequence detected in a pool of *A. sculptum* nymphs was phylogenetically close to *Cytauxzoon felis*. *Rickettsia* sp. closely related to *R. belli* was detected in a pool of *A. sculptum* nymphs. This is the first report of hemoplasmas, *B. machadoae* and *Cytauxzoon* in *A. sculptum*. Wild boars and associated ticks do not seem to participate in the epidemiological cycle of *C. burnetii* in the region studied. Wild boars may act as a potential disperser of ticks infected with *Ehrlichia*, *B. machadoae*, hemoplasmas, and *Cytauxzoon*, and may bring important epidemiological implications in the transmission of bartonellosis, ehrlichiosis, hemoplasmosis, and cytauxzoonosis to humans and animals, more specifically to horses, rodents, pigs, and cats.

EE18 Diversity of *Cercopithifilaria* spp. in ticks of dogs and cats from Southeast Asia and India

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Tick borne filarioids of the genus *Cercopithifilaria* affect several vertebrate hosts with *Cercopithifilaria bainae*, *Cercopithifilaria grassi*, and *Cercopithifilaria* sp. II sensu Otranto et al., 2013 typically found in dogs. Among them, *C. bainae* has a worldwide distribution according to the occurrence of its tick vector, *Rhipicephalus sanguineus* sensu lato. In Asian countries, despite the wide presence of this tick species, data on *Cercopithifilaria* spp. are scant, with few reports on *C. bainae* detected in ticks from Iran and Malaysia, and *C. grassii* in dogs from Pakistan. Therefore, this study aimed to assess the occurrence of these dermal filarioids in ixodid ticks collected on dogs and cats from Southeast Asia and India, providing a better epidemiological picture on their distribution in this region. Ticks (n = 687) collected on dogs and cats under the frame of previous studies in China, India, Indonesia, Malaysia, Singapore, Taiwan, Thailand, the Philippines and Vietnam were molecularly screened for *Cercopithifilaria* spp. by conventional PCR and real-time PCR using two pair of primers targeting partial sequences of cytochrome c oxidase 1 gene. Overall, *Cercopithifilaria* spp. DNA was detected in 9.5% (n = 65/687) of the tick specimens tested, with *C. bainae* being the most prevalent species (i.e., 8.9%), followed by *C. grassii* (0.6%). Most *Cercopithifilaria* spp. positive ticks were collected on dogs (92.3%; 60/65); whereas ticks collected on cats represented 7.7% of the positive specimens. In addition, *Cercopithifilaria* spp. were mostly detected in *R. sanguineus* s.l. ticks (96.9%; 63/65), followed by *Rhipicephalus haemaphysaloides* (3.1%; 2/65). Data herein presented demonstrate the occurrence of dermal tick borne filarioids of the genus *Cercopithifilaria* in several Asian countries, with *C. bainae* being the most prevalent species. We also report for the first time the molecular detection of *C. bainae* in *R. sanguineus* s.l. ticks collected on cats, as well as in *R. haemaphysaloides* ticks collected on dogs, suggesting that the biological cycle of this filarioid species may involve other intermediate and definitive hosts than *R. sanguineus* s.l. and dogs. However, confirmatory studies on the role of other tick species and domestic cats on the biology of *C. bainae* are advocated.

EE19 Geographical distribution, host selection and seasonality of occurrence in bat-specialist ticks of Europe

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To exploit most effectively their host resources, parasites are in constant race to counterbalance and overcome their host's defense mechanisms. They not just actively seek for hosts but they use a number of adaptive strategies to increase their reproductive success and transmission (colonization of new hosts), like host specificity and seasonality in occurrences (synchronizing their reproduction to the hosts' life cycle, etc.). Ticks are important parasites of vertebrates, which may show high prevalence and intensity and not only deprive their hosts from energy but they may vector a number of pathogens. European bat species are host for three specialist hard tick species (Ixodidae: *Ixodes ariadnae*, *I. simplex* and *I. vespertilionis*). Based on a review of published European hard tick records, here we report details on geographical distribution, host range and seasonality of infestation of these ticks on wild caught bats in Europe. Using georeferenced tick-host relationships we tested several hypotheses on host-parasite evolutionary adaptations regulating host-specificity, seasonality and sympatric speciation. While all three bat-specialists show sympatry in distribution, we observed significant differences between host specificity and the seasonality of abundance between the morphologically different bat specialist ticks (*I. simplex* vs. *I. vespertilionis*) likely caused by their host specificity and their respective host seeking behavior. The two highly generalist, but morphologically similar tick species (*I. ariadnae* and *I. vespertilionis*) showed temporal differences in occurrence and activity, thus exploiting significantly different host communities while occurring in local sympatry. Bat-specialist ticks show a wide range of adaptations to their hosts, with differences in specificity, seasonality of occurrence, the prevalence and intensity of infestation and all these contribute to a successful division of temporal niches of ticks sharing morphologically similar hosts occurring in geographic sympatry.

EE20 Citizen science and the DAMA protocol: our tools to prepare for the emergence of ticks and tick-borne pathogens

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Hyalomma ticks are important vectors of Crimean-Congo Haemorrhagic Fever Virus (CCHFV) and other pathogens. They are frequently carried as immatures from Africa, the Middle East and Mediterranean areas to temperate Europe via migratory birds and emergence of adults has been reported in many countries where it has so far been considered non-endemic. By monitoring the potential arrival of adult *Hyalomma* ticks in Hungary applying citizen-science methods this study aimed to implement the first steps of the DAMA (Document, Assess, Monitor, Act) protocol. By continuously recording (Document) the appearance of pathogens, their vectors and reservoir hosts, and performing the appropriate scientific analyses (Assess), we will be able to identify the organisms that pose a threat to us and manage their targeted surveillance (Monitor). Thus, it will be possible to make adequate proposals to decision makers to take appropriate preventive measures (Act). Ticks were collected from April to December 2021 by asking volunteer participants through a self-made website to look for large, quickly moving, striped-legged hard ticks on themselves, their pets and livestock. Owing to an intensive media campaign, the project website had over 31,000 visitors within seven months; 137 specimens and several hundred photos of hard ticks were submitted by citizen scientists from all over the country. Beside *Ixodes ricinus*, *Dermacentor reticulatus*, *Dermacentor marginatus* and *Haemaphysalis inermis*, a specimen from a dog was morphologically identified as a male Hyalomma marginatum and another removed from a cow as a male *Hyalomma rufipes*. The dog and the cattle had never been abroad, lived approximately 280 km apart, so the two *Hyalomma* observations can be considered separate introductions. Amplification of the partial mitochondrial cytochrome C oxidase subunit I gene was successfully run for both specimens. Sequencing confirmed previous morphological identification for both ticks. Based on the phylogenetic analyses, the *Hy. marginatum* individual most likely belongs to the Eurasian population and the *Hy. rufipes* to a clade of mixed sequences from Europe and Africa. Although not infected with CCHFV, another member of Bunyavirales, Volzhskoe tick virus was detected in the *Hy. marginatum* specimen using metagenomic analysis. By summarizing the scattered historical reports about the occurrence of *Hyalomma* ticks and CCHFV in Hungary, we conclude that CCHFV has been present in wildlife and domesticated hosts and even humans. Our data highlight the effectiveness of citizen science programmes combined with the DAMA protocol in the monitoring and risk assessment of CCHFV emergence and preparedness in our region.

EE21 Effect of urbanization on the distribution of *I. ricinus* and the prevalence of *Borrelia burgdorferi* in European green spaces, a meta-analysis

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Green spaces in the city have a positive effect on the overall wellbeing of humans, however, they bring the urban human population in contact with ticks and tick-borne pathogens. In recent years, multiples studies have described tick abundances and *Borrelia burgdorferi* (B.b.) prevalences in cities around Europe. We performed a meta-analysis on available data from eight European studies including relative tick densities and B.b. prevalences of a minimum of seven locations spread across an urban-rural gradient with available landcover data from the European Urban Atlas Project (Heylen et al. 2019, Hansford et al. 2022, Maetzel et al. 2005, Hornok et al. 2014, Borşan et al. 2020, Sormunen et al. 2020, C. Strube unpublished data, A. Bowman unpublished data). The aim of our study is to determine whether urban/rural gradients in tick densities and B.b. prevalences are comparable across cities and to determine what factors drives this gradient (e.g. size of the green space, surrounding landcover, distance to a large forest). Preliminary results show a lower tick density in urban green spaces compared to more rural green spaces. Also in small green spaces with a high percentage of hardening in the surrounding contain a lower tick density, which could be explained by the higher temperatures associated with hardened areas and the lower biodiversity supported by small and isolated green spaces. No difference in B.b. prevalence was found between urban and rural green spaces, but hardening in the surrounding of the green space does have a negative effect on the B.b. prevalence. However, the risk of contracting Lyme disease can still be considerable due to the presence of small reservoir hosts, as for instance rats. Further analyses are still ongoing to confirm these preliminary results.

EE22 Spatial sorting of *Ixodes ricinus* and its associated pathogens near greenspace infrastructure

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Recent years have seen an increase in studies on within-forest variation in Ixodes ricinus density and associated pathogen prevalence. Both local host abundance and vegetation characteristics have been highlighted as potential drivers of this variation. In previous research, conducted in 10 public greenspaces in Flanders, Belgium, we illustrated a consistent correlation between forest infrastructure, such as benches and trails, and the density of questing nymphs (DON). Here, higher tick density was consistently observed in the interior of forest stands than near greenspace infrastructure. In the follow-up analyses reported here, the 7320 nymphs captured in this study were screened for the presence of *Borrelia burgdorferi* s.l., *Rickettsia*, *Neoehrlichia mikurensis*, *Anaplasma phagocytophilum* and *Borrelia miyamotoi*. For the prevalence of each pathogen, with the exception of Neoehrlichia mikurensis, significant variation between benches, trails and the wooded areas of individual forest stands was observed. We explored the drivers of this variation in nymphal infection prevalence (NIP), as well as the variation in DON. Both the role of the local host abundance, measured by camera trapping, and vegetation characteristics were taken into consideration. We observed significant correlation of both herb- and canopy layer vegetation with DON and NIP. The same was true for the local abundance of hosts, which was shown to at least partly drive the intra-forest stand variation in pathogen prevalence. By the time of presentation, we will model the ecological processes driving the density of infected nymphs, as a proxy for tick borne disease risk, in a structural equation model. The inclusion of direct and indirect effects of greenspace infrastructure (through effects on host habitat use and variation in vegetation characteristics) will enable the quantification of both direct and indirect effects of greenspace characteristics on local tick density and pathogen prevalence.

EE23 Ticks and tick borne pathogens in Umbria and Marche in a one health perspective; a five years monitoring with a description of clinical case of SENLAT

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In the framework of diagnostic activities carried out by Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche (IZSUM) and of the actions forecasted by the National Surveillance Plan for Arboviroses in order to gain knowledge on vector-borne diseases, a survey on ticks collected from animals, environment and humans was carried out aimed to identify the tick-borne pathogens circulating in Umbria and Marche regions and to provide diagnostic support in case of tick bite in humans. From 2014 to 2019, 6985 ticks were collected (512 from humans) and identified at species level by morphology. Molecular assays for *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Babesia* spp., *Coxiella burnetii* and Flavivirus were performed in the Bi-Regional center for vectors and vector-borne diseases of IZSUM. The pathogen most frequently detected was Rickettsia spp. (9,9%) followed by *A. phagocytophilum* (9%) and *B. burgdorferi* s.l. (3%), *Babesia venatorum* was also detected, while all samples tested negative for Flavivirus and C. burnetii. Results, even deeply biases affected, confirm the presence of risk for TBD for humans. In the case of *Rickettsia*, it was also confirmed by a clinical case of SENLAT (Scalp Eschar and Neck LymphAdenopathy after Tick bite) diagnosed in a 60 years old woman bitten by a tick in the countryside of Perugia. SENLAT (or DEBONEL-Dermacentor-borne necrotic erythema and lymphadenopathy) is a Rickettsiosis caused mainly by *R. slovaca* and characterized by enlarged neck lymph nodes and scalp eschar after a tick bite. The woman presented asthenia, scalp eschar and lateral-cervical lymphadenopathy. At the onset of symptoms, the disease was not recognized, and local and generic antibiotic treatment with Amoxicillin and Clavulanic acid were prescribed by physicians, resulting ineffective. After two weeks Rickettsiosis was suspected and Doxycycline treatment started, two weeks later lesions were going to recover. The biting tick was delivered to IZSUM Lab and identified as an almost fully engorged *Dermacentor marginatus* female. The tick tested positive for *Rickettsia* sp. by PCR and sequencing had identified *R. slovaca* with a 100% of homology with *R. slovaca* isolated from tick in Corsica in 2018 (MK608660). Sequence has been registered in GenBank (AN ON316844). The study highlights the need of high performing diagnostic tools able to identify tick-borne pathogens at species level in order to assess specific risk for humans, moreover, once more demonstrates the necessity of operating as knowledge's network in a One Health perspective for managing the risk of tick borne diseases.

EE24 Tick co-infestation of rodents in the Netherlands, an emerging area for TBEV

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Tick-borne encephalitis virus (TBEV) is medically the most important arbovirus in Europe. Previous studies have shown that enzootic cycles depend on virus transmission from infected nymphs to uninfected larvae co-feeding on rodent hosts. Essential to this transmission pathway is that large numbers of larvae aggregate with nymphs on the same individual rodents. Such coincident aggregation can only happen when both life stages show coincident seasonal activity, which is thought to occur only in areas characterized by rapidly increasing spring temperatures. However, TBEV has recently emerged in parts of Europe that lack these specific climatic conditions, such as the Netherlands. An important question is therefore to what extent rodents are co-infested by larval and nymphal ticks in these emerging areas. We compiled a large dataset of *Ixodes* spp. ticks (n=37,563) feeding on rodents (n=4,039) from 36 sites across the Netherlands to analyze patterns of tick infestation in relation to host characteristics and environmental factors. We found strong evidence for coincident aggregation of larval and nymphal ticks: the 20% most heavily infested rodents carried 79% of all larvae and 76% of all nymphs. Moreover, rodents with nymphs had three times more larvae than rodents without nymphs. Co-infestation occurred from March – November but peaked in June, when 13% of all rodents carried both life stages and the mean number of larvae per co-infested rodent was 42 (95% CI: 24.5-71.1). The probability of co-infestation was significantly higher for wood mice (*Apodemus sylvaticus*) than any other rodent species, and increased with body weight more strongly for males than for females. In contrast, co-infestation was not associated with habitat type nor spring warming and was not more common in TBEV foci than elsewhere. Our results suggest that the 'rapid spring warming' hypothesis does not hold for emerging areas in Northwestern Europe, where host community composition may be more important for TBEV-transmission than environmental factors.

EE25 Cadavers of free-living species of tick hosts are a valuable source of material and data for tick-borne pathogen surveillance in urban habitats and beyond

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Ticks and tick-borne pathogens (TBPs) have previously been reported to occur in urban habitats. Nevertheless, the transmission cycles between ticks and TBP reservoir hosts have not been elucidated. We focused on four vertebrate species that occur at relatively high densities in urban habitats, are frequently parasitized by ticks, and are considered competent to transmit multiple TBPs. We sampled cadavers of accidentally killed individuals (mostly road killed) of these species instead of labour- and time-intensive animal captures, moreover associated with significant ethical issues. A total of 267 individuals of European hedgehog (*Erinaceus europaeus*; EE), Northern white-breasted hedgehog (*E. roumanicus*, ER), Eurasian red squirrel (*Sciurus vulgaris*, SV), and common blackbird (*Turdus merula*, TM) were found, dissected, and multiple tissues were sampled. A total of 1827 samples were analysed for the presence of TBPs by multiplex real-time qPCR. In general, a surprisingly high prevalence of TBPs was found in host tissues: *Borrelia burgdorferi* sensu lato: 64% in ER, 87% EE, 85% SV, 60% TM, *Anaplasma phagocytophilum*: 98% in ER, 99% EE, 61% SV, 59% TM, *Bartonella* spp.: 43% ER, 24% EE, 76% SV and *Rickettsia helvetica*: 64% ER, 39% EE. The prevalence of TBPs in cadavers found in urban environments was not statistically different from the prevalence determined in cadavers found in rural areas. Considerable diversity within pathogen species, and differences in pathogen detection efficiency between different organs/tissue tropisms were observed. Cadavers of the target animal species were proven to be a suitable and efficient source of biological material for the monitoring of TBP in urban habitats and elsewhere.

EE26 What's hiding under the skin: subcutaneous ticks in wild canid hosts

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Ticks are arthropods that typically attach to the external surface of the skin. In some carnivore hosts, ticks were found in atypical location, such as the subcutaneous tissues. The majority of these hosts are represented by red foxes (*Vulpes vulpes*) followed by other canid carnivores in Europe and USA. The purpose of this research was to evaluate the presence of subcutaneous ticks in wild carnivores from Romania in order to elucidate the association of aberrant localization with canid and non-canid hosts. Over a period of 6 years, 313 carcasses of 12 species of wild carnivores (94 golden jackals *Canis aureus*, 15 gray wolves *Canis lupus*, 32 wild cats *Felis silvestris*, 3 Eurasian lynxes *Lynx lynx*, 109 Eurasian badgers *Meles meles*, 25 beech martens *Martes foina*, 11 European polecats *Mustela putorius*, 4 European pine martens *Martes martes*, 17 Eurasian otters *Lutra lutra*, one stoat *Mustela erminea*, one European mink *Mustela lutreola*, one least weasel *Mustela nivalis*) were examined by parasitological necropsy. Detected subcutaneous ticks were removed with the nodules and stored in ethanol, then morphologically identified to species level. Only one carcass (1.06%) belonging to a golden jackal was infested by a subcutaneous tick, located in the left inguinal area. The detected tick was identified as a female *Ixodes ricinus*. All the other examined animals were negative for subcutaneous ticks. There are several speculations regarding the presence of subcutaneous ticks, however the predisposing factors are not yet known. They could be related to the tick species and development stage, or factors related to the host itself. It is not clear if canids are the only hosts to be parasitized by subcutaneous ticks or it is the lack of studies on this topic that make them seem predisposed. The majority of the published cases are referring to canid hosts, with the exception of one human host.

EE27 Tick-borne pathogens detected in ticks removed from animal hosts in northeastern Italy

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Wild and domestic animals occupy an important role in the enzootic life cycles of many tick-borne pathogens (TBPs). The aim of this study was to investigate the occurrence and prevalence of TBPs in ticks removed from different wildlife and domestic animals and assess the ecological and reservoir role of these hosts. During 2019–2021 period, ticks (adults and nymphs) were collected from both wildlife and domestic hosts through passive surveillance. The samplings were carried out in TBPs endemic regions (northeastern Italy). After collection, ticks were morphologically identified and then molecularly screened for several TBPs individually or in pools. Minimum Infection Rate (MIR) was used to calculate prevalence. A binomial Generalized Linear Model was applied to assess if host species and/or tick stages influenced the *A. phagocytophilum* and *R. helvetica* infection in ticks. A total of 367 ticks were collected and four species were identified: n=215 *Ixodes ricinus*, n=146 *I. hexagonus*, n=1 *Dermacentor marginatus*, n=1 *Rhipicephalus sanguineus*, n=2 Ixodes spp. and n=2 *Dermacentor* spp. Ticks were collected from 71 hosts belonging to 11 species (cattle, chamois, deer, roe deer, wild boar, wolf, fox, golden jackal, badger, hedgehog, and buzzard). *Ixodes ricinus* was collected from all the host species; *I. hexagonus* from badger, hedgehog, and fox; *D. marginatus* and *Dermacentor* spp. from wild boar, and *R. sanguineus* from hedgehog. Eleven TBPs were detected in 321 pools (206 adults, 15 nymphs' pools) analyzed. The 30.2% (65/215) of *I. ricinus* was found positive for at least one TBP and the main TBP were *A. phagocytophilum* (MIR=19.1) *R. helvetica*; (MIR=9.8) and *R. monacensis* (MIR=4.7). One *Dermacentor* spp. specimen was found positive for *R. slovaca* and five *I. hexagonus* for *Rickettsia* spp. A total of 15 ticks (4.1%) removed from chamois and roe deer were found co/multiple infected. *Anaplasma phagocytophilum* prevalence was significantly higher in ticks fed on wolf and jackal ($p<0.001$) and deer and roe deer ($p<0.05$), while *R. helvetica* prevalence was significantly higher in ticks fed on wolf, jackal and roe deer ($p<0.001$) and chamois and deer ($p<0.05$). In conclusion, this study highlights the occurrence of a high diversity of pathogen species in this area. In addition, it was observed a high prevalence of TBPs in *I. ricinus* collected from wildlife and the possibility of multiple pathogen transmission by co-infected ticks. Wolf, jackal and roe deer seem to act as the main host for ticks and reservoir for TBPs in this area.

EE28 *Borrelia burgdorferi* s.l. diversity in ticks and small mammals from grasslands and forests

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Ixodid ticks are important vectors for zoonotic pathogens with *Ixodes ricinus* being the most important in Europe. Rodents are hosts of immature life stages of *I. ricinus* ticks and are considered main reservoirs for tick-borne pathogens, e.g. *Borrelia burgdorferi*. This study aims to analyse the prevalence as well as genospecies and sequence type (ST) diversity of *B. burgdorferi* in ticks and small mammals from Central Germany and to elaborate the influence of environmental and/or individual host and vector factors on *Borrelia* prevalence. Small mammals were snap trapped (2017-2019) and ticks collected from vegetation (2018-2019) in grasslands and forests in Hainich Dün region. After species identification, 1167 mammal skin samples and 1094 ticks were screened by *B. burgdorferi* s.l. qPCR and positive samples characterized by multi-locus sequence typing (MLST). Generalized linear (mixed) models (GLMM/GLM) were used to estimate how seasonality, small mammal species/tick life stage and habitat affect individual infection status. In total, 10 small mammal species (*Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, *Arvicola amphibius*, *Clethrionomys glareolus*, *Microtus agrestis*, *M. arvalis*, *Crocidura russula*, *Sorex araneus*, and *S. minutus*), and 2 tick species belonging to the *I. ricinus* complex and *Dermacentor reticulatus* were investigated. *Borrelia* DNA was detected in 8 of 10 hosts with an average prevalence of 6.2% with 2 genospecies, *B. afzelii* and *B. garinii*, and at least 3 STs which have not been reported in small mammals before. In ticks, *Borrelia* spp. was found only in *I. ricinus* complex ticks with a prevalence of 13% and 6 genospecies (*B. afzelii*, *B. valaisiana*, *B. garinii*, *B. lusitaniae*, *B. spielmanii*, *B. burgdorferi* s.s.) and 25 STs of which 12 have not been described. The GLMM showed that ticks (but not small mammals) from grasslands were significantly more often infected than those from forests. The prevalence between sites varied significantly for ticks and small mammals. The prevalence was lower in *Apodemus* compared to *Microtus*, *Clethrionomys*, and *Sorex*. For ticks, adults were significantly more often positive than nymphs. Seasonality was not affecting prevalence significantly either for ticks or hosts. The GLM suggested that the probability of infection in ticks was positively correlated to the number of small mammal species per site. The results indicate that the variety of genospecies and STs of *B. burgdorferi* s.l. in this region is high. Local factors such as land use and species richness of potential hosts have an impact on *Borrelia* spp. prevalence and diversity in ticks.

EE29 Effect of forest structural complexity on tick-borne diseases in small mammals and ticks

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A positive association between small mammal density and nymphal density in the vegetation has already been demonstrated in forests. To better understand which forest characteristics influence the small mammal density, and as such also the tick density, we carefully selected 19 forests in Flanders (Belgium) for our study. These forests follow a carefully designed gradient in structural complexity and their dominant tree species is either beech (7), oak (5) or poplar (7). We also want to have a better understanding of the relationship between the tick-borne diseases found in the ticks from the vegetation, ticks found on the small mammals and in the small mammals itself. Ticks from the vegetation were collected using drag sampling in June, July and September of 2021. From the small mammals, caught using live traps in the same summer, the ticks were carefully removed and individual ear-punches were taken. All ticks and ear-punches are screened for different tick-borne diseases. Tick life stage and species were determined if possible. The two small mammals most abundantly captured were bank vole (*Myodes glareolus*) and wood mice (*Apodemus sylvaticus*). A total of 991 nymphs were caught in the vegetation and 611 larvae on the 314 small mammals. All ticks from the vegetation were *Ixodes ricinus*, on the small mammals I*. ricinus* was the most prevalent species but also *I. trianguliceps* was found. The results on the prevalence of the tick-borne pathogens will be presented in correlation with the tick density and also in relationship with the forest structural complexity score.

EE30 Tick-borne pathogens in ticks from pasture in Norway

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The aim of the study was to investigate the occurrence and severity of tick-borne pathogens, especially *Anaplasma phagocytophilum*, in ticks collected from pasture in Norway. In September 2021, ticks collected by flagging in south-western areas of Norway were used in an experimental infection trial in sheep. Collected ticks were stored in the refrigerator for one to two weeks before start of the experimental study, including sixteen lambs (5-6 months old). The actual lambs were placed in four groups, each with four lambs. Lambs was infected/infested as follows: Group A: *Anaplasma phagocytophilum* i.v (day 0); group B: *A. phagocytophilum* (i.v.)(day 0) + ticks (day 4); Group C: Ticks (day 0); Group D: Controls. All lambs were followed daily from day 0 to 28 by clinical examination and collection of engorged ticks. Blood (serum and EDTA) were samples frequently for hematological analysis and identification of pathogens both by PCR (*Anaplasma*, *Borrelia*, *Neoehrlichia*, *Babesia*) and serology (*Anaplasma*). In addition, unfed and engorged/partly engorged ticks were investigated for the same pathogens. Altogether, 1118 nymphs and 117 adults *I. ricinus* ticks were collected from pasture. Of these ticks, 998 nymphs and 104 adults (32 males and 72 females) were allowed to feed on sheep, approximately 135 ticks on each lamb. The total number of engorged /partly engorged ticks collected from these lambs were 369 nymphs and 24 adults. All lambs, except for the controls, reacted with fever and typical symptoms of an *A. phagocytophilum* infection, and the bacteria were also detected in the blood of these lambs together with seropositive antibodies. No other pathogens in the blood were detected. More results, including data from the investigated ticks, will be presented and discussed at the meeting.

EE31 Co-infection, reinfection and superinfection with *Anaplasma phagocytophilum* strains in a cattle herd based on ankA gene and multilocus sequence typing

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Anaplasma phagocytophilum is a Gram-negative obligate intracellular bacterium that replicates in neutrophil granulocytes. It is transmitted by ticks of the *Ixodes ricinus* complex and causes febrile illness in humans and animals. We used multilocus sequence typing (MLST) and ankA gene-based typing to study the molecular epidemiology of the *A. phagocytophilum* strains circulating in a German cattle herd over one pasture season. The aim was to investigate whether co-infection with two distinct variants, reinfection with the same and/or superinfection by a different strain occurred during one pasture season. Eight genetic loci were sequenced in 47 PCR-positive samples from 15 animals. Five different sequence types (ST) and four ankA alleles were detected in the cattle herd. Three different ST caused clinically overt tick-borne fever in primary infected animals. The concordance between ST and ankA allele was 100%. Therefore, the housekeeping genes used for MLST and the highly variable ankA gene were concatenated to increase resolution. Co-infection could be proven because samples of chronologically close collection dates were included. Co-infecting *A. phagocytophilum* strains differed by 14 to 18 single nucleotide polymorphisms (SNPs). Most superinfecting variants varied by 14 SNPs from the previous strain and appeared in median after a free interval of 31 days. Thus, it is unlikely that superinfecting strains arose by in-animal evolution. Immunity against re- or superinfection was assumed because the cattle developed clinical signs only during primary infection. The tick-pathogen-vertebrate host interaction is probably much more complex than previously thought taking into account the frequently occurring events of co-infection, reinfection and superinfection. This complex situation could not be easily simulated in an experimental infection and underlines the value of field studies.

EE32 Identification of *Anaplasma* species in wild animal species in the Kruger National Park and surrounding game reserves, Mpumalanga, South Africa

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The rapid advancement of next-generation sequencing technologies has led to the discovery of many novel sequences ascribed to the genus *Anaplasma*, with nearly 20 new species being proposed since the last formal organization of the genus. Most of the 16S rRNA gene surveys for *Anaplasma* were conducted on cattle and to a lesser extent on rodents, dogs, and ticks. Little is known about the occurrence, diversity, or impact of *Anaplasma* species circulating in wildlife species. Therefore, we conducted a 16S rRNA gene survey with the goal of identifying *Anaplasma* species in a variety of wildlife species in the Kruger National Park and neighbouring game reserves in Mpumalanga Province, South Africa, using an unbiased 16S rRNA gene microbiome approach. An *Anaplasma*-genus specific qPCR assay revealed the presence of *Anaplasma* species in 70.0% (21/30) of African buffalo, 86.7% (26/30) of impala, 36.7% (11/30) of greater kudu, 3.2% (1/31) of African wild dog, 40.6% (13/32) of Burchell's zebra, 43.3% (13/30) of warthog, 22.6% (7/31) of spotted hyena, 40.0% (12/30) of leopard, 17.6% (6/34) of lion, 16.7% (5/30) of African elephant and 8.6% (3/35) of white rhinoceros samples. The 16S rRNA gene microbiome sequencing data from the *Anaplasma*-positive wildlife samples revealed genotypes that phylogenetically group with known and previously published *Anaplasma* sequences, as well as novel *Anaplasma* genotypes. Our preliminary results reveal a greater genetic diversity of *Anaplasma* species circulating in wildlife species than currently classified within the genus *Anaplasma*, suggesting potential for transmission to livestock or companion animals. Our findings highlight the need for genetic and genome sequencing of putative species for correct classification.

EE33 Circulation of *Rickettsia aeschlimannii* in ticks and cattle in Côte d'Ivoire

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R. aeschlimannii is an obligate intracellular bacterium that causes Mediterranean spotted fever, which is a zoonosis. It is a rickettsial disease that presents as an acute febrile illness characterized by the appearance of skin pimples and bedsores. It should be noted that *R. aeschlimannii* is mainly transmitted by cattle ticks of the genus *Hyalomma*, which are present only in the northern part of Côte d'Ivoire. In fact, among the factors that contribute to the establishment of this pathogen, cattle occupy an important place. Despite the presence of its potential vectors in Côte d'Ivoire, Mediterranean spotted fever remains little known in cattle and is hardly diagnosed in our health centers. Main objective of the study the aim was to contribute to improving the management of zoonoses, particularly those transmitted by ticks in Côte d'Ivoire. Cattle blood samples and ticks from five regions of Côte d'Ivoire (Abidjan, Bondoukou, Bouaflé, Korhogo and Man) were used to test for *R. aeschlimannii* by molecular biology, notably qPCR. Two tick species were infested by *R. aeschlimannii* namely *H. marginatum* and *A. variegatum*. *A. variegatum* showed a very low prevalence of infection of 0.77% (95% CI: 0.02-4.24). In contrast, *H. marginatum* was very significantly infested with *R. aeschlimannii* at 100% (95% CI: 2.50-100). In cattle, two breeds were infected with *R. aeschlimannii*. These were 6.25% (95% CI: 0.16-30.23) for the Metis and 5.26% (95% CI: 0.13-26.03) for the N'Dama breed. It should be noted that the ticks and the infested cattle all came from the Korhogo region (North of Côte d'Ivoire). The presence of Mediterranean spotted fever in cattle herds in Côte d'Ivoire is now a reality. Veterinary services must be vigilant to contain its spread which for the moment is located in the northern part of Côte d'Ivoire. In the so-called "One Health" approach, the sensitization of health personnel on the circulation of tick borne Mediterranean spotted fever in Côte d'Ivoire is of paramount importance.

EE34 *Rickettsia rickettsii* is not in Asia

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Rickettsia rickettsii, the causative agent of Rocky Mountain spotted fever (RMSF) and Brazilian spotted fever (BSF) is endemic to the Western Hemisphere. The tick vectors of this agent include: *Dermacentor andersoni*, *Dermacentor variabilis*, *Amblyomma americanum*, *Amblyomma cajennense*, and *Rhipicephalus sanguineus*. Other tick species have been found to have evidence of *R. rickettsii* within them. Locations of *R. rickettsii* isolations from humans, dogs and ticks include: North America, Central America, and South America. Interestingly, in a recent review article, it was written that *R. rickettsii* was also endemic to Asia. One of the reports cited in the review mistakenly assumed that since *R. rickettsii* was used as an enzyme-linked immunosorbent assay (ELISA) antigen preparation, that the ELISA-positive results would indicate the IgG antibodies detected were specific to *R. rickettsii*. Because rickettsial serological assays are group-specific, either for typhus group rickettsiae (TGR), spotted fever group rickettsiae (SFGR) or scrub typhus group orientiae, this was unlikely. Moreover, the report in question, did not report the presence of *R. rickettsii* in Asia (Indonesia), but only that SFGR-specific antibodies were present. To determine how much of a problem, the idea of *R. rickettsii* erroneously associated with Asia is, a literature examination of individual reports and review articles was undertaken. To determine the validity of potentially false accounts, an evaluation of reports' methods, results and conclusion sections was conducted. The literature was assessed to determine whether ticks or vertebrate hosts were evaluated and which methods (culture isolation, molecular assays and/or serological studies) led to the determination of the presence of *R. rickettsii*. It was ascertained that no culture isolates of *R. rickettsii* were reported. Moreover, molecular studies utilizing conserved gene fragment sequences were not specific enough to identify *R. rickettsii*. Lastly, group-specific serologic assays containing *R. rickettsii* antigens that were positive were erroneously assumed to be due to species-specific antibodies against *R. rickettsii*, and not the result of another spotted fever group rickettsia. In conclusion, there is no substantial data that currently supports the premise that *R. rickettsii* is in Asia.

EE35 Plasticity in the timing of detachment of an Eurasian-African songbird tick, *Ixodes frontalis*

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In non-permanent parasites, host detachment should take place in an environment that ensures the continuation of their life cycle. Timing of detachment - in combination with the host's space use - affects dispersal and transmission success of the parasites and of the pathogens they vector. Before reaching the adult reproductive stage, ticks need to go through multiple immature developmental stages (larva and nymph), each feeding on host blood. In between the feeding bouts, they often remain in the off-host environment for considerable periods of time. With this study, we aimed to obtain more insight in *Ixodes frontalis* off-host habitat use by comparing its detachment pattern in different life stages with that of two habitat-specialized ticks also found on birds: the endophilic tree-hole tick (*Ixodes arboricola*) and the exophilic sheep tick (*Ixodes ricinus*), the latter living in humid understory vegetation of forests. For this, we artificially infested hole-roosting (Great tits, *Parus major*) and open-roosting (Blackbirds, *Turdus merula*) birds with ticks under laboratory conditions, and recorded whether detachment occurred during the day or the night. We hypothesize that nocturnal detachment improves off-host mating opportunities and host localization, whereas diurnal detachment optimizes tick dispersal. *Ixodes frontalis* nymphs detached during the night, especially when feeding on blackbirds. This behaviour was very similar to that of *I. arboricola* (larva and nymph) feeding on great tits. In contrast, *I. frontalis* larvae detached during the day, especially when feeding on great tits, which resembles that of *I. ricinus* feeding behaviour (larva and nymph). *Ixodes frontalis* left the host within seven days, immediately after completion of the blood meal. This is similar to both developmental stages of *I. ricinus* but contrasts with the very long (up to 20 days) feeding duration in *I. arboricola*. Thus, *I. frontalis* shows strong plasticity, switching from dispersal-centered (larvae) to host-centered (nymphs) detachment behaviour. Findings are discussed with regard to the ticks' habitat use, dispersal, life history and host specificity.

EE36 When do ticks invade?

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Invasive tick species and the pathogens they vector pose increasing threats to human and animal health around the world. Invasion may be expansile, increasing the contiguous range of a tick, or occur due to long distance transport and introduction to a new region. Techniques like niche or movement models highlight locations at risk of novel tick establishment, but less attention has been paid to particular characteristics, and species that have these characteristics, and are thus most likely to invade. Here we briefly overview invasion biology, which is the study of how organisms come to be introduced into a new area and establish themselves with some probability of local persistence. We analyze examples of tick invasion events in North America in order to identify those characteristics of the invasive tick species that facilitated the invasion. Our case studies include brown dog ticks, American dog ticks, Asian longhorned ticks, *Amblyomma* species and others. Ticks with the identified common characteristics are likely to be ones that will invade in the future. Commonalities among invasive ticks are that they thrive in anthropogenically modified habitats, feed on either domestic animals or wildlife that occur in high density, and can survive across a broad range of climatic conditions. Our invasion examples varied widely in life history and reproductive characteristics. We also assess environmental factors that underlie invasion such as climate, habitat, and societal changes. Identifying patterns and key components of the process may help guide surveillance and inform prevention and intervention programs to lessen the consequences associated with invasive tick-borne disease.

EE37 The raccoon, an invasive alien species in Europe: what contribution to the population dynamics of local ticks and their infectious agents?

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The introduction of invasive alien species can cause significant health impacts through the introduction with them of new pathogens or vector species, or the modification of the circulation of pathogens or vector populations already present in the area where they were introduced. Raccoon was introduced into Europe in the 1920-30's: as a result of escapes or releases of individuals from farms, zoos or used as pets, it built up large populations and is currently considered an invasive alien species due to its predatory action on native species. While in its native area - the North American continent, raccoon can be parasitized by large tick loads and is a competent host for *Borrelia burgdorferi* sensu stricto, no study has been conducted in Europe to assess its impact on endemic tick population dynamics and the circulation of tick-borne infectious agents. In this study, we investigate tick infestation and tick-borne pathogen infection of raccoons in two different ecosystems in France: in a North-East deciduous forest area on the French-Belgian border (semi-continental climate) and in a South-West peri-urban area in Gironde dominated by wet meadows and hydrophilic woodlands (oceanic climate). In summary, out of 171 animals trapped at different seasons in 2020 and 2021 and examined for the presence of ticks, 324 ticks, mainly nymphs and females, were collected from 77 individuals (46%). Four tick species were observed - 229 *Ixodes ricinus*, 44 *I. hexagonus*, 23 *Haemaphysalis concinna*, 2 *Dermacentor reticulatus*, as well 25 Ixodes spp. In Gironde, only *H. concinna* and *I. hexagonus* was observed. In the North-east, raccoons were mainly infested by adults of *I. ricinus*. Spleen samples were analysed with high-throughput microfluidic real-time PCR targeting numerous tick-borne pathogens: 3.5% were positive for *A. phagocytophilum* and none were positive for *Borrelia* spp., *Babesia canis*, *Rickettsia* spp. and *Bartonella* spp. The results of the PCR in ear biopsy targeting *B. burgdorferi* s.l. and *A. phagocytophilum* will be presented. The potential influence of raccoon on the local population dynamics of the different tick species and tick-borne pathogens in these ecosystems will be discussed.

EE38 Another ecological trap for ticks: comparison of introduced raccoons and masked palm civets in Japan

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Ticks infest animals as their main source of blood-meal, but host animals vary by species and developmental stage. However, there are known examples that suggest that host specificity is not the only determinant of tick infestation patterns. The grooming and feeding behaviors of Virginia opossums (*Didelphis virginiana*) to remove ectoparasites such as tick, flea, and lice are known to reduce tick infestations. In this study, we conducted a survey on tick infestation on raccoons (*Procyon lotor*) and masked palm civets (*Paguma larvata*) in Japan, which are introduced mammals with similar ecological niches. A total of 18,509 ticks of six species were collected from 60 raccoons and 152 ticks of two species from 41 civets captured in the same area. *Haemaphysalis flava* was the dominant species in both mammals, and its infestation was significantly intense in raccoons (Bootstrap BCa 95 % Confidence Interval (CI) of mean difference: 115.4 < xadult *H. flava* < 368.4, 64.12 < xnymph *H. flava* < 162.59, 2.99 < xlarva *H. flava* < 28.96). We collected gastro-intestinal digesta of raccoons and civets to assess the number of removed ticks by counting the number of ingested ticks. We found 16 and 106 nymphal and larval *Haemaphysalis* spp. ticks in raccoons and civets, respectively, indicating that civets groomed and ingested significantly more ticks than raccoons (Bootstrap BCa 95 % CI of mean difference: −3.27 < xnymph.

EE39 Survey of tick-borne zoonotic agents in wild ungulates and *Ixodes ricinus* ticks in Northern Italy

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In European countries, most vector-borne zoonotic diseases derive from tick bites. Tick-borne encephalitis virus (TBEV) and *Borrelia burgdorferi* sensu lato (s.l.) have a remarkable impact on human health, as well as other neglected but emerging bacteria like *Rickettsia* spp., *Coxiella burnetii*, and *Ehrlichia* spp. Wild ungulates are preferential hosts for tick feeding in sylvatic cycle and thus have an important role in tick biological cycle and a potential to diffuse infected ectoparasites. This research aimed to evaluate the prevalence of TBEV, *B. burgdorferi* s.l., *Rickettsia* spp., *Ehrlichia* spp., and Coxiella spp. in blood samples and ticks collected from wild ungulates. Sampling procedures were carried out in the Alpine and Prealpine areas of Northern Italy, during the 2017-2020 hunting seasons. A total of 274 blood samples were collected from roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), alpine chamois (*Rupicapra rupicapra*) and mouflon (*Ovis orientalis musimon*). A total of 331 *Ixodes ricinus* ticks were sampled from culled animals. DNA and RNA extraction was performed on all blood and tick samples followed by real-time PCR screening of the pathogens mentioned above. Positive samples were then confirmed by classical PCR and Sanger sequencing analysis. *B. burgdorferi* s.l. was detected in 1.1% (3/274) of blood samples and 8.8% (29/331) of *I. ricinus*. The identified species were *Borrelia garinii*, *Borrelia afzelii*, *Borrelia burgdorferi* sensu stricto and *Borrelia valaisiana*. *Rickettsia* spp. was identified in 1.1% (3/274) of blood samples and in 26.6% (88/331) of ticks with detection of two species, *Rickettsia helvetica* and *Rickettsia monacensis*. *Neoehrlichia mikurensis* was found in 1.2% (4/331) of *I. ricinus* but not in blood. Positivity for TBEV and C*. burnetii* were not detected in blood nor tick samples. A remarkable prevalence of zoonotic *B. burgdorferi* s.l. and *Rickettsia* spp. was found in Northern Italy, highlighting a concrete risk of tick-transmitted diseases. Although with a lower prevalence, the detection of *Neoehrlichia mikurensis*, a zoonotic bacteria identified in the last decade, is noteworthy. Lack of detection of TBEV and *C. burnetii* may be related to a low prevalence of these pathogens in the studied area, but their presence cannot be excluded at all. This research outlined the significant diffusion of tick-borne pathogens in *I. ricinus* ticks, together with a much lower prevalence detected in wild ungulates that makes these species suitable feeding hosts but not reservoirs of the studied zoonotic pathogens.

EE40 Zoonotic tick-borne pathogens in ticks from vegetation and Alpine ibex (*Capra ibex*) in the Maritime Alps, Italy

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As part of a project aimed to conservation of the Alpine ibex (*Capra ibex*), we assessed the presence of ticks in the Natural Park of the Maritime Alps (Piedmont, Italy), by collecting them from vegetation and ibex, captured in late spring and early summer. *Ixodes ricinus* was the most abundant and widespread questing species (94.5% of 658 collected ticks), followed by *Haemaphysalis punctata* and *Dermacentor marginatus*. The former was collected from 780 up to 1824 m a.s.l. Beechwoods were the tick preferred habitat, followed by firwoods and stone-pinewoods. Tick abundance significantly decreased with altitude. Twenty-eight of 72 ibexes were infested by *I. ricinus* (87.4% of 143 collected ticks), *H. punctata* (10.5%) and *Haemaphysalis sulcata* (2.1%). By molecular analysis, *Borrelia burgdorferi* s.l. was identified in questing *I. ricinus* (28.3%; 95%CI: 19.4-38.6), namely *B. afzelii*, *B. garinii*, *B. valaisiana* and *B. lusitaniae*. The pathogen was not present in *I. ricinus* collected on animals. *Rickettsia* spp. (*R. helvetica* and *R. monacensis*) was detected in both questing I. ricinus (20.6%; 95%CI: 12.9-30.3) and ticks from ibex (30.2%; 95%CI: 21.2-40.4). *Rickettsia monacensis* was identified in questing *H. punctata*. Finally, Anaplasma *phagocytophilum* was detected in 4.3% (95%CI: 1.2-10.8) of questing *I. ricinus*, and in 45.3% (95%CI: 34.6-56.4) of *I. ricinus* collected from ibex. *Ixodes ricinus* females collected from animals were significantly more infected by *A. phagocytophilum* than females collected from vegetation (OR=11.7; 95%CI: 3.8-48.1). Ecotype I was detected in ticks from animals, ecotype II in questing ticks. Our study indicates that different tick-borne zoonotic agents are present in the Park territory with a wide altitudinal range, as confirmed by ticks found on a typical mountain-dwelling mammal. The significantly higher prevalence of *A. phagocytophilum* in ticks from ibex compared to questing ticks suggests that ibex could have a reservoir role, similar as other wild ungulate species; ecotype I, prevalent in our sample, is considered zoonotic. On the other hand, the detection of *B. burgdorferi* s.l. limited to questing ticks suggests that ibexes, as other wild ruminants, are not competent hosts for this bacterium. Our results confirm the presence of *I. ricinus*, *H. punctata* and *D. marginatus* in the study area, where they had been already reported 30 years ago, and signal *H. sulcata* as an additional species. Moreover, the study further documents the expansion of ticks and associated pathogens to high altitudes in the Alps. Tourists visiting the park and all professionals operating in the investigated area should be made aware of the existing hazard.

EE41 Roe deer as useful animal sentinel to detect the exposure risk to tick-borne pathogens?

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Monitoring changes in the risk of exposure of humans to tick-borne pathogens circulating in the environment is critical in the current context of global change. The circulation of an agent can be assessed by detecting it in vectors, hosts, or by indirect approaches using antibody detection in sampled hosts. When multiple host species are involved, a host that is widely distributed and commonly exposed to the vector could represent a useful sentinel. The roe deer (*Capreolus capreolus*) is a widely distributed mammal in Europe. The species is highly infested by ticks and exhibits a relatively stable spatial behavior. Moreover, roe deer sampling is relatively easy to perform as a by-product of hunting, with 581,000 individuals shot last year in France. Acquiring data on tick-borne pathogens circulating in roe deer may allow mapping the risk of exposure to infected ticks for various host species including humans. Among these pathogens, *Borrelia burgdorferi* sensu lato (Bbsl) is a complex of bacteria transmitted to humans by the bites of Ixodes ticks, causing Lyme disease. Unlike humans, deer do not develop the disease but kill the bacteria through their complement immune system. The presence of antibody against *Borrelia* may nevertheless inform us on their exposure to the bacteria during a time window depending on the temporal persistence of antibody levels. We aim to improve our understanding of the temporal dynamic of the immune response of roe deer to Bbsl and its implications for the use of this species as a sentinel of Bbsl risk in their environment. For this purpose, we estimated the persistence of antibodies levels against Bbsl by analyzing the dynamics of the serological status of roe deer from two populations repeatedly captured each year for 10 years. We estimated the proportion of individuals changing from seronegative to seropositive (seroconversion rate) and from seropositive to seronegative (seroreversion rate) each year using capture-mark-recapture statistical modelling. The seroreversion rate took values that indicate a relative short temporal persistence of anti-Bbsl antibody levels. The relative short persistence suggests that the results of serology provide an information of the risk near to the sampling and evaluate the time interval between samples to update maps. The results confirm the usefulness of the roe deer as sentinel to map spatial variability of risk using 3,500 samples of hunted roe deer between 2019 and 2022 in nineteen French counties.

EE42 Bird species influence more the tick burden of French common birds than environmental conditions during the breeding season

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Tick-borne diseases represent a serious threat for human and animal health worldwide. Host species can differ in their quality as a reservoir, which is their ability for producing infected ticks (i.e. reservoir host potential). Understanding the role of each host species in the production of infected ticks and the main factors that influence it is important to be able to prevent tick-borne diseases. The number of ticks produced by a host species is a key element of its reservoir host potential. Whereas many studies in Europe have focused on the tick burden of mammals (such as deer and rodents), few have considered birds although they also participate in the population dynamics of ticks, during the migration period (with a special importance for long-distance dispersal) and during the breeding period, when they contribute to the multiplication of ticks and pathogens. In this study, we explored the relative importance of bird species and of environmental conditions on the tick burden of birds from a French forest community, during their breeding period. The aim was to investigate the effect of life history characteristics if the bird species had the greatest influence on their tick burden and the effect of environmental factors if it did not. We first used a zero-inflated negative binomial model to test the relative influence of the year (as a proxy of environmental conditions) and of the bird species. We then studied the effect of bird extrinsic (year) and intrinsic factors (species, sex/age, mean body mass and mean foraging height) on the tick burden of birds. Finally, we developed a tick production index per bird species by considering both the mean tick burden and the host density for each bird species. We observed that tick burden of birds varied more between species than between years and the most infested birds belonged to the Turdidae family. Moreover, juveniles, species with a low foraging height in the vegetation and a high mean body mass were significantly more infested by ticks. These results led to the identification of the bird species the most involved in the tick production in the studied ecosystem. In the future and in a perspective of tick-borne disease prevention, this would allow us to determine which bird communities participate the most to acarological risk.

EE43 Individual variation in a tick-bird system: tick life history, fitness, evolutionary potential, and host quality

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Tick-host systems are characterized by dynamic interactions that facilitate reciprocal adaptations and counter-adaptations affecting the life history, ecology and ultimately the evolution of both the host and parasite. To comprehend these systems, it is thus essential to estimate the intraspecific trait variation and evolutionary potential, the genetic and phenotypic covariance between traits across life stages, and the effects that individual host characteristics (host quality) have on ticks. Nevertheless, ticks have mainly been studied as agents of selection and pathogen vectors rather than as evolving species in their own right. Moreover, host-mediated effects on tick traits and performance have mostly been neglected. To shed light on tick individual variation and evolutionary potential we collected a wild population of the nidicolous tree-hole tick (*Ixodes arboricola*) and raised two consecutive generations in semi-natural conditions in our laboratory and in nest boxes in the field. Ticks were individually marked and fed on wild great tits (*Parus major*). Relatedness between individuals was known for both ticks and great tits. For larva, nymph, and adult stages we measured on-host and off-host performance (e.g., attachment, feeding, moulting, survival, and hatching success) as well as life-history traits (e.g., feeding time, engorgement weight, moulting time, and clutch size) at the individual level. Furthermore, we investigated individual variation and heritability of host quality through variation in tick performance and life-history traits. Heritability estimates of tick life-history traits were generally higher in nymphs than in larvae and estimates for engorgement weight and moulting time were consistently higher than those for feeding time. Higher engorgement weights were correlated with shorter moulting and feeding times in larvae and nymphs but not in engorged females. As regards host quality, we found a significant correlation in attachment success between larvae and nymphs on the same host, suggesting consistent among-host variation for this performance measure. We found a strong heritable signal for host quality as measured through tick feeding time, and lower but substantial estimates in other performance variables. Feeding success and survival of larvae was lower on female birds, and nymphal survival was higher on older birds. We discuss the implications of our results for tick evolutionary ecology, co-evolutionary dynamics, acaricide resistance, and disease spread.

Genetics and genomics (GG01-GG10)

GG01 CRISPR-mediated genome editing in *Rhipicephalus microplus* ticks

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Recent years have seen an enormous increase in genomic data on ticks and tick-borne pathogens. Unravelling tick gene function may advance the development of novel control methods to improve human and animal health. Currently, gene silencing by RNA interference (RNAi) is the most widely used tool to examine tick gene function. Despite its many advantages, RNAi does have some limitations, as it is for instance not easily applicable all tick life stages, its knockdown effect is transient and complete gene silencing is rarely achieved. CRISPR-Cas9 based gene editing has the potential to overcome many of these disadvantages and has found wide use in other arthropod species such as mosquitoes. In this proof-of-principle study, we examined the possibility of inducing CRISPR-Cas9 based gene editing in *Rhipicephalus microplus* ticks by delivery of the CRISPR/Cas9 ribonucleoprotein complex (RNP), consisting of the Cas9 protein and single guide RNA (sgRNA), by injection in engorged females followed by electroporation. The distalless (*dll*) gene was selected as a target, as previous studies in other arthropods showed that it is essential for limb development. Successful CRISPR-Cas9 based alteration of the *dll* gene is expected to create frameshift mutations causing aberrant limb development. Transovarial RNAi studies in *R. microplus* as well as *Ixodes ricinus* ticks confirmed that *dll* gene silencing during embryogenesis resulted in aberrant larvae with missing or malformed legs. Five different sgRNAs targeting the first exon of the *dll* gene from *R. microplus* were designed and their cutting activity was confirmed in *in vitro* cleavage assays. Combinations of the Cas9 protein with single sgRNAs or sgRNA mixtures in different mole ratios were injected in groups of engorged *R. microplus* females. These groups were exposed to different electroporation conditions and subsequently allowed to oviposit. Larvae that hatched were screened phenotypically under a stereomicroscope. DNA was extracted from aberrant larvae and subjected to PCR for *dll* sequence analysis. A proportion of the larvae that hatched from females injected with Cas9 and *dll* sgRNAs showed aberrant phenotypes such as missing or malformed legs that were not found in the control groups. DNA sequencing confirmed mosaic mutations including insertions and deletions at the expected cutting sites. These results demonstrated that CRISPR-mediated genome editing can be performed by the injection of Cas9 protein with sgRNAs in engorged female ticks followed by electroporation. Further studies are required in order to optimize this method and to determine its suitability for knock-in editing in ticks.

GG02 South African buffalo-derived *T. parva* is genetically divergent from East African strains

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Theileria parva is an economically important haemoparasite responsible for a high number of cattle mortality in Africa including South Africa. It is associated with three diseases, namely East Coast fever (ECF), January disease and Corridor disease that occur in different African countries. In recent history, Corridor disease, a buffalo to cattle transmitted disease, has been the only endemic disease reported in South Africa. ECF and January disease are cattle-to-cattle transmitted. Knowledge of the genetic diversity of South African *T. parva* population will assist in determining its origin, evolution and identify any cattle-to-cattle transmitted strains. To achieve this, genomic DNA was extracted from blood samples collected from Corridor endemic areas (KwaZulu-Natal, Mpumalanga and Limpopo provinces) and archived tissue culture materials prepared from buffalo-derived isolates. Paired-end whole genome sequencing using Illumina HiSeq 2500 was performed. Data were analysed using BWA and SAMtools variant calling with the cattle-derived *T. parva* Muguga genome used as a reference and publicly available East African cattle- and buffalo-derived *T. parva* strain sequences for comparison purposes. Buffalo-derived strains had more diversity, with twice the number of variants than cattle-derived strains, which confirms that buffaloes are reservoirs of *T. parva*. Phylogenetically, strains tended to cluster by hosts with South African buffalo-derived strains clustering with East African buffalo-derived strains and cattle –derived strains clustering together. Among the buffalo-derived strains, South African strains were genetically divergent from other buffalo-derived strains indicating possible geographic sub-structuring. The knowledge generated from this study indicate that to date, ECF is not circulating in buffalo from South Africa.

GG03 Elucidating tick transmission and sequestration phenotypes in *Babesia bovis* using live vaccines

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Babesia bovis is the causative agent for Asiatic redwater. Current vaccines utilize live attenuated strains that is produced in cell culture or animals. These vaccines are costly to produce and needs a cold chain for distribution and storage. Stable subunit vaccines would be a preferred alternative but one of the bottlenecks are the identification of suitable vaccine candidates. The current study address one possible manner in which vaccine candidates may be identified using current live vaccines. The South African S24 *B. bovis* vaccine derive from a virulent field strain that has been rapidly needle-passaged through 23 passages resulting in phenotypes and genotypes unique to the vaccine. This include low virulence, efficacy against genotypically different virulent field strain challenge, non-transmissibility by *Rhipicephalus* (*B.*) *microplus* as homologous strain, co-transmissibility when combined with heterologous field strains, inability to sequester and propagate in the host when infected by limited dilution and the possession of a single Bv80 A allele of 558 bp. A major question that remain is what mechanism allow co-transmission when present as mixed strains? To investigate this, the genomes were sequenced from parent strains (S24 + 05-100 field strain) as well as clonal lines obtained after tick-transmission and isolation by limiting dilution infection. One clone showed evidence of sexual recombination of S24 and 05-100. This suggests that 05-100 contributed both a tick transmissible and a sequestration phenotype during sexual recombination that allowed co-transmission of the S24 strain. Various potential genes that may be involved in tick transmission and sequestration was present in the recombinant. This opens the possibility to use progressive sexual recombination with the S24 parent strain and selection based on tick transmission and limiting dilution to identify the factors responsible for tick transmission and sequestration. These may then be exploited to produce dual transmission blocking and virulence mitigating vaccines.

GG04 Determining the stage of '*Candidatus*' Mycoplasma haemobos infection by quantitative Real-Time PCR using SYBR green fluorescent dye

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'*Candidatus*' Mycoplasma haemobos is a small, pleomorphic, obligate, gram-negative, uncultivable cell wall-less bacterium transmitted by ixodid ticks and biting flies. It is found attached onto erythrocyte surface or free in the serum of animals. This emerging bacterial pathogen and *Mycoplasma wenyonii* are the major causative agents of bovine haemoplasmosis. In field situations, it is usually difficult to determine the stage of infections hence, the use of quantitative real-time PCR (qRT-PCR) to evaluate pathogen gene copy numbers. A total of 130 blood samples were obtained from Brahman x Kedah-Kelantan and Bali cattle after physical examination. Genomic DNA was extracted from whole blood using the DNeasy® Blood and Tissue kit (Qiagen, Germany) according to the manufacturers' protocol. DNA concentration and purity were measured with a Nanodrop spectrophotometer. DNA samples with A260/A280 ratios between 1.7 – 2.2 were further analysed. Haematology and serum biochemistry analyses were performed using automated haematology and chemistry analysers. Real-time PCR amplification was performed using 2x SensiFast SYBR® Hi-ROX mix, forward and reverse DNA primer sequences (CMhbos F 5`-AGATCCGGCAGTGTGAGAAA-3′ and CMhbos R 5`-TGCAGCAGCAGCTATTGGTA-3`) targeting gapN gene of '*Candidatus*' M. haemobos, 100ng of template DNA and molecular grade water using previously reported thermal cycling conditions. A melt curve was generated to verify the specificity of the amplifications, alongside a standard curve. '*Candidatus*' M. haemobos was detected in 70/130 (40.98%, 95% CI 29.54% – 53.50%) and its melt curve showed a single PCR product with melting temperature at 79.5°C. Cattle showing clinical signs of the disease (haemolytic anaemia, anorexia, dehydration, weight loss and prefemoral lymphadenopathy) had between 17,208 and 4,403,84 pathogen gene copy (GC) numbers per microlitre while subclinical carriers had between 317 and 15,353 pathogen GC numbers per microlitre. Haematobiochemical findings in the cattle showing clinical signs of the disease include macrocytic normochromic anaemia, anisocytosis, poikilocytosis, thrombocytosis, degenerative left shift, hyperkalaemia, hypernatremia, hyperchloridemia, hyperglobulinemia, hyperbilirubinemia due to increase in unconjugated bilirubin level, and significant decreases in serum urea and creatinine levels. Therefore, '*Candidatus*' M. haemobos infected cattle with pathogen gene copy numbers between 17000 to 440000 could be regarded as clinical cases while those with GC numbers between 317 – 15000 could be regarded as subclinical carriers.

GG05 Full length 16S nanopore sequencing reveals the microbiome of *Ixodes ricinus* ticks for varied UK woodland habitats

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Full length 16S nanopore sequencing reveals the microbiome of *Ixodes ricinus* ticks for varied UK woodland habitats, and the development of a novel multiplex nanopore sequencing assay to identify the microbiome, tick species and bloodmeal host of individual ticks. The microbiome of ticks is complex and it is largely unknown how the environment affects its composition. Microbiome studies up until now focused on the targeting small regions of the 16S rRNA gene, potentially missing important bacterial species. For this study, questing ticks were collected during the Spring in 2019, 2020 (during UK COVID lockdown) and 2021 from six different habitats: Beech, Grazed, Pine, Spruce, Oak and Glades. Adults and nymphs were morphologically identified as *Ixodes ricinus* and were pooled according to life stage (5 adults per pool and 10 nymphs per pool). Sequencing of the full 16S gene was carried out using Oxford nanopore full length sequencing and classification of bacteria to genus level was determined via the Epi2me platform. Bacterial microbiome showed significant differences across years with 2019 having the most diverse microbiome and 2020 the least. We report a number of observations, including that *Rickettsia* was more abundant in females across all habitats. *Anaplasma* was consistently detected across all habitats in nymphs. *Borrelia* was detected in 2019 across all lifestages but only detected in females in 2020 and not seen at all in the pools from 2021. In conclusion, ticks from a UK woodland have a diverse microbiome that differs significantly between lifestages and habitats within the same woodland. The bacterial diversity of the tick microbiome also changes over time, marked changes in 2020 possibly due to changes in human behaviour in the woodland during COVID lockdown. Data from the study also informed the design of a PCR panel for rapid nanopore sequencing of targets designed to identify the microbiome, tick species and last bloodmeal host of each tick in a single multiplex sequencing assay which can be performed cost-effectively at high throughput using a portable MinION device. Performance of this assay will be demonstrated on a variety of tick cohorts.

GG06 Novel *Babesia*, *Ehrlichia* and *Hepatozoon* genotypes in white-eared opossums (*Didelphis albiventris*) and associated ticks from Brazil

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White-eared opossums (*Didelphis albiventris*) are well adapted to anthropized areas, which favors their frequent contact with domestic animals and humans, mediating the transmission of arthropod-borne pathogens. Between May and December 2017, 43 *D. albiventris* (27 males and 16 females) were captured in the city of Campo Grande, state of Mato Grosso do Sul, midwestern Brazil. Ticks (n= 46 *Amblyomma dubitatum* nymphs and n= 24 *Amblyomma* spp. larvae) were collected from 14 out 43 (32.5%) of the white-eared opossums. Intra-erythrocytic oval ring–shaped organisms similar to piroplasmid merozoites were detected in blood smears from four. Eleven out of 43 (25.5%) *D. albiventris* sampled in Campo Grande were positive to piroplasmids in the nPCR targeting the 18S rRNA gene. Two (nymphs) out of 28 (7.14%) *A. dubitatum* samples obtained from *D. albiventris* were also positive. The phylogenetic inference performed using the near-complete 18S rRNA, hsp-70, and cox-1 genes positioned the putative novel piroplasmid species detected in *D. albiventris* and associated *A. dubitatum* ticks near to *Babesia* sensu lato clade (Western group—cluster III) and distant from the Australian marsupial-associated piroplasms. An inclusion resembling Anaplasmataceae morulae was found in a white-eared opossum's monocyte. Five (11.63% [5/43]) white-eared opossums' blood samples and 7 (25% [7/28]) ticks' samples (2 pools of *Amblyomma* spp. larvae and 5 pools of *A. dubitatum* nymphs) were positive in the PCR assays based on the rrs gene of Anaplasmataceae. The phylogenetic analysis based on the rrs gene positioned three sequences obtained from opossums and ticks in the same clade, forming a subclade within the *Ehrlichia canis* clade, albeit these samples were negative in a qPCR assay specific for *E. canis* based on the dsb gene. On the other hand, in the phylogenetic analyses based on both the gltA gene and 23S-5S intergenic region, the sequences obtained from opossums' blood samples were positioned in a separate clade from the other *Ehrlichia* sequences. Two opossums were positive for both Anaplasmataceae and piroplasmids. The phylogenetic analysis based on the 18S rRNA gene positioned the *Hepatozoon* sp. sequence obtained from a *D. albiventris* specimen in a clade with sequences previously detected in the marsupial "Monito del monte" (*Dromiciops gliroides*) from Chile. All the other sequences of *Hepatozoon* sp. previously detected in marsupials from Brazil were positioned in a separated clade. The present work showed the occurrence of putative novel genotypes of *Babesia*, *Ehrlichia*, and *Hepatozoon* in white-eared opossums and associated *A. dubitatum* ticks from midwestern Brazil.

GG07 What have we learned from the first 600 mitochondrial genomes of ticks and other Acari?

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Mitochondrial genomes have been remarkably instructive about the evolutionary-history (phylogeny), population-genetics and phylogeography of Acari, particularly the ticks. At present we have entire mt genomes for 125 of the 896+ species of ticks (316 mt genomes in total), and for 146 of the thousands of other species of Acari (296 mt genomes in total). Total number of mt genomes available for the Acari is 612 [we aim to have mt genomes for 400+ species of ticks and other Acari by 2025 in time for the 11th International Congress of Ticks & Tick-borne Disease]. It has never been easier to sequence entire mt genomes. Any lab with basic wet lab capability can do this by using commercial sequencing companies. In 2021, Barker & Kelava precipitated the Tick Mitochondrial Genome Network with a YouTube Channel of the monthly meetings [https://www.youtube.com/channel/UCnBhfhYxjC4rsJmVpBwHT0g/_featured]. Selected insights and outcomes will be discussed in our talk, including: (i) phylogenetic trees that led Ben Mans and us to propose that the genus *Carios* s.l. be dissolved and the subgenera *Alectorobius*, *Antricola*, *Nothoaspis*, *Reticulinasus* and *Subparamatus* be raised to genus level; (ii) an extraordinarily re-arranged mt genome arrangement in *Amblyomma* (*Africaniella*) *transversale* that prompted the re-elevation of the subgenus *Africaniella* to a genus; (iii) recent insights into the phylogeny of the genera *Robertsicus*, *Amblyomma* and *Haemaphysalis*; (iv) the tick-box motif may be involved in the insertions of 132 to 312-bp in two *Haemaphysalis* species (*H. (Al.) inermis* and *H. (Al.) kitaokai*) and *R. (B.) geigyi*; (v) assignment of several ticks to subgenera including: *Ixodes woylie* and *I. barkeri* to *Endopalpiger*; (vi) realization that *Ixodes anatis*, the kiwi tick, may be closely related to the ticks of marsupials of Australia and Papua New Guinea.

GG08 Proteomic insight into the midgut of the hard tick *Ixodes ricinus* during its development

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The tick midgut is the main tissue for storage and digestion of host blood, which serves as the sole source of energy and nutrients for overall tick development and reproduction. During feeding of each developmental stage, dynamic changes of tick midgut epithelium reflect the changes in the physiological processes occurring in this tissue. Furthermore, the midgut serves as the primary interface between the tick and tick-borne pathogens that determines tick vector competence. Several transcriptomics data have been published in *Ixodes ricinus*, however only few studies have examined tick proteomes. Unlike transcriptome data, proteomics enables in-depth understanding of key cellular processes occurring in the investigated tissues. Additionally, potential targets for drug or vaccine treatment might be developed. This work will present proteomics insight into the midgut during tick development for the first time. We used a label-free quantitative proteomics to elucidate changes during the blood meal and development of *I. ricinus*. Midguts from different feeding stages of nymphs (unfed, fed for 2 days, fully-fed, 14 days after detachment, and before molting) and adults (unfed, fed for 1, 3, 5 days, fully-fed, 4 and 6 days after detachment) were dissected, thoroughly washed to remove the excess of the host blood, and homogenized. In-solution digestion using trypsin was followed by the peptide analysis carried out on the timsTOF Pro (Bruker) mass spectrometer coupled to an Ultimate 3000 RSLnano System (Thermo Fisher Scientific). Obtained raw data were submitted to the actual database available on UniProt for the *I. ricinus* and searched in MaxQuant software. Data were further analyzed using Perseus and Blast2GOPro programs or in-house written scripts. Acknowledgement: Supported by GACR 21-08826S and ERDFunds (No.CZ.02.1.01/0.0/0.0/16_019/0000759).

GG09 Genetic diversity of *Babesia divergens* in *Ixodes ricinus* nymphs collected from farm- and woodland sites in Ireland compared to *B. divergens* isolates from humans, cattle and deer

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The tick-borne protozoan parasite, *Babesia divergens* causes redwater fever in cattle and a rare, albeit life-threatening disease in humans. In Ireland, *B. divergens* has always been considered an important pathogen as the high incidence of redwater fever precluded areas of the country from cattle farming. Moreover, a relatively large proportion of human cases were reported here. Much uncertainty remains over the potential role of deer as reservoir hosts for the parasite. While roe deer (*Capreolus capreolus*), which are frequently infected with *Babesia* species other than *B. divergens*, are absent from Ireland, red deer (*Cervus elaphus*), which often harbour babesias that are genetically very similar (if not identical) to *B. divergens*, are relatively widespread. In the present study, 1,369 nymphal *Ixodes ricinus* ticks collected from woodland, farmland, bog and limestone pavement habitats were screened for the presence of *B. divergens* using a species-specific TaqMan PCR (targeted at hsp70) followed by conventional nested PCR (targeted at the 18S rRNA gene locus) and compared against published Irish *B. divergens* isolates from cattle, humans and red deer. Overall just 1% of *I. ricinus* nymphs were infected with *B. divergens*. Most (64%) 18S rRNA gene fragments were 100% identical to sequences derived from cattle and humans, but several isolates from both woodland and farmland ticks differed by between 1 and 3 single nucleotide polymorphisms (SNPs). Genetic heterogeneity was slightly higher in *B. divergens* isolates detected in ticks collected from farmland. Overall our results provide further supporting evidence that red deer may have a role in the transmission cycle of *B. divergens*.

GG10 Differential expression differences between paralysis and non-paralysis inducing strains of *Rhipicephalus evertsi evertsi*

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Ticks, besides being vectors of disease, are also able to induce toxicosis, sweating sickness or tick paralysis. Tick paralysis is generally accepted to be caused by neurotoxins produced in the salivary glands of female or immature ticks that are secreted into the host at a specific time during feeding. Tick paralysis have been neglected in South Africa with no prophylactic treatment for affected animals. No molecular data or the application of more recent technologies, such as next-generation sequencing to investigate tick paralysis for South African species are available. The current study investigated the differences observed between paralysis and non-paralysis inducing strains of *Rhipicephalus evertsi evertsi*, the cause of Spring lamb paralysis in parts of South Africa. The salivary gland transcriptomes of two *R. evertsi evertsi* strains, one causing paralysis, the other not, were sequenced using Illumina® MiSeq and HiSeq2000 next-generation sequencing technology. Salivary glands were dissected from ticks every two days over the feeding period of 6 days. Transcripts were assembled and analysed using CLC genomics, Trinity and Minia software. Assembly and systematic analysis of the transcriptomes confirmed the presence of the predicted major secretory families such as the Kunitz/BPTI, lipocalin, BTSPs, apyrases and metalloproteases with differential expression during the different feeding phases. A description of the transcriptomes of paralysis inducing and a non-paralysis inducing strains gave insight into the general feeding biology of this tick species and allowed comparative analysis to advance our understanding of general tick salivary gland biology. The possibility that some of the differential markers may be paralysis toxins were also considered.

Infection and pathogenesis (IP01-IP06)

IP01 Cholinomimetics-mediated salivary gland secretion in hard ticks - how does it actually work?

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For over four decades, it has been known that injection of the muscarinic acetylcholine receptor (mAChR) agonist, pilocarpine, into partially-fed hard tick females, induces robust long-lasting salivary gland (SG) secretion, whereas it fails to induce saliva from isolated SG. Here, we molecularly characterized two distinct mAChRs (type A and B), both being expressed in *Ixodes* SG and synganglion. Heterologous expression of both receptors, followed by their activation with specific ligands, revealed that only receptor A is sensitive to pilocarpine. Using cholinergic marker, we uncovered a direct cholinergic axonal connection between distinct cells in *Ixodes* synganglion and non-saliva producing type I SG acini and suggested its role in osmoregulation for atmospheric humidity absorption by desiccated ticks. Using epitope-specific antibodies, we localized both mAChR types in *Ixodes* SG and synganglion. Immunostaining confirmed the presence of both receptors in epithelial cells of type I acini, which is not surprising, as these structures are the targets for synaptic cholinergic signals. However, aside from this immunosignal, we spotted strong reactions in two distinct innervations of saliva producing acini type II and III. Specifically, the antibodies recognized mAChR-A in axons innervating exclusively type II acini, while mAChR-B immunoreaction was found in axonal projections innervating both type II and III acini. Furthermore, we revealed that both axonal mAChRs-A or -B are colocalized with different classes of neuropeptides originating from specific neurons in the synganglion. In addition, our results suggest that the *Ixodes* SG is a pool of acetylcholine as both, choline acetyltransferase, the enzyme synthetizing acetylcholine, and vesicular acetylcholine transporter, that loads the acetylcholine to secretory granules, are expressed in this tissue. Based on these findings, we propose that two distinct cholinoceptive neuropeptidergic axonal pathways innervating several hundreds of type II and III acini are sensitive to paracrine acetylcholine, arising from acinar cells to mediate the feedback signal back to peptidergic neurons in the synganglion. Taken together, our results indicate that axonal communication between synganglion and SG is bidirectional, synganglion-to-SG and SG-to-synganglion, regulated by peptidergic and cholinergic signals, respectively.

IP02 Insight into complex serotoninergic system in the ticks *Ixodes ricinus*

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The biogenic amine, serotonin (5-Hydroxytryptamine, 5-HT), and its receptors are critical for regulating physiological and behavioural processes in vertebrates and invertebrates. Herein, we provide a deep insight into the complex serotoninergic system in ticks that can serve as a baseline for developing effective control measures against ticks and tick-borne pathogens. Firstly, we localised the serotonin neurons in the anterior part of the *Ixodes* synganglion protocerebrum. We molecularly identified two serotonin G-protein-coupled receptors (5-HT1A and 5-HT1B) expressed in various *Ixodes ricinus* tissues. To functionally characterise downstream signalling pathways, both receptors were expressed in mammalian heterologous systems. Several agonistic and antagonistic ligands were also used to further characterise receptor affinity. By generating antibodies for immunomapping, we localised 5-HT1A and 5-HT1B in *I. ricinus* synganglion, salivary gland, and midgut. In tick synganglion, the 5-HT1A was localised in small lateral neurons and their projections reaching olfactorial lobes, while 5-HT1B was localised in prominent anterior protocerebral neuronal bodies. In salivary glands, both receptors were localised in distinct granular cells of acini type II and III. Interestingly, the anti-serotonin antibody showed reaction in some of these cells indicating a possible autocrine signalling of this chemical messenger in *Ixodes* salivary glands. For *I. ricinus* midgut, the 5-HT1A receptor was expressed in the digestive cell membranes, with 5-HT1B expressed in outer muscle cells. During a blood-meal, qRT-PCR showed a dramatic increase of both receptor transcripts in *I. ricinus* midgut. Moreover, silencing of 5-HT1A receptor resulted in increased tick nymph weight after the feeding. Based on our results, serotonin may play multiple functions in tick physiology associated with feeding and/or digestion.

IP03 Yezo virus, a novel tick-borne nairovirus associated with acute febrile illness in Japan

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Identification of a causative agent of disease is an initial process to understand the true burden of disease. In the present study, we aim to identify the etiology of an acute febrile illness with thrombocytopenia, leukopenia, and elevated liver enzyme and ferritin levels after tick bite in patients found in 2019 and 2020 in Hokkaido, the northernmost islands of Japan. Patient samples were sequentially passaged through AG129 mice, double knockout mice of interferon α/β and γ receptors, and Vero E6 cells for virus isolation. Illumina sequencing was performed to obtain whole genome sequences of the isolated virus. RT-PCR, RT-qPCR, and enzyme-linked immunosorbent assay were established for the genetic and serological identification of the virus infections in humans, ticks, and wild animals. We discovered and successfully isolated a novel orthonairovirus, designated Yezo virus (YEZV), from the patient samples. YEZV is phylogenetically grouped with an orthonairovirus, Sulina virus, detected in *Ixodes ricinus* ticks in Danube Delta, Romania. YEZV infection was confirmed in seven patients during 2014–2020, and four of these patients were co-infected with *Borrelia* spp. Antibodies to YEZV are found in wild deer and raccoons, and YEZV RNAs are detected in ticks (i.e. *Haemaphysalis megaspinosa*, *Ixodes ovatus*, and *Ixodes persulcatus*) captured in Hokkaido. Our results demonstrate that YEZV is highly likely to be the causative pathogen of febrile illness. This is the first report of an endemic infection associated with an orthonairovirus potentially transmitted by ticks in Japan. Complicated clinical features of co-infections with Yezo virus and *Borrelia* spp. should be carefully examined in the future.

IP04 Analysis of wild rodent gut microbiota as a function of exposure to ticks and tick-borne pathogens

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Host microbiota may impact disease vector behavior and pathogen transmission, but little is known about associations between pathogens, parasites, and microbial communities in wildlife reservoir species. We used Illumina metagenomic sequencing to compare gut microbiome composition by analyzing feces and small intestine samples among three rodent species, *Peromyscus leucopus*, *Sigmodon hispidus*, and *Rattus norvegicus*, that are known reservoirs of zoonotic pathogens. We also explored gut microbial community composition in hosts as a function of parasitism by ticks and infection with tick-borne pathogens. In a second experiment, we compared the fecal microbiota of lab mice (BALB/c) before and after experimental tick infestation. We detected significant variation in gut microbiota among species, but not between sampling sites. Moreover, we found gut microbiome composition, as well as microbial richness, to vary as a function of tick parasitism and infection with the Lyme disease agent, *Borrelia burgdorferi*, although we note that some of these differences were species-specific. We are unable to determine whether variation in host gut microbiome affects propensity of individual rodents to become parasitized by ticks or infected by tick-borne pathogens, or if host microbiome is affected by these factors. However, the apparent link between host gut flora and transmission of vector-borne pathogens adds a new layer of complexity to enzootic transmission dynamics and cycles. Results from experimental infestation experiments are ongoing at this time but up-to-date results will be shared. We expect to use these initial analyses to explore mechanistic links between host microbiota and tick parasitism, as well as with infection with tick-borne agents.

IP05 Pharmacology of two different muscarinic acetylcholine receptors in *Ixodes ricinus* and their localization in the salivary glands

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Previous studies have shown that pilocarpine, a cholinomimetic drug, induces salivation in ticks when injected *in vivo*, but fails to provoke salivation in isolated salivary glands. Therefore, it has been assumed that the activation of muscarinic acetylcholine receptors (mAChR) by this drug mediate saliva secretion. Here, we have molecularly identified two different *Ixodes ricinus* mAChRs (type A and B), and tested their pharmacology in heterologous expression systems. Our reporter systems, monitoring either downstream calcium mobilization in Chinese hamster ovary cells or cAMP elevation in human embryonic kidney cells, revealed that stimulation of mAChR-A triggers intracellular Ca2+ mobilization, whereas that of mAChR-B leads to cAMP elevation. Both mAChRs responded to acetylcholine and muscarine, while only mAChR-A showed sensitivity to pilocarpine. In addition, we screened a high number of putative cholinergic agonists and antagonists to further investigate the pharmacological profiles of these receptors. On the other hand, immunohistochemistry of *I. ricinus* salivary glands uncovered the presence of mAChR-A in axon terminals innervating exclusively type II acini, whereas mAChR-B was detected in axon terminals innervating both type II and III acini. Interestingly, we observed that during different stages of *I. ricinus* female feeding, not all salivary glands were immuno-positive. A likely explanation is that ticks might be capable of regulating the expression of axonal mAChRs depending on their physiological needs. Thus, we suggest that the activities of saliva-producing type II and III acini are under strict cholinergic command mediated by two distinct mAChRs. This study brings a better understanding of neuronal control of salivary glands in hard ticks.

IP06 Comparative mapping of protein-protein interactions between tick-borne flaviviruses and their mammalian hosts reveals virus and mammalian host-specific interactions

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In Europe, two tick-borne flaviviruses, tick-borne encephalitis virus (TBEV) and louping Ill virus (LIV), are responsible for severe neurological disease in humans and sheep, respectively. Like other viruses, TBEV and LIV are obligate intracellular life forms whose survival requires subversion of metabolic circuits and evasion of anti-viral pathways. This feat is achieved in no small part by binary interactions between dedicated viral proteins and host proteins. Such protein-protein interactions (PPI) constitute molecular determinants of critical pathobiologic traits of viruses, including host-range, zoonotic potential and virulence, and represent realistic targets for anti-viral therapies. To shed light on the pathobiology of TBEV and LIV, we have resolved the network of PPI established with human and ruminant hosts by interaction proteomics. High-throughput screens for virus-host PPI were performed involving the complete set of open reading frames of TBEV and LIV and cDNA libraries of Homo sapiens and Bos taurus, by means of yeast two-hybrid methodology. The functional significance of these PPI in viral infection as viral dependency or restriction factors has been characterized *in vitro* by RNA interference, and the interacting mammalian proteins have been annotated by bioinformatic analysis. We have discovered a large set of virus-host PPI, many of which have never been documented in the literature for TBEV and LIV. Some of the PPI appear to be virus- or mammalian-host specific, and may thus underpin the differential host range of the two viruses. Our comparative study should provide molecular explanations for the distinctive pathobiology of TBEV and LIV. More generally, it should illuminate the strategies by which tick-borne flaviviruses control cellular processes and cause disease, and ultimately disclose viral vulnerabilities that can be exploited therapeutically.

Taxonomy and evolution (TE01-TE06)

TE01 Perspectives on Argasid systematics

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Ticks are composed of three extant families: Argasids (soft ticks), Ixodids (hard ticks) and the monotypic Nuttalliellidae. Argasid systematics remain controversial with maintenance of the Hoogstraal (1985) classification scheme, even if this scheme does not reflect evolutionary relationships and results in paraphyly for the major tick genera. The alternative scheme proposed by Klompen and Oliver (1993) has inherent problems of its own, notably the paraphyly of the *Pavlovskyella* and the controversial lumping of the *Alectorobius*, *Antricola*, *Carios*, *Chiropterargas*, *Nothoaspis*, *Parantricola*, and *Subparmatus* subgenera into *Carios*, even though the latter are considered as part of the Argasinae by Hoogstraal (1985). Recent systematic analysis based on 18S/28S rRNA and mitochondrial genomes has broadly confirmed the scheme of Klompen and Oliver (1993), confirming the paraphyly of *Pavlovskyella*, placement of *Alveonasus*, *Proknekalia*, *Ogadenus* and *Secretargas* in the Argasinae and placement of *Carios* and *Chiropterargas* in the Ornithodorinae. The *Carios* clade with its constituent subgenera remain controversial since the systematic position of its type *Carios vespertilionis* has not been determined with confidence. The current study aimed at the resolution of the *Carios* sensu latu Klompen and Oliver, 1993, as well as *Carios* sensu strictu Hoogstraal, 1985 by determining nuclear and mitochondrial markers for *C. vespertilionis*. Both nuclear and mitochondrial markers support placement of *Carios* s.s. within the Ornithodorinae, but outside the clade with the other subgenera that forms part of the *Carios* s.l. Klompen and Oliver (1993). The other subgenera from *Carios* s.l. Klompen and Oliver, 1993, form a monophyletic clade that may either be put in a new taxon within the genus *Alectorobius*, or be kept as subgenera. Given the extensive differences in biology of these sub-genera, it is proposed that these sub-genera should be raised to the genus-level. Given the extensive differences in biology of these sub-genera, it is proposed that these sub-genera should be raised to the genus-level. Solutions to the paraphyletic status of the *Pavlovskyella* will also be presented.

TE02 Tick evolution from the perspective of *Nuttalliella namaqua* phylogenomics

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Ticks are composed of three families: Argasidae (soft ticks), Ixodidae (hard ticks) and the monotypic Nuttalliellidae (*Nuttalliella namaqua*). Systematic relationships among the tick families are important for understanding of tick evolution. Good evidence exist that the tick families are monophyletic to the exclusion of other Parasitiformes, implying monophyly of hematophagy in ticks. However, biological differences related to blood-feeding and modulation of host defense mechanisms in hard and soft ticks suggest independent adaptation. Systematic analysis using 18S/28S rRNA and mitochondrial genomes, suggested that *N. namaqua* group basal to the main families. Alternatively, analysis placed *N. namaqua* as sister group to hard or soft ticks. Sister group status to one of the families may suggest that it is a family outlier and would not resolve the biological differences that exist between the tick families. A basal position suggests that blood-feeding evolved in the ancestral tick lineage and may explain characters unique to each family by modification by descent. The current study considers a total evidence approach. Salivary gland transcriptomes of 1st instar nymphs and of a whole body female indicate that the major secretory protein families are conserved among all tick families, suggesting that these families were present in the salivary glands of the ancestral tick lineage. However, *N. namaqua* do not have many secretory orthologs that correspond with known functions in other tick species. Those with defined orthologs are also found in the major tick families, suggesting that these were present in the ancestral tick lineage. Most other secretory proteins seem to be unique, suggesting that *N. namaqua* evolved these functions independently. The transcriptome data allows phylogenomic analysis based on an extensive number of nuclear genes and may hold the promise of defining the systematic relationship of the three tick families that would allow interpretation of the evolution of blood-feeding characters in this enigmatic group.

TE03 *In vivo* isolation of *Babesia aktas* n. sp. infecting for goats: morphological, molecular characterization and pathogenicity

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Babesia species transmitted by ixodid ticks are common in domestic and wild animals in tropical and subtropical region of the world, including Türkiye, and cause clinical infections with high mortality. In this study, morphological and molecular characterization of a new *Babesia* species infecting goats was carried out. For this purpose, splenectomy was performed to suppress the immune system of the goat, which was determined to be infected with *Babesia* sp. by using molecular tools (PCR-RLB) in the field studies, and the parasite was re-emerged. The two goats selected among the carrier animals that signaled only to *Babesia* sp. specific probe by RLB was immunosuppressed by intramuscular injection of dexamethasone following splenectomy. When the amount of parasite increased in the peripheral blood smear (1.9%), infected blood was taken from the goat and inoculated another 4-month-old goat which immunocompromised by intramuscular injection of dexamethasone, blood stabilate obtained from goat when parasitemia high degree (10%), and cryopreserved for use in experimental infection. The pathogenicity of *Babesia* sp. was performed by experimental studies in 4-8 month old goats with immunosuppressed (n=7) and spleen intact (n=6). Experimental studies showed that severe clinical symptoms such as high fever, anemia, hemoglobinuria, stagnation, anorexia, rapid breathing, lying down and not being able to stand up was observed in immunocompromised individuals. In spleen intact goats, an increase in body temperature and parasitemia up to 0.2% were detected in the days after parasite inoculation. In this group of goats, clinical findings such as loss of appetite, stagnation and lying on the ground for 2-3 days were observed, but typical clinical findings of babesiosis were not encountered, except for increased body temperature. The parasite has defined microscopically in ring, paired pyriform, spectacle frame-like, and line forms. As a result of phylogenetic analysis and sequence comparison based on 18S rRNA and cox1 genes, it was seen that this species is quite different from ovine *Babesia* species (18S rRNA 92-94%, cox1 79-80%) similar and most closely resembles *Babesia* sp. deer which is described in deer. In addition, it was found to be more similar to *B. venatorum*, *B. divergens*, *Babesia* sp. FR1 and *Babesia* sp. MO1 species, which are known to be zoonotic. Further studies are needed on the vector capacity of this parasite and its pathogenicity in different hosts (mountain goat, sheep, calf). This work was supported by founding from the Scientific and Technological Research Council of Türkiye (TUBITAK) (project no. 118O871).

TE04 *Ixodes inopinatus* cannot be distinguished from *I. ricinus* by the sole use of the 16S ribosomal gene

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The most common and studied tick species in Europe is by far *Ixodes ricinus*, an exophilous species with catholic feeding behaviour and a major vector for numerous tick-borne diseases. The development of molecular markers has recently improved our knowledge of the intra- and inter-specific genetic variability for the different *Ixodes* species within the ricinus complex and their systematics. In particular, North African populations have revealed marked genetic divergences with European populations of *I. ricinus* using the sequences from 6 different genes (Noureddine et al. 2011). By using both morphological and molecular data (albeit restricted to partial sequence of the 16S gene only), Estrada-Peña et al. (2014) have described a new species, *I. inopinatus*, mostly occurring in north Africa but also considered to have been found in a few other locations in Europe. Since this description of a new *Ixodes* species in western Palearctic, a growing number of publications have reported *I. inopinatus* in additional locations in Europe (Germany, Romania) identified using 16S sequences. Using the dataset provided in Noureddine et al. 2011, we demonstrate that the 16S gene does not allow recognizing a distinct and robust monophyletic clade gathering all the north African individuals to the exclusion of all European samples. By contrast, the other coding genes (Co1, but even more efficiently Defensin, TROSPA and EF1α) allow clustering all the North African individuals to the exclusion of all European ones. More recently, a set of more than 100 SNPs spread all over the genome (Quillery et al. 2014) has confirmed the marked genetic divergence between *I. inopinatus* and *I. ricinus* and suggests that there is no introgression of the genes of the first species into Europe (Poli et al. 2020). Although additional investigations remain to be conducted to identify morphological differences between the two taxa as well as their potential interfertility, we argue that *I. inopinatus* cannot be distinguished from *I. ricinus* by the use of the sole 16S ribosomal gene. Likewise, the existing 16S sequences assigned to inopinatus available in GenBank should be taken with caution, especially the European ones.

TE05 *Theileria* sensu stricto parasites of cervids: host-related evolution and revised taxonomy

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Recently, it has been shown that piroplasmids represent at least ten different lineages as demonstrated by molecular phylogeny (Clade I to X, Jalovecka et al 2019). Furthermore, most piroplasmid lineages are associated with defined mammalian and/or bird vertebrate host taxa, suggesting that their evolution is driven by cospeciation (Fahrenholz's rule). Correspondingly, piroplasmids pertaining to *Theileria* sensu stricto (Clade IX) infect exclusively bovids and cervids, strongly suggesting that their evolution depends on these vertebrate host taxa. Notorious species of *Theileria* s.s. *T. parva*, *T. annulata*, and *T. lestoquardi* are scrutinized as they cause high economic losses in livestock worldwide. In contrast, *Theileria* species that infect cervids are understudied: phylogenetic placement within their clade is often unclear, and there are incongruences with respect to their specific epithet. To address this, we sequenced and compared nearly complete 18S RNA genes of *Theileria* spp. isolated from red deer, fallow deer, and roe deer and compared them with available relevant *Theileria* s.s. sequences. Only long 18S RNA gene sequences were included, since, due to their relatively short evolutionary history, phylogenetic relations of species of *Theileria* s.s. are characterized by small genetic distances. This approach ensured maximizing the recovered phylogenetic signal and verifying whether 18S RNA genes are principally able to define species within this clade. Based on the inferred tree the following cervid-isolates can be clearly delineated and we suggest describing these taxa as novel species of *Theileria* s.s.: *Theileria cervi* isolated from Wapiti (*Cervus canadensis*), *Theileria* sp. (Nippon) isolated from sika deer, *Cervus nippon*; *Theileria* sp. (Sika) isolated from sika deer (*Cervus nippon*); *Theileria* sp. (Pecora) isolated from sheep, *Ovis aries*, red deer, *Cervus elaphus*, roe deer, *Capreolus capreolus*, and Pyrenaen chamois, *Rupicapra pyrenaica*; *Theileria* sp. (Crispus) isolated from Japanese serow, *Capricornis crispus*; *Theileria* sp. (Elaphus) isolated from red deer, *Cervus elaphus* and fallow deer, *Dama dama*. Although moderately supported, *Theileria* sp. (Qilian) has been identified exclusively in the threatened Tibetan red deer (*Cervus canadensis wallichi*), confined to the Qilian mountains, China, which justifies to describe it as a novel species. In contrast, sequences of *Theileria* spp. of roe deer (*Capreolus capreolus*) do not segregate into a single well-defined clade. Although identity of cervid-infecting species is strongly supported, their placement with regard to each other is not supported by 18S RNA gene comparison. Thus, cospeciation between piroplasmid parasite and corresponding cervid host is suggested, but cannot be conclusively confirmed by this approach.

TE06 The Piroplasmida *Babesia*, *Cytauxzoon*, and *Theileria* in farm and companion animals: species compilation, molecular phylogeny, and evolutionary insights

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The order Piroplasmida, including the genera *Babesia*, *Cytauxzoon*, and *Theileria*, is often referred to as piroplasmids and comprises of dixenous hemoprotozoans transmitted by ticks to a mammalian or avian host. In domestic animals, piroplasmid infections often have serious or life-threatening consequences resulting in fatalities. Piroplasmids are particularly notorious for the enormous economic loss they cause worldwide in livestock production, the restrictions they pose on horse trade, and the negative health impact they have on dogs and cats. Furthermore, an increasing number of reported human babesiosis cases are of growing concern. In this study, we present a compilation of all piroplasmid species, isolates, and species complexes that infect domestic mammals and which have been well defined by molecular phylogenetic markers. Altogether, 57 taxonomic piroplasmid entities were compiled, comprising of 43 piroplasmid species, 12 well-defined isolates awaiting formal species description, and two species complexes that possibly mask additional species. The extrapolation of the finding of at least 57 piroplasmid species in only six domestic mammalian groups (cattle, sheep, goat, horse, dog, and cat) allows us to predict that a substantially higher number of piroplasmid parasites than vertebrate host species exist. Accordingly, the infection of a vertebrate host species by multiple piroplasmid species from the same and/or different phylogenetic lineages is commonly observed. Molecular phylogeny using 18S rRNA genes of piroplasmids infecting domestic mammals results in the formation of six clades, which emerge due to an anthropocentric research scope, but not due to a possibly assumed biological priority position. Scrutinizing the topology of inferred trees reveals stunning insights into evolutionary patterns exhibited by this intriguing parasite group. Contrary to expectations, diversification of parasite species appears to be dominated by host-parasite cospeciation (Fahrenholz's rule), and, except for piroplasmids that segregate into Clade VI, host switching is rarely observed. In contrast, the unique ability of transovarial transmission of *Babesia* s.s. piroplasmids of Clade VI appears to facilitate species diversification by host switching to other host vertebrate species. Thus, when exclusively natural infections of domestic mammalian hosts are considered and accidental infections disregarded, *Babesia* sensu lato (s.l.) parasites of Clades I and II infect only dogs and cats, respectively, *Cytauxzoon* spp. placed into Clade III only infect cats, *Theileria* placed into Clade IV exclusively infect horses, whereas *Theileria* sensu stricto (s.s.) of Clade V infects only cattle and small ruminants. In contrast, *Babesia* s.s. parasites of Clade VI infect all farm and companion animal species.

Immunity and vaccines (IV01-IV09)

IV01 Anti-microbiota vaccines, concepts and applications for Lyme and malaria control

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Ticks harbor microbial communities including pathogenic and non-pathogenic microbes. The relation between pathogens and the microbiome is bidirectional. Empirical evidence shows that pathogen acquisition modulates the tick gut microbiome, while at the same time the tick microbiome is a gatekeeper for pathogen colonization of tick tissues. Increased knowledge of microbial ecology and vector-host interactions is driving the emergence of new concepts and tools for vector and pathogen control. In 2020, the concept of anti-microbiota vaccines was presented to the community. Anti-microbiota vaccines modulate the taxonomic and functional profiles of the tick microbiome, as host antibodies taken in the blood meal target microbiota bacteria within hematophagous arthropods. Our initial studies show that host antibodies against a single bacterial species trigger cascading ecological effects on the whole microbiome with consequences for vector physiology and vector-pathogen interactions. In this keynote, we'll present current knowledge and paradigms in tick microbiota research. We'll also present our results on the use of anti-microbiota vaccines to block *Borrelia* and *Plasmodium* colonization in *Ixodes* ticks and *Culex* mosquitoes, respectively. Vector microbiota manipulation by host antibodies offers an alternative to develop effective transmission-blocking vaccines.

IV02 Anti-tick vaccines as a practicable alternative to control ticks

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There are increasingly frequent reports of multi-resistant tick strains to chemical acaricides. In this context, vaccination becomes in a very attractive alternative as control measure for these ectoparasites. However, the challenge of the research community working on anti - tick vaccines is to get effective antigens, with a broad action spectrum, in spite of the biochemical complexity of these multicellular parasites, and their contact with the host immune system only during feeding. Bm86 vaccination against *Rhipicephalus microplus* has demonstrated the feasibility of the tick immunological control under field conditions, when used as part of an integrated management strategy. The universal character of this program is given by the wide possibilities for full adaptation of autochthonous practices, in different regions, to the main vaccine backbone. The most important consequences of these Programs applied in Cuba and other countries have been: tick infestation reduction after two or three generations feed on vaccinated animals, diminution in the incidence of hemoparasitic diseases and a dramatic reduction in the use of chemicals. However, obtaining new effective antigens against other tick species becomes of great relevance in order to improve the practical application of these vaccines. Although a successful proof of concept in laboratory conditions against different tick species has been obtained by host vaccination with an antigen based on a peptide from the tick P0 ribosomal protein, in order to get an anti-tick vaccine, a development pathway needs to be performed, in which effective formulations for different host species and their scale up production processes will be set up. In this development process, the establishment of a validated and robust analytic system to guarantee the vaccine consistence and quality is also addressed before assuming clinical trials for the sanitary register.

IV03 Comparative study of serological methods for tick-borne encephalitis virus-specific antibody detection in animals: impact of the screening approach on the estimated seroprevalence

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Tick-borne encephalitis (TBE) is an emerging tick-borne disease, caused by a flavivirus. TBE virus (TBEV) is transmitted by tick bites to humans and animals. Humans are dead-end hosts which occasionally can develop serious complications. Animals mostly remain asymptomatic. Several countries monitor the presence and spread of the virus by serological screenings in different animal species. Such screenings often consist of a primary screening by ELISA, followed by confirmation of positive samples by a virus neutralization test (VNT) which is considered to be the golden standard method. In this study, we tested 406 wild boar sera collected in the framework of the Flemish wildlife surveillance in 2020 with two routinely used commercial ELISAs for TBEV screening in animals (Immunozym FSME (TBEV) IgG All Species (Progen) and ID Screen West Nile Competition (IDVet)) and VNTs for TBEV and USUTU virus. The results showed that the Progen and IDVet ELISAs have a relative sensitivity of only 23% and 20%, respectively, and a relative specificity of 88% and 84% compared to the results obtained with the VNT. Most of the false positives in ELISA were due to the detection of USUTU virus antibody positive samples. The minimal TBEV prevalence in our sample set was 8,6% when determined by VNT. When the routinely used screening approach of ELISA testing followed by confirmation in VNT would have been followed, a TBEV seroprevalence of only 2,0% and 1,7% would have been found after a primary screening with the Progen ELISA or the IDVet ELISA, respectively. To verify whether the suboptimal performance of the ELISAs was specific for wild boar sera, we also tested sera from experimentally TBEV infected sheep. Similar results as in the wild boar sera were obtained. While the VNT detected TBEV specific antibodies in 94% of the sheep sera collected between 7 to 18 days post infection (p.i.), the ELISAs detected only a part (50% in the Progen and 31% in the IDVet ELISA) of the sera as positive. This study shows that is important to only compare seroprevalence data obtained with the same methodology and that the same methodology should be used to track changes in seroprevalence over time. Importantly, the routinely used ELISAs to screen animal sera for TBEV antibodies showed to have low sensitivity, making that they can lead to an underestimation of the true prevalence and thus the risk for humans becoming infected with TBEV after a tick bite. Finally, the results also highlight that the ELISAs are not flavivirus specific, making that confirmatory testing of positive sera remains indispensable.

IV04 Biochemical characterization and immunosuppressive activity of Ricistatin, a new salivary cystatin from *Ixodes ricinus*

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Ticks are ranked among the most important pathogen vectors affecting humans, domestic and wild animals. For a successful feeding, ticks inject their saliva in order to subvert the immune response at the host level. Tick saliva represents a cocktail of pharmacologically and immunologically bioactive components such as cysteine protease inhibitors referred as cystatins. In this work, we report the biochemical features and the immunosuppressive activity of Ricistatin, a cystatin from the salivary glands of *Ixodes ricinus*. Purified Ricistatin inhibited a wide range of cathepsins such as Cathepsin B, C, H, L and S with different affinities. It also inhibited Cytokines (IL-1β, IL-6 and TNF-α) secretion in activated macrophages. Furthermore, Ricistatin had a significant effect on nitric oxide production by macrophages and inhibited neutrophil migration *in vitro*. Our findings highlight the numerous anti-tick responses affected by Ricistatin and pave the way for its exploitation as potential immunotherapeutic.

IV05 The Tick Cell Biobank – new resources for tick and tick-borne microorganism research

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Many aspects of research into tick biology and physiology, endosymbionts and endogenous viruses, tick-pathogen interactions and control of ticks and tick-borne diseases are greatly facilitated through use of tick cell lines. The Tick Cell Biobank, founded thirteen years ago, has distributed tick cell lines to nearly 100 institutes in Europe, Africa, Asia and North and South America. During this time, we have trained more than 130 scientists from 37 countries in generation and/or maintenance of tick and other arthropod cell lines. The Tick Cell Biobank houses around 70 cell lines derived from 19 ixodid and three argasid tick species, as well as a smaller number of cell lines derived from sand flies, biting midges, mosquitoes, triatomine bugs, tsetse flies and honey bees. New tick species represented in the cell line collection include *Amblyomma sculptum*, *Argas reflexus*, *Dermacentor reticulatus*, *Hyalomma lusitanicum*, *Hyalomma scupense* and *Rhipicephalus bursa*. The Tick Cell Biobank also houses a small collection of intracellular tick-borne bacterial pathogens and endosymbionts; these include newly-isolated strains of *Rickettsia raoultii*, '*Candidatus*' Rickettsia vini and *Spiroplasma* spp. from *Ixodes* and *Dermacentor* spp. ticks. To enhance the applicability of the cell lines in a broad range of research areas, we are currently sequencing the genomes of selected cell lines derived from *Amblyomma*, *Hyalomma*, *Ixodes* and *Rhipicephalus* spp. ticks. The Tick Cell Biobank Outposts in Asia (Malaysia), Africa (Kenya) and South America (Brazil) are already contributing to capacity-building in these regions through local training and distribution of tick cell lines. We anticipate that the Tick Cell Biobank will continue to underpin many areas of global research into biology and control of ticks, other arthropods and vector-borne viral, bacterial and protozoan pathogens for many years to come.

IV06 Trials and tribulations of discovering tick vaccine antigens - the Australian story

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The *Rhipicephalus microplu*s species complex transmits babesiosis and anaplasmosis to cattle in tropical and sub-tropical regions of the world costing ~$US22-30b losses per annum. A cattle tick vaccine program commencing in 2005 used a reverse vaccinology approach to identify antigens from a *R. microplus* EST library and candidate discovery using subtractive hybridization and microarray analysis comparing tick expression when feeding/sensing resistant vs susceptible breeds of cattle. Candidates were pre-screened *in vitro* using antibody feeding of semi-engorged females. Several *in vivo* trials were undertaken using mixtures of peptides, polypeptide recombinant proteins and single antigens. Antigens associated with large protein families such as metalloproteases, cystatins and lipocalins where not found to be successful vaccine candidates. The final vaccine candidates are single copy genes showing good conservation across tick species. A dual vaccination consisting of two whole recombinant proteins administered twice prior to tick challenge were subsequently challenged a second time six months later. After the first challenge the efficacy was 83% and following second challenge 90% (overall 87%) while Bm86 efficacies were 74% and 64%, overall, 70%. The adult female ticks from the dual challenge presented as a black phenotype which did not lay eggs. Transcriptome analysis revealed the up regulation of mitochondrial genes and the down regulation of cytochrome 450 genes correlating with studies associated with chemical knockdowns. Although the results are excellent, pharma are looking for larval knockdown and/or similar knockdown of adult ticks as observed when using acaricides.

IV07 Bovine host biomarkers for tick resistance – the discovery phase

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Rhipicephalus microplus species complex impacts in cattle in the world's tropical and sub-tropical regions cost ~$US22-30b per annum. Although hybrids of *Bos taurus* and *Bos indicus* have improved tick resistance over *Bos taurus* breeds, they also vary in tick susceptibility. Selective breeding requires phenotyping for tick susceptibility/resistance, which can be subjective between herds and is difficult with extensively grazed cattle. We investigated the abundance and expression of serum proteins and microRNAs before, during and after 12 weekly larval tick challenges using extreme phenotypes within Brangus cattle (Angus x Brahman, 25-75% Brahman content) to identify potential biomarkers for tick resistance. Peripheral blood leukocyte and skin miRNAs were analyzed using a gene expression linear model incorporating the phenotype (high resistance/HR, low resistance/LR), RNA Integrity Number and genomic *Bos indicus* content. TargetScan database was used to determine the biological targets of 10 miRNAs with differential expression (DE) of one skin miRNA (bta-mir-210) correlating with mRNA DE analyses. Five miRNAs were upregulated in HR steers prior to tick challenge, a further four downregulated post tick challenge. Following LC-MS/MS measurement of serum and skin peptides and ProteinPilot analysis, SWATH-MS was used to measure the relative abundances in HR and LR steers. HR naïve cattle carried significantly highly abundant proteins associated with immune response, blood coagulation and wound healing compared with LR cattle. Additionally, the proteins showing a significantly different abundance in HR cattle following tick exposure compared to naïve were associated with immune response, coagulation, homeostasis, and wound healing, whereas LR cattle developed only some of these responses. Further evaluation of putative biomarkers is underway.

IV08 A strain of *Borrelia burgdorferi* sensu stricto may be resistant to anti-OspA vaccines

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Lyme borreliosis is the most prevalent tick borne disease of the Northern hemisphere. It is caused by the transmission of *Borrelia burgdorferi* s.l. a complex of pathogen bacteria transmitted to the host by *Ixodes* ticks. In Europe, nearly 85,000 cases are reported each year, an underestimated figure due to the many undeclared or underdiagnosed cases. European *Ixodes ricinus* ticks are able to transmit various *B. burgdorferi* species (*B. burgdorferi* s.s., *B. afzelii*, *B. garinii*), pathogenic to humans contrary to United states situation where the most prevalent species is *B. burgdorferi* s.s. Bacteria are principally present in unfed tick midgut, then migrate to salivary glands during blood meal and infect a new host via saliva. In this study, efficiency of transmission in a mouse model of three strains of *Borrelia burgdorferi* s.s. (B31, N40 and BRE-13) was examined in order to evaluate infection risk after tick bite and needle injection. These three strains are invasive and belong to different OspC groups of invasivity. B31 was isolated from *Ixodes scapularis*, N40 from *Ixodes damminii* and BRE-13 from the CSF of a patient in France. Location in the ticks and transmission to mice were also determined for the three strains by following infection kinetics. After inoculation, we found a significant prevalence in the brain for BRE-13 compared to the other strains. N40 was never found in the heart. After tick bite, N40 was found only in the skin and joint whereas the other strains were identified in all organs tested. We then tested the presence of *Borrelia burgdorferi* s.s. in the tick after several times of blood feeding. In females, BRE-13 was found in salivary glands of *Ixodes ricinus* before blood feeding. N40 was found after 24H of blood feeding and B31 was identified after 72H of blood feeding. In good agreement, mice were infected as early as 24H post tick bite with BRE-13 and N40. We determined the sequence of OspA of BRE-13 and found several amino acids mutations compared to N40 and B31. Interestingly, some mutations were present in the LA-2 epitope, which is the target of neutralizing antibodies on OspA. These data may call into question the efficacy of a vaccine directed against OspA during transmission of a *Borrelia burgdorferi* s.s. strain such as BRE-13.

IV09 Anti-tick microbiota vaccine reduces *Borrelia afzelii* infection in the tick vector *Ixodes ricinus*

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The tick microbiota consists of an ensemble of commensals and symbionts that cohabit in different tissues and interact with each other forming a complex microbial network. Studies on tick microbiota increased in the last decade due to the pivotal role of microbes in tick physiology and vector competence. Modulation of the tick microbiota can impact tick fitness, molting and egg hatching rates. Shifts in the community composition or the abundance of specific microbial members of the tick microbiota can also affect the colonization of pathogens within the tick. This suggests that tick microbiota modulation can be used as a strategy to impair tick physiology and more importantly, vector competence. Currently, most studies use broad-spectrum antibiotics, that target several bacterial taxa, for the modulation of tick microbiota rendering it difficult to establish causal links between specific bacteria and its impact on tick physiology or the vector competence. Recently, our lab introduced anti-microbiota vaccines as a tool for the precise manipulation of tick microbiota. We demonstrated that targeting keystone taxa in the tick microbiota through host antibodies can impact tick performance and modulate the tick microbiome in a taxon-specific manner. Nevertheless, the impact of anti-tick microbiota vaccines on tick-borne pathogens development within the vector has not been tested. In this study, we immunized C3H/HeN mice with a live *Escherichia coli* vaccine to target bacteria of the keystone taxon *Escherichia*, a common resident of the tick microbiota. The mice were also experimentally infected with *Borrelia afzelii*, the causative agent of Lyme disease. *Ixodes ricinus* larvae were placed on *E. coli*-immunized/*B. afzelii*-infected mice and on mock-immunized/*B. afzelii*-infected mice and allowed to feed until repletion. Levels of anti-*E. coli* antibodies were measured in mice sera and the pathogen was quantified in *Ixodes* larvae. Immunization with a live vaccine elicited antibody response specific to *E. coli*. Notably, a significant decrease of *B. afzelii* infection was found in fed larvae. These findings suggest that antibodies targeting keystone taxa in the tick microbiota can disrupt pathogen development within the vector.

Diagnosis and treatment (DT01-DT06)

DT01 Essential oils can prevent tick attachment to humans and companion animals

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Plant essential oils show promise as natural alternatives to synthetic tick repellents and could make a valuable contribution to integrated tick management programmes for both humans and their companion animals. However, while many studies report their efficacy in the laboratory, few extend these investigations to *in vivo* or field trials. Here we present the results of studies which examined the use of essential oils to prevent tick bites on both humans and dogs. First, for humans, simultaneous blanket-drags and standardised walks were employed to evaluate the acquisition of Ixodes ricinus by 1 m^2 cotton blankets or cotton trousers, in woodland edge habitats of known high tick abundance. Blankets and trousers had been treated with one of 5% oregano, rosemary, spearmint or thyme oils, 20% DEET (N,N-Diethyl-3-methylbenzamide) (positive control) or ethanol excipient-only (negative control). The number of ticks present on the blankets or trousers differed significantly between treatments: spearmint oil treatments resulted in significantly fewer ticks than the negative controls for both blankets and trousers and significantly fewer ticks were present on the oregano oil treated blankets. No reduction in repellence was detected over a 24 h period between treatment and testing. For dogs, spearmint, turmeric, thyme, ginger and geranium were able to abolish the orientation and taxis of *Ixodes ricinus* towards sebum extracted from dog hair in initial laboratory assays. Subsequently blanket-drag field assays were used to show that tick acquisition rates were as low on blankets impregnated with turmeric oil as with 20% DEET. Finally, in a participatory *in vivo* trial, tick acquisition by untreated control dogs was compared with dogs sprayed with turmeric oil and 16 dogs sprayed with orange oil (both 2.5 % v/v diluted in water with a 1% coco glucoside excipient) before each walk, in known tick infested areas. The percentage of dogs with ticks attached to the legs or belly when sprayed with turmeric oil suspension was significantly lower than that of ticks attached to the same areas of dogs sprayed with a positive control or untreated dogs. The results suggest that some essential oils, particularly thyme and spearmint, exhibit considerable potential as effective natural tick repellents for application to clothing or animals, with effective equivalence to 20% DEET.

DT02 First detection of Jingmen tick virus in Corsica: Development and validation of a real time assay to face a potential emergence

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The last ten years have witnessed a number of public health burden of emerging infectious diseases. Therefore, investigate the sources of infections to develop surveillance systems and create preparedness and response strategies is crucial for future epidemics. Corsica is a sentinel area for the study of tick-borne diseases. Its geolocation, the presence of avian migration corridors and a warm climate facilitate the emergence of new vector species and associated vector-borne pathogens. Through monitoring vector-borne viral agents on the island with developing rapid diagnostic systems, we can implement preparedness and response of potential epidemics in Europe. A diagnostic system is therefore important to understand the dynamics of an epidemic as a whole through a One Heath approach. Jingmen Tick virus group, new segmented viruses discovered in China, is showing worrying epidemiological results. There are a number of clues to its potential as an emerging arbovirus, including experimental replication and trans-ovarial and trans-stadial passage of the virus in ticks and viremia and virus isolation in humans associated with symptoms. The aim of this study was to develop a rapid diagnostic system for the JMTV genome detection and to investigate the circulation of this virus in ticks collected from animals in Corsica. A new Real-time PCR system was designed and evaluated for the detection of JMTV group. Ticks collected from domestic and wild animals have been tested with this new diagnostic assay. Complete genome of the virus was sequenced by NGS method. Infection rates were calculated as the maximum-likelihood estimation (MLE) with 95% confidence intervals (CI). A total of 6,269 ticks collected during 2018-2020 from cattle, horses, wild boars and sheep were grouped into 1,715 pools of 1-6 ticks. Jingmen tick virus DNA was detected in 21 tick pools collected from three cattle and in one tick pool collected from a sheep. The highest JMTV DNA prevalence (MLE=0.58%, 95% CI: 0.35%-0.6%) was observed for *Rhipicephalus* genus. *Hyalomma marginatum* and *Rhipicephalus bursa* collected from the same host were detected positive to the JMTV DNA. Sequencing showed that Corsican JMTV strains were closed to Kosovo JMTV strains. This study describes the first detection of JMTV in Corsica and more widely in the south-western Mediterranean and allowed to the development of a diagnosis system assay. Future research aimed at defining the origin, the ecology and the spillover potential of JMTV will be critical to understand its relevance to public health.

DT03 Comparison of digital and nested PCR assays for rapid and accurate detection of *Borrelia burgdorferi* in tissues from experimentally infected mice

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A nested polymerase chain reaction (n-PCR) is routinely used in our laboratory for detection of *Borrelia burgdorferi* in biological specimens. Here, we evaluated the QIAcuity Digital PCR (d-PCR, Qiagen) system for the detection *B. burgdorferi* DNA in multiple tissue samples collected from 96 experimentally inoculated mice which were included in another study. Ear biopsies were collected from each mouse on Day 21, 28, and 35 post-inoculation (PI) and screened against *B. burgdorferi* using n-PCR and d-PCR. Furthermore, tissue samples including hearts and meninges were collected and tested for the presence of *B. burgdorferi*. The diagnostic efficiency was compared with that of the "gold standard" *in vitro* culture. *B. burgdorferi* DNA was detected using n-PCR and d-PCR in 0 (0%), 2 (2.1%) and 16 (16.7%) and 11 (11.5%), 25 (83.3%; tested n= 30) and 55 (57.3%) on Day 21, 28 and 35 PI, respectively. Out of 96 tested heart tissues, 10 (10.4%) and 25 (26%) were n-PCR and d-PCR positive, respectively. Out of 48 tested meninges, only one was n-PCR positive, while 15 (31%) were d-PCR positive. All culture-positive specimens were d-PCR positive. In conclusion, compared with spirochete cultivation, d-PCR assays had high sensitivity in the detection of *B. burgdorferi* DNA from biological samples.

DT04 A One Health approach to tackle tick-borne diseases - analysis of surveillance initiatives in selected EU countries: The Netherlands, Spain and Italy

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Ixodid ticks and tick-borne diseases (TBD) are expanding their geographical range. At European level, EFSA and ECDC are involved in TBD surveillance and reporting, but surveillance activities vary among countries. To identify ideal elements for TBD monitoring and prevention, considering a One Health (OH) approach, we analysed the surveillance systems in place in some European countries. We applied the semi-quantitative evaluation protocol developed by the NEOH COST Action, to identify outcomes and assess the degree of OH implementation within the initiatives. At first, we analysed the surveillance system in The Netherlands, a country that has implemented a consultative structure to monitor and report zoonoses; the National Institute for Public Health & Environment coordinates the different project-based monitoring, research and educational activities on TBD. The level of transdisciplinary and trans-sectoral collaboration is high, regular meetings and on-line platforms enable communication and data sharing among actors; moreover, the non-scientific community is actively involved. The surveillance plan has yielded measurable outcomes (e.g., reduction in tick bites) and early detection of unexpected events (e.g., discovery of new TBD and vectors). In other European countries, such as Italy and Spain, TBD surveillance and reporting systems are based on compulsory notification. Although legislation seems quite relevant within these initiatives, law enforcement, alongside dedicated time and availability of economic resources, is rather fragmented and limited to the most severe health issues (e.g., TBE in Italy and CCHF in Spain). Veterinary and human medicine are the most involved disciplines, with the first prevailing in some local/regional contexts. Stakeholders are marginally considered and collaborations are mostly limited to local initiatives. Despite the existence of good communication channels, data sharing is somehow compartmentalized and mainly restricted to specific actors. Even so, the efficiency and preparedness of the health system from Spain was proven with the early detection of new emerging pathogens in ticks (e.g., CCHFv) and the subsequent detection of human cases. Research activities in Italy and Spain have mostly contributed to gain knowledge on the distribution of tick vectors at national level (e.g., ticks expanding their geographic range) and the discovery of new pathogens (e.g., *Borrelia miyamotoi*, *Neoehrlichia mikurensis*, '*Candidatus*' Rickettsia rioja, etc.). Differences emerge in the TBD surveillance plans of the 3 countries, as well as the OH-scores. Although all TBD surveillance plans comply with the EU regulations, the initiatives characterized by trans-disciplinary collaboration may be more effective for the surveillance and prevention of TBD.

DT05 Humans infested with *Ixodes ricinus* are exposed to multiple tick-borne pathogens in Romania

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Tick-borne pathogens have a major threat to human health in some temperate regions of the Northern Hemisphere. *Ixodes ricinus* tick species is the primary vector for several pathogens for humans in Europe. The present study was conducted between 2014-2015 on patients who presented themselves to the Infectious Diseases Clinic and Emergency Hospital from Cluj-Napoca. Ticks (n=116) feeding on humans (n=115) were collected and high-throughput microfluidic real-time PCR system was performed to screen 36 species of tick-borne pathogens in Romania. Blood samples (n=54, 47%) from the same patients were serologically screened to evaluate the seroprevalence for *Borrelia burgdorferi* s.l. by wo-tiered testing strategy: positive and equivocal immunoenzymatic test results for IgG and IgM antibodies were confirmed by Western-Blot. Ticks were molecularly identified and belonged to two species of the genera *Ixodes* (90%) and *Dermacentor* (4%), whereas 6% of the ticks were unidentifiable. 68.7% of the ticks were infected with at least one pathogen, being *Rickettsia helvetica* (47.4%) the most common pathogen, followed by *B. burgdorferi* s.l. (22.4%), *Anaplasma phagocytophilum* (9.5%), *R. felis* (4.3%), respectively *Bartonella* spp., *B. miyamotoi*, *N. mikurensis*, and *R. slovaca* with the same prevalence (2.6%), and *R. conorii*, *R. massiliae*, *Babesia microti*, and *B. vogeli* (0.9%). Despite to the high proportion of infected ticks, only 7 (13%) blood samples have positive and 2 (3.7%) have equivocal IgM antibody index, whereas 16 (29.6%) have positive IgG antibody index by Western-Blot. Our study highlights the importance of tick-borne pathogens that have a clinical impact in the investigated cohort from Romania.

DT06 Selective targeting of proteasome(s) - an innovative strategy to combat ticks and tick-borne diseases

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Proteasome is a multi-component protein complex serving as the degrading arm of the proteasome-ubiquitin system in eukaryotic cells. Inhibitors of the 20S proteasome catalytic core represent approved therapeutics for human oncological disorders and selective inhibition of parasite over host proteasomes has recently been validated as one of the most relevant novel therapeutic strategies for infectious diseases including malaria, leishmaniasis, Chagas diseases and schistosomiasis. In this work we adopt this strategy for ticks and tick-borne diseases. We have recently validated the proteasome of two zoonotic *Babesia* species as great targets for inhibitor-based drug development. This was done ex vivo in *Babesia divergens* infected bovine erythrocyte cultures and *in vivo* in *Babesia microti* infected mice using approved and commercially available proteasome inhibitors carfilzomib, ONX-0914, epoxomicin, bortezomib and ixazomib. We are aiming our current efforts to increase the selectivity index of *Babesia* targeting proteasome inhibitors by screening compounds primarily developed to target the proteasome of the related malaria parasite *Plasmodium falciparum*. Our current approach uses derivates of a naturally derived carmaphycin B - a potent inhibitor against both asexual and sexual blood stages of malaria - for their effect against *B. divergens*. These compounds effectively target the β5 subunit of *Babesia* proteasome at low nanomolar concentrations and, interestingly, display differential efficacy against *B. divergens* and *P. falciparum*. They also have reduced toxicity to the host (human cell lines) which provides new insights into the development of small proteasome inhibitors as selective drugs for babesiosis. Additionally, some proteasome inhibitors have been also found effective against the Lyme spirochetes *Borrelia afzelii* and surprisingly, even against the tick vectors themselves. This is demonstrated via membrane feeding of tick *Ixodes ricinus* on proteasome inhibitor containing blood. Out of the three tested compounds carfilzomib (epoxyketone based), bortezomib and MLN9708 (both boronic acid based), the last two listed lead to 100% mortality of adults occurring within days post their detachment from the host in adults, while tick nymphs were blocked in feeding (kept comparable weight with unfed nymphs) but remained alive. Overall, our results allow for an interesting comparison of proteasome inhibitors individually developed for related apicomplexan parasites and clearly demonstrate the multiple potential of selective proteasome inhibition for the development of novel interventions against ticks and tick-borne pathogens. Acknowledgements: The Czech Science Foundation (GACR) projects No. 21-11299, No.17-14631 and the ERDF/ESF Centre for research of pathogenicity, and virulence of parasites (No. CZ.02.1.01/0.0/0.0/16_019/0000759).

Miscellaneous (MI01-MI04)

MI01 Scenes from tick physiology: proteins of sialome talk about their biological processes

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Ticks are blood-sucking parasites with different strategies of feeding depending on the tick family. The major families are Ixodidae or Argasidae, being slow or fast feeders, respectively. In the recent years, the advances in molecular sequencing techniques have enabled to gain knowledge about the proteome of the tick's salivary glands. But a holistic view of the biological processes underlying the expression of the sialome has been neglected. In this study we propose the use of standard biological processes as a tool to draw the physiology of the tick's salivary glands. We used published data on the sialome of *Rhipicephalus sanguineus* s.l. (Ixodidae) and *Ornithodoros rostratus* (Argasidae). A partial set of proteins obtained by these studies were used to define the biological process(es) in which proteins are involved. We used a directed network construction in which the nodes are proteins (source) and biological processes (target), separately for the low-level processes ("children") and the top-level ones ("parents"). We applied the method to feeding *R. sanguineus* at different time slices, and to different organs of *O. rostratus*. The network connects the proteins and the processes with a strength directly proportional to the transcript per millions of each protein. We used PageRank as a measure of the importance of each biological process. As suggested in previous studies, the sialome of unfed *R. sanguineus* express about 30% less biological processes than feeding ticks. Another decrease (25%) is noticed at the middle of the feeding and before detachment. However, top-level processes are deeply affected only at the onset of feeding, demonstrating a redundancy in the feeding. When ixodid-argasid are compared, large differences were observed: they do not share 91% of proteins, but share 90% of the biological processes. However, caution must be observed when examining these results. The hypothesis of different proteins linked to similar biological process(es) in both ticks is an extreme not confirmed in this study. Considering the limitations of this study, carried out with a selected set of proteins, we propose the networks of proteins of sialome linked to their biological processes as a tool aimed to explain the biological processes behind families of proteins.

MI02 Long-term *in vitro* propagation of *Babesia microti*

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Babesia microti and *microti*-like species are tick-borne Apicomplexan hemoparasites, which have been associated with human disease since at least 1970. Propagation of this organism *in vitro* has been limited to short-term cultures of infected hamster ex vivo blood lasting commonly 3 days in growth phase, followed closely by a rapid crash due to the inability of trophozoites to further penetrate uninfected erythrocytes. We noted that the most obvious change during this rapid growth cycle was the non-renewal of both IgM antibody and C3 complement coating the merozoites as they divide intracellularly. Initially all ex vivo merozoites were fully IgM-coated while a majority were also C3 complement-coated, depending upon the time factor. Since the trophozoite search for an uninfected cell is extremely brief, the *in vitro* system must minimally approximate ongoing host production of specific IgM and the alternative complement pathway. Seeking to maintain the propagation we supplemented cultures with both components on a very regular basis, at least once or twice per day. The IgM supplement was purified from an acute phase babesiosis human donor, while both guinea pig and snap-frozen human serum successfully maintained the supply of C3 complement. As both supplements are rather heat-labile, the daily routine demanded in-culture synthesis of these short-lived factors. Mouse IgM hybridomas were developed using infected mouse spleen cells, screened for specific reactivity by IFA and for the ability to promote growth in co-culture. Eight hybridomas were selected and tested in various combinations with infected human erythrocytes. Although all hybridomas are specifically reactive with the p39 protein (BmSA1), mixtures of hybridomas produced a useful oligoclonal effect. A complement source (THP-1) was chosen from available cell types as both hardy and reproducible. The model supports long-term *in vitro* propagation, in sequence producing the stable attachment complex of IgM anti-BmSA1 to "fix" multiple molecules of C3. C3 is then rapidly cleaved to C3b and this complex can attach tightly to erythrocyte CR1 membrane receptors to trigger entry of the trophozoite. This work is ongoing.

MI03 A preliminary account on the history of the Italian acarology-ixodology

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During ancient Rome, Cato the Elder in "*De Rustica*" provided the first account on methods for the prevention and treatment of mange/scabies, which were deemed useful also to prevent the attachment of ticks. During Middle Ages, various authors referred on ectoparasites, although without quoting explicitly ticks. During the Renaissance, many "hippiatrics" and physicians were committed to fight against ectoparasites in humans and animals. In the 16th century, Ulisse Aldrovandi (1522–1605) established entomology as a science, giving the first systematic account on ectoparasites: the chapter five of Treatise VII, "*De animalibus insectis*", named "*De Ricino*" is dedicated to ticks or other ectoparasites supposed to be –or accounted for being- ticks. Aldrovandi narrates on ticks –similar to ricinus seeds- attached to skin, sucking blood "sanguine satur est" and born in the grass "in herbis nascuntur", referring also that ticks are called "garapatas" by Spaniards. With the introduction of the microscope and experimental medicine, Francesco Redi (1626– 1697), in his book "*Esperienze intorno alla generazione degli insetti*" (1668) described ectoparasites as reproducing by eggs fertilized by "coitus"; he provided 29 engraved plates of insects; outstanding are those relevant to the first rendition of ticks with eight legs. Successively, great scientists made contributions in parasitology, but not specifically in acarology/ixodology. During late '800s and early '900s, acarology became a well-established branch of entomology. Even if ixodological studies remained quite limited in Italy, we must remember Giovanni Canestrini who, in 1887, first described *Boophilus microplus*. During and between the two world wars, the interest in veterinary parasitology –and ixodology- diminished. Oleg Starkoff, a Russian émigré, professor of medical parasitology at the Institute of Parasitology in Rome, published in 1958 a comprehensive monograph on ticks of Italy. Between the '60s and '80s, Lorenzo Sobrero, at Zooprophylactic Institute of Apulia, conducted pioneering research on Ixodidae ticks; he published with Giulio Manilla, a comprehensive monograph on the occurrence and geographical distribution of ticks in Italy. From late '80s to-date, a growing number of scientists –from different research groups and disciplines, throughout Italy and from abroad- became actively involved in transdisciplinary researches on ticks and tick-borne diseases, adopting an integrated One Health approach. The Authors apologize for having probably overlooked some "historical" and "modern" Italian ixodologists. This work is dedicated to Raffaele Roncalli-Amici, an Italian scientist naturalized U.S. citizen, veterinarian, and passionate historian.

MI04 The Peer Community In (PCI) project, PCI Infections, and the Peer Community Journal

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The Peer Community In (PCI,) project (https://peercommunityin.org) offers an alternative to the current system of publication - which is expensive and not transparent. PCI is a non-profit scientific organization consisting of communities of researchers who handle the evaluation (through peer-review) and recommendation of preprints in their scientific field. Each PCI is a group of recommenders (60-400) playing the role of editors who recommend preprints based on rigorous peer-reviews to make them complete, reliable and citable articles, without the need for publication in 'traditional' journals (although the authors can submit their recommended preprints to a journal if they choose to). The recommendation process by PCIs is completely free of charge. When a recommender decides to recommend a preprint, she or he writes a recommendation text that is published along with all the editorial correspondence (reviews, recommender's decisions, and authors' replies) on the PCI website. The preprint itself is not published by PCI: it remains on the preprint server where it has been posted by the authors. It can be submitted to a traditional journal or published in the all-new "Peer Community Journal" (PCJ) (https://peercommunityjournal.org), launched in Fall 2021. PCJ directly accepts all articles recommended by any of the existing PCIs. It thus represents the first generalist diamond open access journal to date. The first Peer Community In was started in 2017: Peer Community in Evolutionary Biology (PCI Evol Biol), and now there are 15 functioning PCIs, with more than 1600 scientists from around the world as PCI recommenders. The PCI initiative won the 2020 LIBER award for library innovation of the European League of Research Libraries. PCI Infections (https://infections.peercommunityin.org/) was launched in August 2021. It welcomes all manuscripts dealing with host-pathogen-vector systems as well as with symbiotic organisms in the widest sense and for all the tree of life. Given the width of the topics covered in PCJ and the quality of the editorial process leading to a recommendation, we are expecting a very high impact of this journal. PCIJ is indexed by DOAJ and Google Scholar. To date, the PCJ has published 109 papers in 8 months, and already exhibits very good citation statistics (340 citations, i.e. 4.7 citations/year/paper). Here we would like to make a call. PCI Infections needs more recommenders from around the world and as many preprint submissions as possible. We hope the Tick and Tick-Borne Pathogen community will massively join the initiative.

Symposium “Diversity of symbiotic interactions in ticks” (SY1.1-SY1.6)

SY1.1 Nutritional endosymbiosis in ticks

Duron O

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Ticks rely exclusively on vertebrate blood at all stages in their development. They are consistently exposed to nutritional deficiencies: Blood is rich in some nutrients but relatively poor in others, like B vitamins. Almost all tick species examined harbour a nutritive obligate endosymbiont, *Coxiella*-like endosymbionts (CLE) or *Francisella*-LE (FLE) in most cases, or Rickettsiales endosymbionts in few cases like in the *Ixodes* genus. No other bacterium is so uniformly present in ticks. Each of these bacterial endosymbionts is able to synthesize at least two (for Rickettsiales) or three (for CLE and FLE) B vitamin types and to further provision their tick hosts. Elimination of these nutritive endosymbionts negatively impacted tick life history traits and prevented the development of viable adult females. An oral supplement of B vitamins restored these deficiencies showing the central role of these vitamins for the tick life cycle. However, despite the co-evolved and obligate nature of these mutualistic interactions, the structure of tick's microbiomes does not mirror the tick phylogeny with a clear exclusion pattern between CLE and FLE across tick species. CLE, but not FLE, commonly form evolutionarily stable associations with ticks commonly leading to co-cladogenesis as observed in the *Rhipicephalus* and *Amblyomma* genera. Symbiont replacements is yet obvious during radiation of Amblyomma, with recent, and likely ongoing, invasions by FLE and subsequent replacements of ancestral CLE through transient co-infections. Nutritional endosymbiosis in ticks is thus not a stable evolutionary state, but instead arises from conflicting origins between unrelated but competing bacterial endosymbionts with similar metabolic capabilities. This conflicting processes underscores the important contribution of nutritional endosymbiosis to the first appearance of ticks and their later diversification to current species.

SY1.2 *Rickettsia lusitaniae*, a widespread maternally inherited symbiont of *Ornithodoros* soft ticks

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Rickettsia are obligate intracellular bacteria best known as pathogens of vertebrates and transmitted by blood-feeding arthropods, mainly ticks. However, new *Rickettsia* species and strains with no apparent pathogenicity are continually uncovering from a variety of arthropod groups as ladybirds, spiders, wasps, book lice and even ticks. These *Rickettsia* are endosymbionts which live exclusively within arthropod cells and typically undergo high efficiency maternal (transovarial) transmission to offspring. In this context, the recent discovery of *R. lusitaniae* in several soft tick species belonging to the genus *Ornithodoros* is worthy of interest. *R. lusitaniae* is closely related to the causative agent of flea-borne spotted fever, *R. felis*. It can successfully infect cell lines in lab conditions but was only reported once in organs of a bat in China. In our study, we have investigated on the mechanisms used by *R. lusitaniae* to spread and persist in *Ornithodoros* species. We have first characterized important variations of prevalence (from 0 to 100%) between natural populations and laboratory colonies for six *Ornithodoros* species: *O. maritimus*, *O. sonrai*, *O. capensis*, *O. costalis*, *O. moubata* and *O. erraticus*. Multilocus genetic typing and complete genome sequencing further revealed a low level of genetic differentiation between multiple strains of *R. lusitaniae* across the Old and New Worlds: all *R. lusitaniae* strains cluster in a robust phylogenetic clade well distinct to other species (including *R. felis*, *R. asembonensis* and *R. hoogstraalii*) of the *Rickettsia* transitional phylogenetic group. We further examined the tissue tropism of *R. lusitaniae* using real-time quantitative PCR and Fluorescence in situ Hybridization (FISH): we found this bacterium highly abundant in Malpighian tubules and ovaries, and less abundant in salivary glands and gut. Subsequent surveys of tick progeny revealed a high level of maternal transmission in several *Ornithodoros* species: from 83 to 100% of larvae from an infected mother are themselves infected. Experimental investigations lastly showed that *O. erraticus* does not transmit *R. lusitaniae* to mice on which they have feed. Examination of several mices' organs (skin, heart, spleen and blood) through real-time quantitative PCR assays revealed absence infection. While current view in rickettsiology has a strong anthropocentric bias and tends to describe novel *Rickettsia* species as pathogenic forms, our observations rather establish that *R. lusitaniae* is a common endosymbiont of *Ornithodoros* ticks that largely relies on maternal inheritance to spread and persist in tick populations.

SY1.3 Exploring the physiological role of maternally-inherited endosymbionts in *Rhipicephalus microplus*, *Ixodes ricinus* and *Ixodes scapularis* ticks

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Exclusively blood-feeding arthropods are assumed to rely on obligate mutualist symbionts to provide a supplement of B vitamins and cofactors deficient in their unbalanced diet. However, while the genomes of obligate blood feeders' symbionts converge to be able to produce biotin, the capacity of synthesizing other B vitamins is variable. This biological feature is suggested to be correlated to individual nutritional requirements, based on the host needs and the symbiont's lifestyle. Ticks are obligatory blood-feeders which harbor vertically transmitted endosymbionts that encode genes for the biosynthetic pathways of varied B vitamins. In the past decade, several of these tick-symbiont interactions have been identified, and a few studies have characterized these relationships functionally. Our previous study showed that *Coxiella* symbiont of *Rhipicephalus microplus* is essential for tick self-perpetuation, since under reduced levels of the symbiont ticks were arrested at the metanymph life stage and did not molt into adults. The transcriptomic differential analysis of the *R. microplus* metanymphs in the presence and absence of its mutualist endosymbiont revealed an altered expression profile of transcripts from several functional categories, highlighting a significant underexpression in those involved in blood feeding capacity. On the other hand, with the elimination of *Midichloria mitochondrii*, symbiont of *Ixodes ricinus*, we have found that the symbiont was not essential for tick immediate development and fitness. Nevertheless, the engorgement success of aposymbiotic larvae from two consecutive generations were negatively impacted. Therefore, we suggest that in the long-term the absence of *M. mitochondrii* might be critical for *I. ricinus* self-perpetuation. Taking these results together, we hypothesize that there is a correlation between the repertoire of provided vitamins and the tick-symbiont immediate dependence. While symbionts providing several B vitamins such as *Coxiella* sp. (B2, B5, B6, B7, B9) are essential for tick development and fitness in the short-term, those providing a more limited repertoire of vitamins, such as *M. mitochondrii* (B7, B9), provide the tick host with a nutritional complement during specific points of the development, being important for the species persistence in the long-term. Our preliminary results on the study of the interaction between *Rickettsia buchneri*, B7 provider, and its tick host, *I. scapularis*, corroborate this hypothesis since aposymbiotic ticks do not rely on *R. buchneri* to complete their life cycle. Interestingly, preliminary data suggest that *R. buchneri* contributes to the persistence of *Borrelia burgdorferi*, causative agent of Lyme disease, on the tick host.

SY1.4 Characterization of the bacterial flora in Swedish *Ixodes ricinus* and *I. persulcatus* using 16S amplicon sequencing

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The present study was aimed at characterizing the bacterial microbiome in blood-feeding Swedish ticks, to reveal organ-specific bacterial communities and correlate variation with feeding status and tick-borne pathogen (TBP) carriage. Ticks were collected from animals and humans in Norrland (above latitude 60°N) through a citizen study in 2018. Ticks were washed and morphological identification of tick species was performed before extraction of DNA. Malpighian tubules, midgut, ovaries, and salivary glands were analysed separately following dissection of a second set of *Ixodes ricinus* ticks. 16S ribosomal RNA community profiling was performed with the Illumina standard protocol on a MiSeq instrument. Microbiome analysis on the resulting data was performed using QIIME 2.0 on the Nephele platform and continued using Phyloseq and DESeq2 in R 4.0.4. Community diversity was low in whole ticks, with most samples dominated by likely endosymbiont species with *Midichloria* prevalent in *I. ricinus* ticks and occurring together with *Lariskella* in most *I. persulcatus*. Additionally, there were several *Rickettsia* that could not be determined to species level. The microbiota of all categories of organ samples were more diverse. Most organ samples contained *Midichloria*, but this genus was only the most abundant in a limited number of samples primarily from ovaries and salivary glands. *Rickettsiella* was also observed in a few samples. The most common observations overall in organs included *Enhydrobacter*, *Acinetobacter*, *Stenotrophomonas*, *Streptococcus*, *Pseudomonas*, and bacteria of the Chitinophagaceae and Neisseriaceae families. Most samples from the guts of engorged ticks as well as several samples from other organs collected from engorged ticks presented a remarkably stable composition of bacteria both in terms of presence and relative abundance. This community was dominated by Lachnospirales, Oscillospirales, Bacteroidales, and Lactobacillales, but contained representatives of many other orders. Known or putative TBPs were detected in both whole ticks and organ samples. *Borrelia* was most common in ovaries and salivary glands. *Neoehrlichia* was detected in all sample types. *Anaplasma* was not detected in any organ sample but in whole ticks. *Borrelia* was significantly positively correlated with *Pedobacter*, Neisseriaceae, and Aerococcaceae, negatively with *Rhizobium*, *Paracoccus*, and *Pseudomonas*. *Neoehrlichia* positive associations were observed for *Pseudoxanthomonas*, *Devosia*, and *Pseudomonas*, and negative for Comamonadaceae, *Granulicatella* and *Roseomonas*. The presented data adds knowledge to our understanding of the association between the tick microbiome and TBPs and shows the value of targeted community profiling of individual tissues. The present work was partially founded by the Swedish Research Council, grant TICKBIOCON, 2018-03830.

SY1.5 Isolation of bacterial endosymbionts (*Spiroplasma* spp.) from *Ixodes ricinus* ticks collected in Sweden

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Ixodes ricinus is the most common tick in Sweden. It harbors many human and animal pathogens such as *Borrelia* spp., tick-borne encephalitis virus and *Babesia* spp., as well many symbiotic bacterial microorganisms, some of which are beneficial for the survival and fitness of the tick. *Spiroplasma* are vertically-transmitted endosymbionts of arthropods, including ticks, and pathogens of some arthropods and plants. They are helical mycoplasmas belonging to the class Mollicutes. In ticks, they can be found in the salivary glands, gut, and reproductive organs. *Spiroplasma* spp. have been detected in ticks in Germany, Slovakia, Norway and many other European countries; however, to our knowledge, there are no reports suggesting their presence in Swedish ticks. The aim of this study was to isolate *Spiroplasma* spp. from Swedish *I. ricinus* ticks using tick cell lines. Live ticks were collected from dogs and cats in different regions of Sweden (mainly Uppsala and Skåne counties) and their internal organs were used to infect two tick cell lines derived from *Rhipicephalus microplus* (BME/CTVM23) and *I. ricinus* (IRE/CTVM19) embryos. A conventional cytocentrifugation technique combined with Giemsa staining was used to visualize the presence of bacteria. PCR was also performed on DNA extracted from the material left from ticks after the isolation attempts, using *Spiroplasma* genus-specific primers targeting the 16S rRNA and rpoB genes. Examination of cytocentrifuged cells led to a preliminary confirmation of the isolation of the bacteria from some tick specimens, but data from PCR analysis will give a definitive proof of the successful isolation. Isolation of *Spiroplasma* spp. (and therefore potentially other tick-associated organisms) using tick cell lines confirms that tick cells represent an excellent substrate in tick research, and we plan to use them to further explore the interactions (i.e. synergistic, antagonistic) between different components of tick microbial flora (both symbionts and pathogens) and to shed light on unknown aspects of tick biology, like tick fitness and reproduction.

The present work was partially founded by the Swedish Research Council, grant TICKBIOCON, 2018-03830.

SY1.6 Metabolic interactions between *Coxiella* like endosymbionts and the brown dog tick

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Rhipicephalus sanguineus (Ixodidae) ticks have a nutritional symbiosis with *Coxiella* like endosymbionts (CLEs). CLEs are essential for fitness and fecundity of ticks presumably by supplementing B vitamins that are lacking in blood diet. Computational evidences show that in addition to B vitamins, CLEs can synthesize and export l-proline, however, no experimental evidence for B vitamins and l-proline contribution to the hosts are available. To determine the contribution of these metabolites to the reproductive fitness of aposymbiotic, CLEs deprived, ticks we used rescue treatments by microinjection of B vitamins (B), and B vitamins and l-proline (BP) cocktail before feeding and after repletion. Since rescuing treatments in hard ticks cannot be done continuously, the treatment timing is crucial to observe an effect. We found that BP treatment is more effective in both treatment time point, however it is prominently effective only in the after-repletion time point. Among various reproductive fitness parameters tested, the rescue treatment results were significantly successful for the hatching rates. Our findings suggest that the effect of rescue treatment depend on the time of treatment (before or after feeding), and that both B vitamins and l-proline supplemented after repletion are needed for embryonic development. These finding together with recent other works support the hypothesis that CLEs are essential to ticks during high metabolic demands such as feeding and development.

Symposium “Tick-borne encephalitis” (SY2.1-SY2.6)

SY2.1 The phylo-epidemiology of TBE virus in Central Europe

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Tick-borne encephalitis (TBE) is the most important tick-borne viral infection in Europe and Asia. It is caused by TBE virus, a member of the genus Flavivirus in the family Flaviviridae. So far, at least five different subtypes of TBE virus can be distinguished using molecular genetic analysis. In Central Europe, so far only the European subtype has been detected. During the last years, more than 200 TBE virus strains from > 50 different TBE virus natural foci have been isolated and genetically characterized. These data show that many different strains circulate in Germany and countries of Central Europe. So far, at least nine different genetic clades within the European subtype of TBE virus have be detected. Further analyses show that the virus is genetically stable. Comparative studies of TBE virus strains from the same natural focus over a period of 40 years show the genetic identity of TBE virus in this time frame. Even in long established natural foci, which are located close to each other, the TBE virus strains may be genetically different. This implies that each of these foci was established by a separate introduction of a different TBE virus strain or evolved into a distinct TBE virus genotype over time. However, meanwhile also a continuous spread of virus over larger distances could be documented. Comparing the TBE virus strains on a local and regional level, the data show that TBE viruses in Germany are imported in Southern Germany mainly from the South-eastern part of Europe (Austria, Czech Republic, Slovak Republic). Recent isolates in Northern Germany show a close genetic relationship to TBE virus strains from Finland and from Poland. The data imply that there is a continuous influx of TBE virus into Germany from different directions. As a long-distance way of spread bird migration is implicated. In some instances, a continuous spread from one location to a neighbouring place was demonstrated implying migration of terrestrial wild animals or transport of domestic animals as ways of spread.

SY2.2 Comparative pathogenesis of tick-borne encephalitis virus and louping ill virus in experimentally infected sheep

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Tick-borne encephalitis virus (TBEV) and louping ill virus (LIV) are genetically and antigenically closely-related zoonotic flaviviruses that are mainly transmitted by ticks. Rodents are the main reservoir host for TBEV while sheep and red grouse are major reservoirs for LIV. Sheep play an important role in the epidemiology of both diseases but their susceptibility to both viruses is different. LIV causes a febrile illness in sheep that can progress to fatal encephalitis whereas TBEV infection is usually asymptomatic. Studies addressing the pathogenesis of TBEV and LIV in sheep and the associated immune responses are limited. Here, these aspects were studied after intradermal inoculation of 8 month-old sheep with a dose of 10^5 $TCID_{50}$ of TBEV Neudoerfl strain or LIV LI/31 strain. Clinical signs and rectal temperature were monitored daily. Two mock-infected sheep were euthanized on 0 dpi. At 1,2,3,5,7,10,14 and 18 dpi, 2 TBEV and 2 LIV infected sheep and 1 control animal were euthanized. Blood, visceral and lymphoid organs, brain tissues and skin biopsies at the site of inoculation were collected at each time point. Although sheep seroconverted at 7 dpi with TBEV, no TBEV RNA was detected in serum and in the examined tissues except for the skin, the lymph nodes and the spleen. TBEV viral loads in the latter remained however relatively low. In contrast, LIV RNA was detected in serum from 2 dpi and peak viremia was reached at 5 dpi. The latter dropped by 7 dpi correlating with the first detection and subsequent increase of neutralizing antibodies from 7 dpi onwards. The skin, lymphoid and visceral organs were LIV RNA-positive starting 1 dpi, 2 dpi and 3 dpi, respectively, and at 7 dpi high viral loads were found in the lymphoid organs (10^6-10^7 $TCID_{50}$/g). LIV RNA was detected in all CNS regions starting from 5 dpi with an increase till 10 dpi which seems indicative for a local replication. The medulla oblongata and the pons harbored the highest viral loads of LIV RNA at 10 dpi compared to the others CNS regions. These results confirm the difference in disease outcome after TBEV and LIV infection in sheep and suggest that TBEV replication and spread is counteracted early upon infection whereas LIV tends to replicate efficiently in sheep and disseminates to the brain. mRNA expression profiles of cytokines and chemokines will be determined over time to analyze which immune responses are potentially involved in the observed differences.

SY2.3 Tick behaviour in a TBE natural focus during higher temperature

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During the last years and decades, a rapid change in climate has been noticed, mainly caused by anthropogenic activities. The higher average temperatures also cause changes in fauna diversity and phenology. Also, for *Ixodes ricinus* distribution and activity dramatic changes have been forecast. Tick-borne encephalitis, the most important virus infection may also be dramatically influenced by the rapid change in climate. The years 2018 to 2020 were the years with the highest average temperature in Germany since 1960, with average temperatures higher than 10°C. We studied the abundance of *Ixodes ricinus* during the three years and compared it with the years 2015 to 2017, which were 0.7 to 1.0°C cooler than the following three-year period. Ticks were sampled monthly in a well-characterized TBE natural focus in south-eastern Germany. Numbers of ticks collected from the ecotone zone and from the forest were separately sampled and analysed. The abundance of *Ixodes ricinus* differed significantly in the three-year periods with cooler and higher temperatures. Significantly higher nymphal numbers were found in hotter years while the number of adult tick stages stayed stable. Nymphal numbers were found especially high in the months April and May in hotter years. There was a significant shift of tick abundance from the ecotone into the forest. A temperature increase of 0.7°C had no negative effect on the total numbers nor on the activity of *Ixodes ricinus*. Also, no differences in the prevalence of TBE virus in *Ixodes ricinus* was detected. *Ixodes ricinus* seems to well tolerate the higher temperatures of average 0.7°C (10.4°C versus 9.7°C). There are significant changes in the phenology of the tick species with a migration from the ecotone into the forest, probably from worse to better environmental conditions for the tick. The higher temperature seems not to have any influence on the circulation of TBE virus. The earlier models on changes of TBE distribution in times of climate change have to be revisited.

SY2.4 Utility of eco-epidemiological studies for the surveillance of tick-borne encephalitis virus in non-endemic areas – a UK example

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New tick-borne encephalitis virus (TBEV) foci are often detected through occurrences of one or more diagnosed TBE cases in an area; however, in the UK TBEV was thought to be absent, with no autochthonous cases reported. Utilising deer as sentinels for TBEV, a nationwide serosurveillance study was undertaken, that involved collecting and testing 1,309 deer serum samples from volunteers undertaking routine deer management. Two areas of possible TBEV presence were detected, in Thetford Forest and Hampshire, with 47.7% and 14% of deer samples seropositive, respectively. Screening of 2,041 ticks collected off deer in areas where seropositivity was observed revealed five PCR positive ticks - one of which yielded a full genome sequence. This was the first instance of TBEV recorded in the UK with significant homology to the Mandal strain (Norway, 2009). In addition, questing tick surveys conducted in areas in Thetford Forest and the New Forest area where seropositive deer were recorded over two years - comprising of 10,290 ticks - revealed nineteen positive pools in Thetford Forest and one positive pool New Forest border area. Sequence analysis from the latter showed homology to the TBEV-Salland strain (Netherlands, 2017). This indicates two separate importation events and the most western record of TBEV in Europe. Subsequently, with increased awareness of TBEV presence in the UK the first two probable cases were reported bordering the New Forest area. This shows use of deer as sentinel species for tick borne diseases has great utility in the UK where previously TBEV presence was unknown.

SY2.5 TBE pathogenesis study based on human blood-brain barrier model

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Disruption of the blood-brain barrier (BBB) is a hallmark of TBE development. The BBB is formed by neurovascular unit (NVU) cells, but the direct interaction of TBEV with these cells should be clarified. We investigated the pathology that occurs after TBEV infection in the cellular components of the NVU, ie, primary human brain microvascular endothelial cells (HBMEC), pericytes, astrocytes, and neurons. In addition to NVU cells, we also tested human microglial cells. All of these cell types are susceptible to TBEV infection, but the replication kinetics of the virus and the percentage of virus-infected cells in culture are different. An *in vitro* model using HBMEC on a microporous filter membrane of the transwell system, as well as tests of replication kinetics and cell monolayer integrity, demonstrated that virus can pass through the BBB via the transcellular pathway without compromising integrity. Correlative immunofluorescence and scanning electron microscopy confirmed the presence of an unaltered and well-organized TJ between TBE-infected or infected and uninfected cells. Infection of astrocytes and pericytes led to their activation and induced the expression of pro-inflammatory cytokines. Neurons are very sensitive to infection and produced high viral titers. Electron tomographic (ET) reconstructions yielded high-resolution 3D images of the proliferating endoplasmic reticulum and individual tubule-like structures in both neuronal and nonneuronal cells. In addition, ET revealed connections between cellular microtubules and vacuoles that harbored virions in outgrowths of infected neurons. The 3D topographic organization of membranous whorls and autophagic vacuoles in TBEV-infected neurons was mapped. The results provide new insights into the interactions between TBEV and cellular components of the neurovascular unit that may contribute to TBEV-induced neurotoxicity and BBB breakdown.

SY2.6 Seroprevalence against Tick-borne encephalitis (TBE) virus in wild rodents in two TBE natural foci in Bavaria, Germany

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Tick-borne encephalitis is the most important tick-borne viral disease in Europe. After the record year 2020 with more than 700 TBE cases reported by the Robert Koch Institute in Germany and the annual spread of risk areas to currently 175 counties, it is more important than ever to recognize and understand the spatial distribution of natural foci in order to successfully apply precautionary measures. It is still unknown how seroprevalence in wild rodents, the reservoir hosts of TBE virus, is distributed throughout the year in a natural focus and what their role is in maintaining TBE virus in nature. Therefore, rodents of the species bank vole (*Clethrionomys glareolus*) and yellow-necked vole (*Apodemus flavicollis*) were trapped and serologically analyzed once per month for two consecutive nights from March to October 2019 to 2021 in two, known natural TBE foci in Haselmühl and in Heselbach in the county Oberpfalz, Bavaria, Germany. Rodents were captured using 50 live traps per site, anesthetized with isoflurane, and tagged with an RFID transponder. A blood sample was then collected and serum was analyzed for antibodies against TBE virus using an indirect immunofluorescence test (IIFT). Thereafter, the animals were released back at the capture site. Furthermore, generalized linear mixed model (GLMM) analysis was performed to investigate ecological as well as individual factors for the probability of infection in the rodents. A total of 602 samples from Haselmühl (195 *C. glareolus*, 63 *A. flavicollis*) and Heselbach (240 *C. glareolus*, 104 *A. flavicollis*) were examined. In Haselmühl, *C. glareolus* (19.0%) had a significantly higher incidence rate of specific antibodies than *A. flavicollis* (1.6%). In Heselbach, the difference was not significant, yet a higher incidence rate was found in *C. glareolus* (20.4%) than in *A. flavicollis* (16.3%). A significant difference between seasons was not detected at either site. Male bank voles (41.2%) had a significantly higher incidence rate than females (17.0%) and juveniles (9.5%). In contrast, female yellow-necked mice (20.4%) had significantly higher incidence rates than males (6.4%) and juveniles (3.6%). The high incidence rates of rodents, particularly the bank vole, highlight their important role in the maintenance of TBE virus in a natural focus and demonstrate how reliably serologically positive rodents can be detected in a natural focus regardless of season.

Symposium “Tick vector competence: challenges for the future” (SY3.1-SY3.6)

SY3.1 Vector competence of ticks for zoonotic agents: The basics and where are we now?

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Ticks take up various microbial agents during their large blood meals on vertebrate hosts. Some species are capable of maintaining their infection and transmitting certain agent(s) to another host when feeding again in a later life stage. They act as vectors. Tick-borne pathogens circulate between vector tick species and (vertebrate) reservoir hosts in so-called natural foci, an eminent concept developed by E. Pavlovsky in the late 1930s. They are usually harmless for wild and adapted animals, but they can be pathogenic to humans and/or domestic animals and may cause more or less serious disease in them. It is therefore important to know which tick species are involved in such natural foci. This knowledge opens up the possibility to investigate the specific tick-pathogen relationships and to learn about the ecological conditions under which a given tick-borne pathogen can perpetuate, a prerequisite to initiate effective control measures. What seems rather simple at first glance – but usually is not – is to check the possible vector competence of a given tick species for a certain pathogen. This makes it necessary, as a rule, to carry out transmission experiments, which can be difficult and costly to perform, especially if the pathogen is dangerous and protected vertebrate species are involved in its circulation. Thus far the theory, simple and clear. Due to the increasing availability of modern, very powerful detection techniques, however, a multitude of microbial agents and parasites (new species or new genotypes) have been described in recent years only by identification of relatively small parts of their DNA/RNA in questing or feeding ticks in recent years. On this vague basis authors often erroneously call these ticks vectors and not carriers, which would be the correct term. As a result, it is extremely difficult to maintain a clear overview of proved vector tick species on the one hand versus only incriminated vector tick species on the other hand, a highly unsatisfactory situation. This introductory contribution shall set the scene in the symposium on vector competence and the following discussion.

SY3.2 Proteomics: A novel alternative to molecular biology in vector competence research?

Boulanger N

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Ticks, representing the most important vectors in human and veterinary medicines, harbor numerous microorganisms, potentially pathogens. The availability of effective tools to test the vector competence for these microorganisms is therefore essential. Identification of ticks and associated pathogens to determine this competence is generally performed by microscopic observation and/or molecular biology. These approaches are often tedious for tick species determination and not always satisfactory for pathogen identification. Detection of microorganism DNA in tick does not always mean that they are alive or potentially infectious for vertebrate hosts. In addition, the proof of a transmission to the vertebrate host is often missing. The well-known Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) has been successfully developed for tick species identification. Unfortunately, this proteomic technique did not succeed in distinguishing MS spectra of pathogen-free *Ixodes ricinus* specimens from species infected by *Borrelia burgdorferi* sensu lato, the Lyme disease agent. Therefore, large-scale proteomic approach was investigated on Ixodes ticks collected in a region of France, which is known to be endemic for various tick-borne pathogens, to determine the infection status of ticks. Tick proteins were prefractionated by gel electrophoresis, then digested and resulting peptides were analyzed by liquid chromatography hyphenated to tandem mass spectrometry (LC-MS/MS). Bioinformatic searches were then performed using homemade databases including *Ixodes* and several pathogens known to be transmitted by *Ixodes* ticks. This strategy allowed the identification of *Borrelia* proteins, as well as *Anaplasma*, *Rickettsia* and *Babesia* proteins, in this tick population.
Finally, we measured the *Ixodes* vector competence to transmit pathogens to the vertebrate host by this same proteomic technique. First, we tested the method on mouse models infected either with *Borrelia burgdorferi* sensu lato or with field-collected ticks. We detected pathogen proteins in the skin of infected mice. We also tested proteomics on patients presenting early stage (erythema migrans) of Lyme Borreliosis. Comparison and analyses of the data of this proteomic strategy to well-known molecular methods reveal that the two methods are complementary in tick-borne diseases.

SY3.3 Vectors of *Babesia* species

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Babesia species are apicomplexan parasites of the red blood cells of mammals and birds, transmitted almost exclusively by ixodid ticks. In ticks they undergo a complex development involving the formation of zygotes in the gut, followed by multiple fission in various tissues and culminating in the development of infective stages in the salivary glands. *Babesia* sensu stricto [s.s.] can also undergo transovarial (vertical) transmission while *Babesia microti*–like species can only be transmitted transstadially (horizontally). The association between *Babesia* spp. and their vectors is highly specific. While it has long been understood that there are behavioural barriers to pathogen transmission because many ixodid ticks have strong host preferences, there is growing evidence that the ability of a tick species and life cycle stage to acquire, maintain and transmit *Babesia* parasites is determined by a variety of microbiological, immunological and molecular factors. Probably as a result of this highly specific interaction, most tick species transmit just one significant *Babesia* species and most *Babesia* spp. are transmitted by relatively few tick species. Yet numerous ixodid tick species have been listed as vectors in the literature. Many of these reports have been found to be inaccurate, and others are doubtful as they are chiefly based on perceived associations with observed disease or the result of misidentification of vectors and parasites. More recently overreliance on the detection of parasite DNA in ticks has also led to misleading conclusions. When criteria such as epidemiological relevance together with controlled transmission experiments are strictly applied, the number of previously supposed vectors for particular *Babesia* spp. can be reduced by more than half. Rules are required to make sensible use of DNA detection technology to determine the vector status of ticks for *Babesia* spp.

SY3.4 Identification of candidate molecular determinants of the vector competence of *Ixodes ricinus* for members of the tick-borne encephalitis virus complex

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Ixodes ricinus, the main tick species in Europe, has been known to transmit the tick-borne encephalitis virus (TBEV) and the louping ill virus (LIV) for over a century. However, the mechanisms underlying vector competence in ticks are not as yet understood. Viruses are obligate intracellular life forms, whose survival requires subversion of the host cell's metabolic pathway and evasion of not only innate and adaptive immunity, but also cell-intrinsic anti-viral defenses. Since successful tick-borne transmission of viruses requires survival of ticks between bloodmeals, antiviral countermeasures in vector-competent tick species preserve the life history traits of the tick without eliminating the virus. Such a cohabitation requires a molecular dialogue that is presumed to be largely governed by binary protein-protein interactions (PPIs) established between viruses and vector cells. We have thus established the network of PPIs between viral proteins of TBEV and LIV and tick proteins encoded by a cDNA library of *I. ricinus*, by using yeast two-hybrid methodology. Twenty-two tick proteins that engage in physical interactions with viral proteins were discovered, and in all cases, the tick proteins interacted with both orthologous viral proteins. This network of PPIs revealed that TBEV and LIV proteins target multiple protein modules in tick cells, with roles in activation of JAK/STAT pathways, transcription, protein degradation and cytoskeletal functions. This work reports the first PPI network to be described for TBEV and LIV with *I. ricinus*. It provides multiple leads regarding potential molecular determinants of vector competence of *I. ricinus* for TBEV and LIV and potentially other flaviviruses. An understanding of vector competence at the molecular level could be used for analysis of the risk that exotic viruses, if introduced into Europe, could be transmitted by autochthonous ticks.

SY3.5 Evaluation of two artificial infection methods of live ticks as tools for studying interactions between tick-borne viruses and their tick vectors

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Ticks represent significant risks for human and animal health. Because they are obligate hematophagous ectoparasites and feed on diverse vertebrate hosts, they are considered as one of the most important vectors of zoonotic pathogens. Ticks can transmit a wide variety of bacteria, parasites and viruses. In human and veterinary medicine, most tick-borne pathogens are transmitted by various hard ticks belonging to genera *Ixodes*, *Haemaphysalis*, *Dermacentor*, *Hyalomma* and *Rhipicephalus* and by certain soft ticks belonging to genera *Argas* and *Ornithodoros*. Among tick-borne pathogens, 170 tick-borne viruses (TBVs) were identified and belong to nine virus families and twelve virus genera. Viruses transmitted via tick bites can cause various symptoms in humans and animals, ranging from mild febrile illness to neurological disorders or even haemorrhagic fevers. The oversite and existing gaps in our knowledge of ticks and TBV are partly due to the difficulty of setting effective experimental models to assess vector competence or study virus-tick interactions in general. To overcome this gap of knowledge, it is essential to reproduce transmission cycles under controlled laboratory conditions. In our study, we used viruses belonging to genera Flavivirus or Orbivirus to infect *I. ricinus*. TBEV is known to be transmitted by *I. ricinus* and responsible for severe neurological illness in humans in Europe and Asia. It was used as a positive control to assess the efficacy of both artificial feeding system (AFS) and immersion technique (IT) as infection methods. Kemerovo virus (KEMV, genus Orbivirus) is suspected to be the causative agent of encephalitis cases in humans in central Europe and Russia. AFS and IT were used to assess for the first time vector competence of *I. ricinus* for KEMV. The virus has been isolated/detected in *I. persulcatus* and *I. ricinus*. Assessing the efficacy of both infection techniques was based on the three criteria of vector competence: (i) virus acquisition by ticks, (ii) trans-stadial transmission, and (iii) transmission of the viruses to a vertebrate host. Both methods permitted TBEV acquisition by ticks and we further confirmed virus trans-stadial transmission and onward transmission to a vertebrate host. However, only artificial feeding system allowed to demonstrate both acquisition by ticks and trans-stadial transmission for KEMV. Yet we did not observe transmission of KEMV to mice (IFNAR-/- or BALB/c). Artificial infection methods of ticks are important tools to study tick-virus interactions. When optimally used under laboratory settings, they provide important insights into tick-borne virus transmission cycles.

SY3.6 The conundrum of one *Ornithodoros* species-one *Borrelia* species - where should we look?

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Ticks belonging to the *Ornithodoros* genus are vectors of medical and veterinary important pathogens. Tick–borne relapsing fever (TBRF) spirochetes are primarily transmitted by *Ornithodoros* ticks and the competence of ticks to vector the spirochetes is specifically set for a single TBRF spirochete species to a single or possibly a couple of tick species. Some recent studies have suggested the possibility that there could be specific factors at play in the salivary glands leading to this one tick species–one *Borrelia* species setup. Here I review what we currently know and question the various other possibilities including that the microbiota of the tick may be having a say on which species is successful in the tick, spirochete gene expression in the salivary glands and the possibility of certain species being possibly resistant to particular antimicrobial immune factors within the tick.

Symposium “Tick-host-pathogen interactions: getting closer to disease prevention and control” (SY4.1-SY4.8)

SY4.1 Tick-host-pathogen interactions: a vaccinomics approach to control tick-borne diseases

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Tick-borne diseases (TBDs) represent a growing burden for human and animal health worldwide. Several approaches including the use of chemicals with repellency and parasiticidal activity, habitat management, genetic selection of hosts with higher resistance to ticks, and vaccines have been implemented for reducing the risk of TBDs. However, the application of latest gene editing technologies in combination with vaccines likely combining tick and pathogen derived antigens and other control measures would result in the development of effective, safe, and environmentally sound integrated control programs for the prevention and control of TBDs. This approach in combination with latest omics technologies and focusing on biological processes involved in tick-host, tick-pathogen and host-pathogen interactions would allow the identification and combination of tick-derived and pathogen-derived protective antigens affecting tick infestations, tick pathogen infection and transmission, tick attachment and feeding, and/or host pathogen infection. However, major challenges such as host immunity, pathogen and environmental factors and vaccine efficacy and safety need to be addressed. Vaccinomics provides a platform to address these challenges and improve vaccine efficacy and safety. The immune system contains random processes such as immunoglobulin recombination events and the direct correlation between atomic coordination and peptide immunogenicity that support quantum immunology. In similarity with Albert Einstein's definition of the photon as a quantum of light, the immune protective epitopes were proposed as the immunological quantum. Quantum vaccinomics is the platform proposed for the identification and combination of antigen protective epitopes, the immunological quantum, for vaccine development. Quantum vaccinomics will contribute to vaccine development, efficacy and safety by facilitating antigen combinations to target pathogen infection and transmission in emerging infectious diseases.

SY4.2 *Rickettsia helvetica*: shedding light on the interplay between symbiosis and pathogenicity

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Tick symbionts, microorganisms whose survival depends on ticks, are being explored for their role in the tick's life cycle and for their pathogenic potential. *Rickettsia helvetica* is a gram negative obligate intracellular bacteria previously thought to belong to the Spotted Fever Group Rickettsiae (SFGR). *R. helvetica* can be transmitted through tick bites and has been associated with human disease, however the extent of its pathogenicity is still being studied and knowledge about its eco-epidemiology is scarce. *R. helvetica* can be transovarially transmitted in ticks and has been repeatedly isolated from questing *Ixodes ricinus* in European countries. A survey performed in the Netherlands found a varying prevalence in questing ticks from different biotypes ranging from 6% in a forest area to 66% in a dune area raising questions regarding the enzootic cycle of *R. helvetica* and the species genetic composition. We performed whole genome sequencing of *R. helvetica* infected and uninfected *I. ricinus* ticks from two different biotypes in the Netherlands, using high-end hybrid sequencing technologies. This approach allowed us to investigate the whole genomes of *I. ricinus* ticks as well as those of its microbial tenants in order to gain insights regarding biosynthetic pathways and evolutionary processes that may hint to shared or individualistic survival strategies as well as transmission dynamics in which humans become infected. Moreover we performed murine experiments to glean information regarding *R. helvetica*'s pathogenicity as well as immunofluorescence of infected ticks to learn about the symbiont-host interactions at play.
This one of a kind in-depth study provides a comprehensive look on *R. helvetica*, filling knowledge gaps hitherto explored.

SY4.3 *Rickettsia helvetica* infection is associated with microbiome modulation in *Ixodes ricinus* collected from humans in Serbia

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Rickettsia helvetica is an emerging pathogen in various European countries, and it is one of the agents causing spotted fever diseases. This tick-borne pathogen replicates in the tick organs, but its potential interactions with the vector microbiota are poorly understood. The vector microbiome plays a pivotal role in tick-pathogen interactions, and some microbiota member facilitates or competes with tick-borne pathogen colonization in tick tissues. Manipulations of the tick microbiome have led to reduced pathogen colonization in the tick vector. However, translating these findings into disease control applications requires a thorough characterization of vector microbiota response to different pathogen infections. In this study, we analyzed and compared the microbiota of *Ixodes ricinus* ticks collected on humans in Serbia. Ticks were either positive for *R. helvetica*, or negative (referred hereafter as 'pathogen-free') for 34 major tick-borne pathogens. Richness and evenness in samples of the two datasets were compared using alpha-diversity indexes. Microbial community structure and interaction patterns in response to pathogen infection were characterized using co-occurrence networks. The Network Construction and comparison for Microbiome (Net CoMi) method was used to compare the connectivity of *R. helvetica*-infected and 'pathogen-free' networks. The hierarchical organization, based on keystone taxa, and the functional profiles of *R. helvetica*-positive and 'pathogen-free' samples were also compared. Our results show that *R. helvetica* infection has a significant impact on tick microbiota. The presence of the pathogen was associated with decreased richness and variability. The networks comparison demonstrated a significant change in the hierarchical organization of *I. ricinus* microbiota and some taxa were identified as positively associated with *R. helvetica*. The reconstruction of microbial metabolic pathways shows that the presence of *R. helvetica* might have major impact on the metabolic functions of the tick microbiome. The characterization of the tick microbiome in ticks collected from humans can reveal novel targets to prevent tick-borne pathogen infection, as the tick microbiome has a critical role on vector competence. This study highlighted a potential target for an anti-microbiota vaccine. These results can inform novel interventions for the prevention of *R. helvetica* infection in humans.

SY4.4 Identifying vertebrate host species contributing to Lyme disease emergence in an atypical hotspot

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In Europe, ticks from the genus *Ixodes* are the main vector of many tick-borne pathogens causing disease in humans and livestock. The most common human vector-borne disease in Europe, Lyme disease (LD,) is a potentially debilitating condition caused by bacteria in the *Borrelia burgdorferi* sensu lato (Bbsl) species complex. The main LD vector in Europe, *I. ricinus*, is a generalist species, so the composition of the local vertebrate host community available for ticks to feed on, plays a key role in LD emergence. The Uists' in the Western Isles, Scotland, have a recorded annual LD incidence of nearly 600 cases/100,000 population, around 30x the average in Western Europe, whereas neighbouring islands have standard incidence levels. Lyme disease hotspots are typically linked with woodlands, which are largely absent from the Western Isles. This raises the question, what do the vertebrate host communities look like that are driving LD emergence in this setting.
This study aimed to assess which combination of tick reproduction host and pathogen maintenance host species are necessary for pathogen persistence in this atypical LD hotspot. To answer this, we collected data on nymph and adult tick density as well as indices of deer, livestock, and small mammal abundance across 42 sites on three islands over three years. Tick burdens and Bbsl prevalence for live-trapped small mammals were also to be determined. Questing ticks were highly abundant on all islands, whether deer were present or not. Questing ticks on the Uists showed a prevalence of Bbsl of 6.6%. Among positive ticks, 96% were infected with rodent associated genospecies *B. afzelii*. In contrast, on neighbouring islands <1% questing ticks were positive for Bbsl, and *B. afzelii* was not detected. Species of small mammals tested include pygmy shrew (*Sorex minutus*), field vole (*Microtus agrestis*), wood mouse (*Apodemus sylvaticus*), European hedgehog (*Erinaceus europaeus*) and brown rat (*Rattus norvegicus*). Of these species, brown rats had substantially higher tick burdens than any other small mammal species. Results show deer are not required for high levels of tick abundance on the Western Isles. Questing ticks on the Uists have a high prevalence of *B. afzelii*, implicating small mammals as pathogen maintenance hosts. So far results suggest brown rats may be an important maintenance host for Bbsl in this system.

SY4.5 Degrade to survive: the intricate world of piroplasmid peptidases

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Piroplasmids belonging to the *Babesia* and *Theileria* genera are tick-transmitted parasites with high impact on men and animals. These parasites possess sexual and asexual phases occurring in the definitive arthropod and vertebrate hosts, respectively. Fulfilling their complex life cycles requires the coordinated execution of numerous metabolic pathways of degradation and synthesis. As well as other parasitic protozoa, piroplasmids are equipped with different types of peptidases to fulfill many of such essential processes. Despite their perceived importance, the functional significance of these peptidases remains poorly characterized. Typically, piroplasmids contain around 60 putatively active serine, metallo, cysteine, aspartic and threonine proteases, which are present soluble in the cytosol, inside organelles, bound to the membrane or secreted to the extracellular medium, and are well conserved among species. These parasites also express a similar amount of non-functional protease homologues, with as yet unknown roles. In the vertebrate host, piroplasmid functional peptidases intervene in entry and exit to and from host cells, hemoglobin degradation, and intracellular protein degradation in the proteasome, while other possible functions include immune modulation, organelle maturation, apoptosis and virulence. Additionally, some proteases appear to be highly relevant for the development of parasite stages in the tick vectors. Consistent with their essential roles, blockade of some key proteases using inhibitors or antibodies hampers parasite growth, highlighting their potential usefulness in drug therapies and vaccine development against piroplasmid infections of domestic animals and men. It can be concluded that a better understanding of the functional significance of piroplasmid peptidases will certainly contribute to the improved control of many devastating human and animal diseases. (Supported by INTA projects I102 and I105).

SY4.6 Tick-borne encephalitis virus – host in the skin interface

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Tick-borne encephalitis virus (TBEV), a small enveloped neurotropic flavivirus transmitted by ticks, is the most prevalent arbovirus in Europe and Asia constituting a major threat for humans by the accelerated spread of infection. The virus causes tick-borne encephalitis (TBE), one of the most serious infections affecting the human central nervous system (CNS). So far, no specific treatment exists for this infectious disease with different TBEV subtype-depending clinical outcome. Although brain represents a targeting organ for TBEV, the skin become a first interface where TBEV enters into the host during feeding of tick vectors. The human skin hosts a number of different cell types that play a crucial role in fending off invading pathogens. It´s generally known that upon inoculation of TBEV into the human skin, initial infection and replication occurs in local dendritic cells, macrophages and neutrophils causing primary viremia. However, molecular details of the interaction between keratinocytes, the most abundant cellular component of the epidermis, and TBEV are scarce leading to a poor understanding of the antiviral pathways and viral countermeasures. In this study we focused on interaction between HaCaT cells (human keratinocytes) and TBEV (strain Hypr) *in vitro*, primarily in association with interferon (IFN) system. We have studied gene expression of human IFNs of type I and III, their receptors and chosen IFN-stimulated genes after TBEV infection using basic molecular biology methods (RT-PCR, RT-qPCR); at protein level, IFN production was detected using immunocytochemistry (ICC). Furthermore, infection of HaCaT cells did not lead to visible cytopathic effect even though we proved a replication of TBEV in this cell line. Thus, we aimed on study of TBEV infection on human keratinocytes at an ultrastructural level using transmission electron microscopy (TEM). A better understanding of the processes in the skin during virus transmission should help to identify the mechanism of skin immunity against this invader and to characterize the involved key molecules of the host and the virus. This could become a core for the design of better vaccines. Acknowledgements: This study was produced with financial support of projects VEGA 2/0172/19 and VEGA 2/0108/22.

SY4.7 *Borrelia burgdorferi* strains with high abundance in host tissues have higher transmission to *Ixodes scapularis* ticks

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Borrelia burgdorferi sensu stricto (Bb) is a tick-borne spirochete that causes Lyme borreliosis. In nature, populations of Bb consist of genetically distinct strains that co-exist in the same vertebrate reservoir host and tick populations. These Bb strains differ in their frequency, but the reasons why some strains are more common than others are not well understood. The purpose of this study was to investigate whether strains that establish high abundance in host tissues have higher transmission success to feeding ticks. For each of 11 strains of Bb, we experimentally infected 8 C3H/HeJ mice via nymphal tick bite. Mice were infested with *Ixodes scapularis* larvae on days 30, 60, and 90 post-infection (PI) to determine the lifetime host-to-tick transmission success of each strain. The engorged larvae were allowed to moult into nymphs and the nymphs were frozen at 4 weeks post-moult. The mice were euthanized at 97 days PI and 7 organs were dissected. All organs and xenodiagnostic nymphs were tested for the presence and abundance of Bb using qPCR. We found significant differences among the 11 strains in the abundance of Bb in the mouse organs; this phenotype differed 4.8-fold between the highest and the lowest strains. We also found significant differences in host-to-tick transmission success among the 11 strains, the best strain infected 1.8 times more ticks than the weakest strain (98.7% versus 54.3%). Most importantly, we found that strains with the highest abundance of Bb in the mouse organs also had the highest transmission success to feeding ticks. Our study suggests that strains of Bb are under strong selection to maintain high abundance in those mouse tissues that facilitate transmission to feeding *Ixodes* ticks.

SY4.8 N-glycosylation in piroplasmids: diversity within simplicity

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N-glycosylation is a co-translational process that takes place in the endoplasmic reticulum (ER). In a first step, a glycan with a varying number of branching sugar residues is synthesized onto a dolichol-P molecule inserted into the ER membrane. In a second step, the generated glycan is transferred and linked to an asparagine (N) residue of a nascent protein. Parasite N-glycans participate in host-pathogen interactions and may constitute therapeutic targets. In piroplasmids, in silico analysis of N-glycosylation pathways revealed three distinct situations. *Babesia* sensu stricto parasites (*B. bovis*, *B. bigemina*, *B. divergens* and *Babesia* sp. Xinjang) are able to form dolichol-P-linked chitobiose (NAcGlc)2 and transfer it to proteins. *B. microti* and *Theileria* sensu stricto (*T. parva*, *T. annulata*, and *T. orientalis*) can build dolichol-P-linked NAcGlc or chitobiose but cannot transfer them to proteins. In *T. parva* schizonts, the corresponding genes are transcriptionally active and it can be hypothesized that dolichol-P-NAcGlc and dolichol-P-chitobiose might fulfill biological roles in these and other piroplasmids other than participating in the formation of N-glycans. A third scenario is present in *T. equi* and *Cytauxzoon felis*. These piroplasmids show the most complex N-glycans, since they are able to synthesize $(NAcGlc)_2$-Man and transfer it to proteins. The particular N-glycosylation scenario of *T. equi* adds to the list of differences observed with respect to *Theileria* sensu stricto species, emphasizing its distinct taxonomic placement. The occurrence of N-glycans in *B. bovis* merozoites was analyzed by incubation of fixed smears with a biotinylated lectin that specifically binds NAcGlc residues present in the terminal end of glycans. Detection of lectin binding by incubation with streptavidin-fluorescein and observation by epifluorescence microscopy demonstrated that *B. bovis* merozoites indeed synthesize the N-glycan that was bioinformatically predicted. The relevance of N-glycosylation for *B. bovis* growth and survival was analyzed by incubation with tunicamycin (0.3 to 30 µM), which inhibits the synthesis of dolichol-P-P-N-acetylglucosamine. Although a significant reduction in growth was observed, parasites could still reproduce at the highest drug concentration tested, reaching about 30% parasitized erythrocytes as compared to the control. These results suggest that N-glycosylation facilitates but is not essential for erythrocyte invasion and/or multiplication of *B. bovis* merozoites. Finally, RNAseq studies showed that the genes coding for N-glycosylation enzymes are transcribed in *B. bovis* blood and tick stages, although at relatively low rates, suggesting that this pathway might be biologically relevant throughout the parasite life cycle. (Supported by INTA projects I102 and I105).

Symposium “Rethinking our approach to understanding tick-borne hemorrhagic fever viruses: why we need a trans-disciplinary approach” (SY5.1-SY5.4)

SY5.1 Crimean-Congo hemorrhagic fever enzootic cycle and factors favouring virus transmission: special focus on France, an apparently free-disease area

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Crimean-Congo hemorrhagic fever (CCHF) is a viral zoonotic disease resulting in hemorrhagic syndrome in humans. Its causative agent is naturally transmitted by ticks to non-human vertebrate hosts within an enzootic sylvatic cycle. Ticks are considered as biological vectors but also reservoirs for CCHF virus (CCHFv), as they are able to maintain the virus for several months or years and to transmit CCHFv from ticks to ticks. Although animals are not symptomatic, some of them can sufficiently replicate the virus to become a source of infection for both ticks, as well as humans through contact with contaminated body fluids. The recent emergence of CCHF in Spain indicates that the geographic range of the virus is expanding. In other European countries like France, the presence of its main tick vector and the detection of CCHFv antibodies in animals, without necessarily human cases, suggest that CCHFv has been continuing to spread silently. Based on a systematic review of the literature, we investigated the different CCHF epidemiological cycles already known in endemic countries and determined the one as hypothesized in the French local context. This work made it possible to point out tick species that seem to be the best candidate vectors of CCHFv in France, but also to highlight the importance of the abundance and composition of local host communities on the infection prevalence of vectors. We also identified parameters that may influence the virus transmission among tick vectors and non-human vertebrate hosts. Considering all these components, we understand why tick vectors may remain weakly infected in France and predict a low probability of disease emergence in humans in the current situation. The likelihood of factors that can modify this equilibrium is discussed.

SY5.2 Crimean-Congo hemorrhagic fever: Where are we? Where do we need to go?

Bente D

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Crimean-Congo hemorrhagic fever virus (CCHFV) infection is identified in the 2018 World Health Organization Research and Development Blueprint and has been identified as a European and American priority disease due to its high risk to public health. Tick-borne CCHFV is widespread, found in Europe, Asia, Africa, the Middle East, and the Indian subcontinent. Recently, new foci of CCHF and its tick vector have been identified in several parts of the world, including the Balkan countries, southwest Russia, the Middle East, India, and Spain. Potential reasons for the emergence or re-emergence of CCHF include anthropogenic factors, such as changes in agricultural activities, habitat fragmentation, and importation of infected animals and ticks. Seventy-eight years have passed since the discovery of the disease, yet little progress has been made understanding the factors in the transmission dynamics of CCHFV, especially the tick vector. In this talk, we will examine gaps in knowledge and discuss considerations for a more sustainable approach to disease control.

SY5.3 Genetic background of adaptation of Crimean-Congo haemorrhagic fever virus to the different tick hosts

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Crimean-Congo Hemorrhagic Fever Virus (CCHFV - genus *Orthonairovirus*, family Nairoviridae, order Bunyavirales) is a negative-sense, single-stranded RNA virus. Its genome is approximately 19.2 kb long containing the S (small), M (medium), and L (large) segments. CCHFV causes a severe disease namely Crimean-Congo Haemorrhagic Fever (CCHF). It has become one of the most geographically widely distributed tick-borne viral diseases in the world. The virus is transmitted mainly by tick species in *Hyalomma* genus but other tick species such as representatives of genera *Dermacentor* and *Rhipicephalus* are also involved in virus transmission. The aim of this study is to determine if viruses collected from the same (or closely related) tick species phylogenetically cluster together and if they show higher values of codon adaptation index (CAI) for the particular tick host. To better understand the evolutionary characteristics of the virus, analysis of the codon usage pattern in CCHFV strains isolated from different tick hosts that have been confirmed as vectors (*H. anatolicum*, *H. asiaticum*, *H. dromedarii*, *H. excavatum*, *H. lusitanicum*, *H. marginatum*, *H. rufipes*, *H. truncatum*, *R. bursa*, and *R. sanguineus*) was performed by calculating CAI, effective number of codons (ENC), and other parameters. These calculations were used to analyze codon usage bias in order to measure the adaptation of the virus and predict the virus' behaviour on different tick hosts. CAIcal and CodonW programs were utilized for calculating codon usage bias. The results of the analyses indicate that *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains use different codons. CAI analysis shows that both *Hyalomma*- and *Rhipicephalus*-isolated strains display higher adaptation to use codons that are preferred by *Hyalomma* species. These results suggest that CCHFV genome has optimized its codon usage patterns to utilize the translational resources of *Hyalomma* species more efficiently than that of *Rhipicephalus* species. Higher selection pressure from *Hyalomma* spp. can affect the codon usage of CCHFV and that the evolution of codon usage in CCHFV has allowed it to use the translation resource of species of *Hyalomma* genus more efficiently. Overall, the results of this study show the strong effect of evolutionary processes on codon usage patterns. This research not only provided knowledge about the variation in CCHFV codon usage patterns in relation to their two vectors but also contributed to analyzing the factors that influence the adaptation of the virus to the hosts. In silico studies are very important in the case of CCHFV as it is regarded to be a biosafety level-4 pathogen, and therefore, studies on the virus are very limited.

SY5.4 Better insight into the epidemiology of Crimean-Congo hemorrhagic fever virus in Corsica (France): a One Health approach

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Among pathogens responsible for emerging infectious diseases, those with vector transmission are of major importance. Corsica, a Mediterranean island, is characterized by several factors supporting the spread of vector-borne diseases. Mass tourism and extensive agriculture favor interactions between humans, livestock and wild fauna. Moreover, Corsica, located on the avian migration route, allows transport of new invasive tick species. Favored by global warming, ticks succeed in settling and circulating new diseases including Crimean Congo hemorrhagic fever (CCHF). CCHF is a common zoonotic viral infection transmitted by ticks. In 2016, human cases were detected in Spain. CCHFV was also detected in ticks in Spain and Italy. In Corsica, CCHF virus antibodies were detected in domestic animal with a seroprevalence of 13% in cattle and 2,5% in sheep. In 2016, A surveillance study on Corsican ticks was set up however no CCHFV were detected despite the large circulation of its main vector, and till date no human cases were reported. The objective of this thesis is to strengthen the preparation for tick-borne diseases emergence, especially CCHF emergence. Virus circulation will be examined via a One Health approach and studied in all compartments involved in its epidemiological cycle. A molecular detection of CCHF virus in ticks collected from domestic animals will be conducted in parallel with an 18-month epidemiological survey targeting populations at high risk for CCHF infection due to their proximity to livestock and wild animals (Farmers, slaughterhouse workers, hunters and veterinarian). To this end, a questionnaire will be conducted to assess exposure factors in these populations and serum samples will be collected to calculate the seroprevalence of anti-CCHFV antibodies and compare it to the general population. This transdisciplinary study is necessary to evaluate the source of a possible epidemic and thus monitor its possible emergence by putting in place preventive measurements and raising public health awareness in Corsica and neighboring regions.

POSTERS (P01-P55)

P01 The distribution of Ixodidae ticks on domestic animals in south-west part of Bangladesh

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Livestock plays a crucial role in the agricultural economy of Bangladesh. The annual distribution of ixodid ticks depends on the seasonality of the country. The study was carried out in the Khulna division, south-west part of the country, which has an area of 22,285 km^2. Sampling was carried out during the summer and monsoon in year 2014. During the study period a total of 264 domestic animals (cattle and goats) were examined in 23 veterinary hospitals and the adjacent areas of five districts. Both local and cross breed cattle were susceptible to tick infestation (34.24%). Cows (85.61%) were much more susceptible than goats (14.39%). Compared between cattle breeds, the crossbreed (58.41%) was more susceptible to ticks than the local breed (41.59%). A total of 4,137 ticks (95.46% adult ticks and 4.54% immature ticks) were used for taxonomic identification. Three tick genera were collected on livestock, namely *Rhipicephalus*, *Haemaphysalis* and *Hyalomma*. The overall prevalence of *Haemaphysalis* (80.06%) was higher than *Rhipicephalus* (18.59%) and *Hyalomma* (1.35%). A total of 188 (4.54%) immature ticks (larvae and nymphs) were collected, of which 4.47% was *Haemaphysalis* and 0.07% *Hyalomma*. *Hyalomma* ticks prevalence was only recorded in two districts, the prevalence of both male and female ticks were higher in Meherpur (4.24% and 3.79%) than Chuadanga (1.29% and 1.1.5%). The present study revealed that *Hyalomma* ticks have been found in Meherpur (Mujibnagar) and Chuadanga (Jibannagar & Damurhuda) while *Hyalomma* is restricted to the steppe climatic area of the northern part (Barind Tract) of Bangladesh. These are the border zones, so cattle usually pass through this area from India to Bangladesh. There is no quarantine system active to protect parasites and pathogens. So far Indian cattle are one of the main causes of *Hyalomma* tick infestation and tick-transmitted pathogens infection in this region. This research work provides basic data on tick occurrence and distribution in the south-west part of Bangladesh which warrants further ground investigations to be confirmed. This study was supported by Ministry of Science and Technology (MOST), Bangladesh.

P02 Seroepidemiological survey and a comparative study of tick-borne pathogens in different cattle breeds in the Barind Tract of Bangladesh

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The regional distribution of ticks and tick-borne pathogens in the Barind Tract of Bangladesh were studied during the first phase from 2002 to 2003. During this study period a total of 249 tick samples and 550 blood samples of infected or suspected animals were collected. Among the 249 collected ticks, 105 belonged to *Rhipicephalus*, 33 to *Hyalomma* and 111 to *Haemaphysalis*. In the second phase of sampling, July 2008 to June 2009, the annual distribution of pathogens was studied in five sites (Gomostapur, Shahjadpur, Nimgachi, Kahaloo. Jaipurhat). At each site the blood samples from 15 animals were randomly taken (a total of 900 blood samples during the one-year study period). In total 249 tick samples, 210 blood samples of infected /suspected animal (which cover 47 sites) and 600 blood samples of randomized samplings were tested for pathogens using PCR and RLB-PCR. Identified pathogens were *A. marginale*, *A. bovis*, *Ehrlichia* spp., *Babesia bovis*, *Theileria annulata*, *T buffeli/orientalis* group, catch all BT (*Babesia-Theileria*) and catch all AE (*Anaplasma-Ehrlichia*). *B. bovis* and *T buffeli/orientalis* group co-infection were very common in bovine blood samples. Comparing the results of infected and randomly collected blood samples from five places *A. bovis* (6%), *T buffeli/orientalis* group (91%) and catch all AE (26%) infection was higher in random samples than the infected samples 4%, 79% and 23% respectively. *A. marginale*, *B. bovis*, *T. annulata*, catch all-BT infection however increased in infected samples. This comparison clearly indicates that *Ehrlichia* spp., *T. buffeli/orientalis* group and catch all-AE infection rates gradually increased during this time period of 2002-2003 to 2008-2009. Blood samples collected from normal, non-infected cattle for the study of the seasonality of pathogens also indicate that most of the cattle are carrier of several tick-borne pathogens. This could be from the impact of climate change on numerous disease vectors including ticks and tick-borne pathogens. This study was supported by MOST, Bangladesh and KFPE, Switzerland.

P03 Seasonal activity of Ixodidae ticks on domestic livestock in north-eastern Barind area of Bangladesh

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Barind Tract is the largest Pleistocene physiographic unit of the Bengal basin, covering an area of about 7,770 sq km. The agro-ecologically Barind tract is divided into High Barind, Level Barind and North-eastern Barind. Ticks are the most important ectoparasites of livestock in Bangladesh. Annual distribution of ixodid ticks were studied over the four seasons Monsoon, Autumn, Winter and Summer in five places of north-eastern Barind area: Bochaganj, Baliadangi, Boda, Gangachora and Sadullahpur. A total of 2451 ticks from 268 cattle were collected to study the influence of seasonality in year 2013-2014. Both local breed (56.51%) and cross breed (43.49%) cattle were susceptible to tick infestation (14.42%). Cows (63.43%) were more susceptible than bullocks (36.57%). The overall tick density was always higher on cows (82.27%) than bullocks (17.82%) in the Monsoon rather than the three other seasons. Compared between the seasons, the highest tick burden recorded was during Monsoon (29.82%). Four tick species were identified: *Rhipicephalus microplus*, *R. annulatus*, *R. decoloratus* and *Haemaphysalis bispinosa*. The overall prevalence of *H. bispinosa* (47.41%) was higher than *R. microplus* (40.84%). The distribution of diverse tick species and their respective presence varied among the different sites and seasons. The lowest tick infestation occurred during summer (13.59%). The highest prevalence of *H. bispinosa* was observed in winter (58.13%), whereas *R. microplus* (44.53%) was in autumn. On the other hand, *R. annulatus* was less frequent in all seasons. In this study tick infestations increased during the monsoon and gradually decreased from winter to summer. Our preliminary observation may contribute to the increased understanding of the seasonality and ecology of ixodid ticks affecting livestock animals in the north–eastern Barind area of Bangladesh. Such information is relevant in the planning and implementation of veterinary and livestock extension programmes, and is pertinent for climate adaptation programmes. This study was supported by University Grant Commission (UGC), Bangladesh.

P04 Epidemiological survey and a comparative study of the distribution and seasonal activity of cattle (Ixodidae) ticks in the Barind Tract of Bangladesh

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Ticks and tick-borne pathogens are known to be present in Bangladesh. The regional distribution of ticks in the Barind Tract of Bangladesh has been studied, during the first phase, from 2002–2003. A total of 3,305 animals were inspected, of which 1,489 (45%) were infested by ticks. Compared between different host species, tick density was always higher (21%) in goats as compared to cattle. Female cattle were two times more infested than male cattle. A total of 20,242 ticks were collected. The highest infestation rate was always recorded in female livestock. Three different genera of ticks were identified: *Rhipicephalus*, *Haemaphysalis* and *Hyalomma*, with *R. microplus*, and *H. bispinosa* being the most abundant. Other species identified were *R. annulatus*, *R. decoloratus*, *H. anatolicum*, *H. excavatum*. The distribution of diverse ticks and their respective presence varied among different sites. The overall prevalence of *Haemaphysalis* spp. (58%) was higher than *Rhipicephalus* spp. (28%) and *Hyalomma* spp. (12%) in all study sites. Among 123 sites, *Hyalomma* spp. were recorded from only 20 sites. *Hyalomma* spp. tick infestation rate was higher (46%) than *Haemaphysalis* spp. (41%) and *Rhipicephalus* spp. (13%). *Hyalomma* spp. ticks were for the first time recorded in fewer locations, though *Haemaphysalis* spp. (69%) prevalence was higher in this area, *Hyalomma* spp. (20%) and *Rhipicephalus* spp. (5%). A total of 697 (3%) immature ticks were obtained during the field sampling. The immature ticks burden was higher in *Hyalomma* (75.47%) than *Haemaphysalis* (21.66%). In the second phase, from July 2008 to June 2009, five sites (Gomostapur, Shahjadpur, Nimgachi, Kahaloo. Jaipurhat) were selected to study the prevalence and seasonality. A total of 11,148 ticks were collected from 758 cattle. Both local and cross breed cattle are susceptible to tick infestation. Cows (76%) were more susceptible than bullocks. Young cattle (1-3 years) were much more parasitized by ticks, followed by other age groups: 3-5years, below 1 year, 5-7years. The highest tick burden was recorded in cows at Nimgachi (81%), wheras in Gomostapur it was in bullocks (42%). Overall tick density was two times higher in cows (72%) than bullocks (28%). The annual distribution of ticks varied from site to site. The prevalence of *Haemaphysalis* spp. was higher (75%) than *Hyalomma* spp. (12%) and *Rhipicephalus* spp. (5%). Immature stages (7%), both larvae and nymphs, were also collected. The species *R. microplus* and *H. bispinosa* were the most abundantly distributed all over the sites, whereas *Hyalomma* spp. were restricted to the steppe area of Bangladesh. This study was supported by MOST, Bangladesh and KFPE, Switzerland.

P05 Molecular detection and phylogenetic positioning of *Theileria* sp. in lowland tapirs (*Tapirus terrestris*) from Brazil

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The lowland tapir (*Tapirus terrestris*), a vulnerable species for extinction, is the largest land mammal from Brazil and distributed through all the five biomes in the country. Recently, the lowland tapir was reported as a host for the equid-infective *Theileria equi* by molecular amplification and sequencing of partial piroplasmid 18S rRNA gene. Recent studies have demonstrated that large fragments of the conservative 18S rRNA gene may provide phylogenetic trees with more resolutive topologies for Piroplasmida. The aim of the present study was to detect and characterize piroplasmid genetic fragments in blood samples from free-ranging tapirs from two Brazilian biomes (Cerrado and Pantanal wetlands) and to understand if the endemic horse parasite *T. equi* is occurring in tapir hosts. A total of 122 blood samples collected from 102 tapirs from the Cerrado (n=41) and Pantanal (n=61) were submitted to DNA extraction and molecular amplification of a partial fragment (800 bp) of the 18S rRNA gene by nested PCR. Out of 122 samples, 64 (52.45% CI: 43.66-61.11%) presented bands of expected size on agarose gel electrophoresis. Regarding positive samples, 16 (25% CI: 16.01-36.82%) were collected from tapirs from Cerrado biome and 48 (75% CI: 63.18-83.99%) from tapirs from Pantanal biome. Positive samples were also submitted to PCR protocols aiming to amplify additional molecular markers for Piroplasmida (hsp70, cox1, β-tubulin, and intragenic spacer 1/ITS1). Furthermore, these samples were submitted to a protocol aiming to amplify a fragment of the ema1 gene from *T. equi*. Fifteen short 18S rRNA-positive samples were submitted to sequencing to confirm sequence identity. These 15 samples were submitted to a protocol targeting a larger fragment from the same gene, with approximately 1500 bp. Sequences (1182 pb to 1518 pb) obtained from six different samples revealed identity ranging from 95.23% to 95,53% with sequences of *Theileria* sp. obtained from waterbucks (*Kobus defassa*) from Kenya and horses from Brazil, Chile, USA, and Israel by BLASTn analysis. Three obtained cox-1 sequences showed 76.09% to 77.11% identity with sequences from *Babesia bigemina* from China. Five obtained hsp-70 sequences showed identity ranging from 81.07% to 81.69% to *T. equi* obtained from Mongolia. One obtained ITS-1 sequence showed 96.55% identity with *T. annulata* from Turkey. In the Bayesian Inference (GTR+G evolutionary model with 107 MCMC and burn-in of 10%) based on 1650 bp alignment of the 18S rRNA gene and *Theileria* sp. found in tapirs from Brazil clustered separately from *T. equi* and formed a unique branch. This phylogenetic positioning was corroborated by phylogenetic inferences based on the cox1 and hsp70 genes and ITS-1 intergenic region. These findings reinforce the hypothesis that tapir-infective *Theileria* may represent a putative novel species. The consequences of infection by this putative novel *Theileria* species on tapirs' health and vectors involved in the transmission are still unknown.

P06 Prevalence and geographical distribution of ticks and tick-borne encephalitis virus in questing *Ixodes ricinus* ticks in Norway

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The tick-borne encephalitis virus (TBEV), a zoonotic flavivirus, is endemic in large parts of Norway and Eurasia and is spread via TBEV infected tick bites. The purpose of this study was to present data on the distribution of *Ixodes ricinus* and the prevalence of TBEV along the Norwegian coastline. Surveillance and risk evaluation of TBEV is an important part of the national TBE-vaccine recommendations. This is one of the largest studies in Europe that includes 47 000 questing ticks from 62 sites. In Norway, the geographical distribution of ticks and TBEV are mainly along the Norwegian coastline; from the southeast coast (~59°N) and up to Brønnøy municipality in the southern parts of Nordland County (~65°N). Few ticks were found in locations above ~66°N, and no ticks were found following survey at several locations up to 67.5°N. *I. ricinus* was the only tick species found to be present, confirmed by whole genome sequencing of the mitochondrial genome of six tick samples. The phylogenetic analysis indicated a limited phylogeographic structure for *I. ricinus* in Norway and Europe as a whole. This was based on the finding that the Norwegian *I. ricinus* sequences were clustered together in a clade with sequences from Denmark, Italy and Finland, indicating heterogeneous gene flow between different regions. The overall TBEV prevalence was 0.3% for nymphs and 4.3% for adults. The highest estimated TBEV prevalence in adult ticks was detected in Rogaland and Vestfold County, while for nymphs it was highest in Vestfold, Vest-Agder and Rogaland. The present work shows that the virus is more widely distributed in Norway than previously anticipated.

P07 The situation of theileriosis in the Republic of Armenia in 2019-2020

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Food Safety Inspection Body of the Republic of Armenia

Theileriosis is a disease transmitted by ticks and caused by intracellular parasites, which causes major economic losses to livestock. Large ruminants (LR) are most susceptible, but some species can infect small ruminants (SR). The aim of this study was to determine the general prevalence of theileriosis in the Republic of Armenia and determine at what time of year and which species are most affected. From 2019 to 2020, parenchymatous organs were collected from animals presenting with a preliminary suspicion of disease. Samples were examined in the Infectious-Invasive Research Department of Reference Laboratory for Especially Dangerous Pathogens of the Food Safety Inspection Body by microscopic examination. In 2019 a total of 167 samples were examined for suspected theileriosis (103 LR and 64 SR). In 2020 a total of 214 samples were examined (117 LR and 97 SR). Samples were collected from all 10 regions of the Republic of Armenia . In 2019, *Theileria* parasites were identified in 14 samples of LR (13.6%) and 2 SR (3.1%); in 2020, 21 LR (17.9%) and 8 SR (8.2%) were positive. A total of 381 samples were examined, of which 45 (11.8%) were diagnosed with the presence of *Theileria* in 2019-2020. Out of 45 positive cases, 35 (77.7%) were detected in LR and 10 (22.3%) were detected in SR. Most samples were collected between June-September, including 108 samples in 2019, 150 samples in 2020. Based on our results, theileriosis is prevalent in all regions of the Republic of Armenia and is more common in LR and between the months of June to September. These results will be utilized to prioritize the implementation of anti-parasitic measures and to continue surveillance in areas of high prevalence with additional samples collected from slaughterhouses to prevent missing cases of the disease. Further analysis of additional samples and use of updated methods of ELISA and PCR to identify the species present will contribute to the full knowledge of theileriosis in the Republic of Armenia and inform where control measures will be implemented.

P08 Mathematical and statistical methods for TBPs prevalence calculation in pooled ticks

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The surveillance of pathogens or their molecular markers in ticks is emerging as an alternative or additional method to animal-based or human-based surveillance of tick-borne pathogens (TBPs). Tick surveillance usually consists of collecting and testing thousands of specimens. It is not always feasible to individually test every specimen, so ticks are routinely pooled to reduce cost and improve efficiency. When pathogen prevalence is <10%, pooled testing is comparable to or better than individual testing. However, the screening of pooled samples is complicated since it is impossible to determine if a positive result is due to one or more infected ticks and different mathematical and statistical methods could be used. Through a comprehensive literature research (keywords: tick-borne pathogen*, prevalence, infection rate*, positivity rate*, pool*) in different databases all the possible approaches to calculate TBPs pooled prevalence in tick populations have been retrieved, described and compared. Sampled ticks could be screened as individual or pools (fixed or variable size). Usually larvae and nymphs are pooled and the pool size (number of specimens) depends on the type of ongoing research project. Both individual and pool screening are often applied in the same paper and a high heterogeneity in tick sample size was found among papers. Three pooled indices are mainly used: pool positivity rate (PPR), minimum infection rate (MIR) and maximum-likelihood estimates of pooled prevalence and confidence limits (EPP) calculated by frequentist method. MIR is the most widely used method followed by PPR. Less than 15% of papers used the EPP approach and only five papers used both MIR and EPP methods. PPR and MIR assume that a positive pool only contains a single infected tick, they are highly influenced by pool size and real prevalence of TBPs. The frequentist methods (EPP) provides a measurement of the uncertainty contained in the confidence interval associated with the apparent prevalence estimations instead. The difference between the point prevalence estimations of MIR and EPP is negligible in case of low TBP prevalence but, if it is greater than >0.1%, MIR can significantly underestimate the tick infection rate and EPP is to be preferred. Different TBPs could be detected in tick population of a certain area and each TBPs could be more or less prevalent depending on a wide range of factors. For this reason, EPP should be adopted to describe pooled prevalence of TBPs in case of variable pool size, unknown or high circulation of TBPs.

P09 Tick-borne zoonotic severe fever with thrombocytopenia syndrome virus in the Republic of Korea

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Severe fever with thrombocytopenia syndrome (SFTS) is caused by a tick-borne *Dabie bandavirus* in the Phenuiviridae family. The vector of SFTSV virus (SFTSV) is an Ixodid ticks, and the main vector is *Haemaphysalis longicornis*, and the virus has been detected from *H. flava*, *Ixodes nipponensis*, and *Amblyomma testudinarium*. The SFTS is an emerging infectious disease first discovered in China in 2009. In 2011, the first human infection with SFTS virus was officially reported in western China, and then reported from Japan and the Republic of Korea (ROK) in 2013. SFTS is mainly characterized by fever, leukopenia, thrombocytopenia, and elevated liver function values in human and few animal species, dog, cat and cheetah. Since 2013, total 1,497 people have been infected with the SFTS virus and 274 people have died in the ROK. The case fatality rate was 18.3% in human, it is higher than any other infectious diseases. The *H. longicornis* tick is a competent vector to transmit SFTS virus in both transovarial and transstadial modes. The SFTS virus primary infects humans and animals through tick-biting. Secondary infections have been confirmed by human to human transmission of SFTS virus, and also, reported about secondary transmission from companion animals, dog and cat, to human. According to the epidemiological study of serologic and molecular prevalence of SFTS virus from companion animals, livestock and wild animals, viral antigen and antibody were detected in 14 animal species (dog, cat, cow, goat, horse, chicken, duck, alpaca, geese, wild boar, Korean water deer, white tail deer, raccoon dog, rodents) in the ROK. These results indicate that SFTS virus is circulated in ticks and animals in natural environments. It is very important issue of the public health. The government should establish guidelines for the infection of the animal SFTS virus to ensure that there are no secondary infections of animal-related workers or guardians of companion animals.

P10 Temporal dynamics of *Anaplasma marginale* infection in calves at the wildlife-livestock interface in the Mnisi communal area, Mpumalanga, South Africa

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Bovine anaplasmosis, caused by *Anaplasma marginale*, is one of the most important tick-borne diseases of cattle in South Africa. Data collected through the Health and Demographic Surveillance System in Livestock in the study area of the Mnisi Community in the Mpumalanga Province, indicates the presence of *A. marginale*, with occasional bovine anaplasmosis cases being reported at villages close to the wildlife-livestock interface. This study aimed to investigate the infection dynamics in calves (n=10) in two areas of the Mnisi community during a 12-month period, and the diversity of *A. marginale* strains in the calves. Blood samples were collected monthly from five calves each in a peri-urban area and at a wildlife-livestock interface. A duplex real-time PCR assay confirmed the presence of *A. marginale* in all five calves in the peri-urban area from the first month, but in only two calves at the wildlife-livestock interface and only after six months. *Anaplasma* centrale was not detected in any of the calves. These results were confirmed by 16S rRNA microbiome analysis of the blood samples taken at the last time point. *Anaplasma marginale* sequences were detected in all of the calves from the peri-urban area, but only in the two calves from the wildlife-livestock interface that tested positive. The blood microbiome of nine of the ten calves also contained other *Anaplasma* species sequences. Preliminary results of *A. marginale* strain diversity as determined by *msp1α* genotype analysis revealed >50 genotypes circulating in the calves during the 12-month study period (>10 genotypes from the wildlife-livestock interface and >40 genotypes in the peri-urban area), with five Msp1a repeats that have not been previously reported. Calves in the peri-urban area were more likely to be exposed to and infected with *A. marginale* than calves in the wildlife-livestock interface, resulting in endemic stability in the peri-urban area. This finding correlates with the occasional bovine anaplasmosis cases that have been observed at the wildlife-livestock interface. Cattle in both areas might also benefit from cross-protection afforded by infection with other *Anaplasma* species. Methods of cattle management, acaricide treatment and cattle density could explain differences in exposure to *A. marginale* in the two areas. Our results revealed that most calves were superinfected by distinct *A. marginale* strains within the 12-month study period, indicating a continuous challenge with multiple strains that should lead to robust immunity in the calves and endemic stability in the area.

P11 First study on piroplasms of bats in Africa reveals the presence in fruit bats of a *Babesia* sp. closely related to *Babesia vesperuginis* in fruit bats

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Bats are one of the most diverse groups of mammals in the world and they are divided in two suborders: Yinpterochiroptera and Yangochiroptera with almost 1300 species with a worldwide distribution. During the last few years they were studied very intensively in order to detect different pathogens. Most reports focus on viruses with a zoonotic potential, but the detection of parasites and bacteria is scarce, especially in the African continent. The aim of this study was to investigate the presence of parasites (piroplasms and filarial nematodes) as well as different vector-borne bacteria in tissues of bats from three different countries across Africa. Bats were captured from six different locations from Ivory Coast, Kenya and South Africa. Collections were generally at sunset, as accidental by-catch during bird monitoring using mist-nest trapping projects and in case of samples collected from South Africa freshly dead bats. A total of 53 heart samples were collected from seven different bat families. With the PCR targeting the 18S rRNA gene, we found 3 positive samples, from which a *Babesia vesperuginis*-related sequence was successfully amplified, but we could not detect the same parasite targeting the cox1 gene. We did not find any other samples positive for vector-borne bacteria or for nematodes. To our knowledge, this is the first study reporting a *B. vesperuginis*-like piroplasm in heart tissues of bats from the genera *Epomops* and *Hipposideros* from Ivory Coast, the only positive location for this parasite.

P12 Update on the interbreeding experiments between European and north-African populations of *Ixodes ricinus* (Acari: Ixodidae)

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Ixodes ricinus is the vector of several infectious agents of medical and veterinary importance. Previous studies on this tick species have suggested a lack of genetic structure at the European level, contrasting with a marked divergence from north-African populations. Furthermore, a new species belonging to the *I. ricinus* complex was recently described, but its genetic differentiation and geographical range relative to *I. ricinus* sensu stricto is still under debate. This study was designed to assess the interbreeding capacity of European and north-African populations of *I. ricinus* under laboratory conditions, providing valuable information on the putative divergence and species isolation between these tick populations. Colonies of *I. ricinus* from Mafra (PT, Portugal) and El Jouza (TU, Tunisia) were established from field-collected ticks and maintained under laboratory conditions for three generations. F0 females and their offspring were genetically characterized by sequencing of the mitochondrial gene 16S. For intercross experiments, 30 adults from both colonies were used in each of the four possible combinations of geographic origin and gender. F1 were then reared to the adult stage. Backcrosses were performed using 60 adults of the hybrid line A (originated from the crossing of females TU with males PT) with the three parental colonies (PT, TU and hybrid line A). The results show that both intercrosses and backcrosses between Portuguese and Tunisian *I. ricinus* populations were successful and produced viable offspring under laboratory conditions. These results, together with information on morphological, genetic and biological parameters of the generated colonies, allow a better understanding of the degree of divergence between these two populations, and contribute to the clarification of their taxonomic status. Supported by FCT (project TICKGENOMI PTDC/SAU-PAR/28947/2017).

P13 Development and testing of microsatellite loci for *Ixodes ricinus* population genetics studies

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Ixodes ricinus (Acari: Ixodida) is a major vector of several infectious agents of medical and veterinary concern. This tick is present in most of Europe and North Africa, where several questions regarding the genetic differentiation of ecologically-distinct tick phenotypes remain unresolved. In 2014, the new species Ixodes inopinatus was added to the *I. ricinus* complex. Being partially sympatric and morphologically similar to *I. ricinus*, with only few genetic differences identified between the two species, its description urged the development of a new, more informative set of genetic markers to clearly distinguish between sub-populations of the *I. ricinus* complex. For this purpose, 36 microsatellite markers previously described for this tick species, and 20 new potentially amplifiable loci (PAL), identified by screening *I. ricinus* genome sequences available online with the open-source software Palfinder Galaxy Service, were tested. For genetic marker testing purposes, DNA from questing *I. ricinus* males collected from two different sites within *I. ricinus*' geographic range in Portugal (N=20, Parque Nacional da Peneda Gerês) and Tunisia (N=20, El Jouza) were used. Amplification of pre-developed markers and new PAL was optimized using a touch-down PCR protocol followed by further PCR testing with fixed annealing temperatures. For sample scoring, forward primers were either directly labelled with fluorophores or a fluorophore-tagged M13 tail was added to the PCR reaction and fragment sizes were determined by capillary electrophoresis. Twenty-nine previously described loci and 11 newly developed ones were used to genotype the 40 tick samples. Consistent sample scoring, with a reliable rate of heterozygotes were obtained for 19 loci (three newly developed). This work resumes the genotyping and quality testing results obtained for selected loci, and discusses the utility of those markers for differentiating sub-populations of the *I. ricinus* complex. Supported by FCT (Project PTDC/SAU-PAR/28947/2017).

P14 Invertebrate predators and parasitoids of the ixodid ticks in action

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Ticks (Acari: Ixodidae) are worldwide spread hematophagous parasites that are, without exception, enemies of wildlife and domestic animals as well as of humans. On the other hand, ticks also have their natural foes as they can simply turn to prey being predated by other animals. Among invertebrate predators, ants, beetles, spiders, and parasitic wasps play an important role in limiting tick populations in nature. Despite its importance, the information and knowledge of this natural anti-tick action are rather scarce and limited, mainly based on in-nature observations. The research on the potential use of pathogens, parasitoids, or predators for the tick biological control lags far behind that for plant pests. To get a better insight into this fascinating phenomenon, the condition for laboratory observations of the predatory activity of wolf spiders (Araneae: Lycosidae) and parasitoid activity of the wasp *Ixodiphagus hookeri* against *Ixodes* sp. ticks have been set up. Since *I. hookeri* causes direct tick mortality, this wasp appeared to be a promising candidate as a biological agent for *Ixodes* sp. control. However, the published experimental attempts in releasing a large number of wasps into nature reportedly showed only a temporary effect on the local tick population. In this work, the predatory behaviour of four wolf spiders (*Alopecosa accentuata*, *Alopecosa trabalis*, *Cheiracanthium mildei*, *Trochosa* sp.) against *I. ricinus*, *I. hexagonus*, and *I. persulcatus* has been investigated and documented. The other part of the work was focused on the parasitoid flying wasp *I. hookeri* (Hymenoptera: Encyrtidae), invading solely juvenile tick stages. We present results of the biological observation of the parasitoid wasp - tick interrelationships. In addition, we report a successful laboratory rearing of *I. hookeri* adults from the parasitoid eggs found in field-collected infected *I. ricinus* nymphs. Overall, our data further support the concept of the potential use of these natural predators and parasitoids in the biological control of ticks. Acknowledgement: This work was supported by the Institute of Parasitology, Biology Centre CAS covered by RVO 60077344.

P15 Improved direct detection of *Babesia bovis* and *B. bigemina* by nested PCR in asymptomatic carrier animals

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Bovine babesiosis, a tick-transmitted disease caused by the hemoprotozoans *Babesia bovis* and *B. bigemina*, results in significant morbidity and mortality of cattle and is associated with substantial economic losses in tropical and subtropical regions worldwide. Clinical signs are presented during the acute phase of infection. However, after recovery, animals are asymptomatic yet remain carriers acting as a continuous source of transmission. Due to the markedly low parasitemia of carrier animals, *B. bovis* and *B. bigemina* parasites often escape detection. An improved direct diagnosis of carrier animals can be achieved by the development and use of molecular diagnostics with improved sensitivity. To this end, two nested PCR assays based on species-specific regions of *B. bovis* and *B. bigemina* mitochondrial cytochrome b genes (cytb-nPCR) were developed and showed increased sensitivity with respect to reference protocols. In a first step of the present study, the specificity of cytb-nPCR assays against a panel of hemoparasites that potentially co-occur with *B. bovis* and *B. bigemina* was confirmed demonstrating their applicability in a wide range of geographic regions where bovine babesiosis is endemic. In a second step both cytb-nPCR assays were compared with reference nPCR and qPCR protocols (i) for their capability to detect field sampled carrier animals, and (ii) for their reproducibility when performed in different laboratories by independent operators. We show that in a panel of bovine field samples (n=100), the cytb-nPCR assays detected a considerably higher number of 25% *B. bovis* and 61% *B. bigemina*-positive animals compared to 7 and 20% *B. bovis* and 55 and 49% *B. bigemina*-positive animals when tested by reference nPCR and qPCR protocols, respectively. In addition, cytb-nPCR was found superior in the detection of carrier animals when field samples from Africa were analyzed. Finally, the *B. bovis* and *B. bigemina* cytb-nPCR assays were independently validated in a single blinded study in three laboratories of which two were situated in Argentina (Buenos Aires and Santa Fe) and one in South Africa (Pretoria). Importantly, no significant differences in the number of infected animals was observed between the three laboratories. In summary, both cytb-nPCR assays are specific and showed a higher sensitivity compared to reference nPCR and qPCR protocols as evidenced by the detection of a substantially higher number of chronically infected carrier animals in the context of different epidemiological field situations. Furthermore, a high reproducibility between laboratories could be demonstrated. (Financed by INTA projects I103 and I109).

P16 Metagenomic analysis of bacterial 16s rRNA in female *Rhipicephalus microplus* ticks from selected provinces in Luzon, Philippines

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The tick microbiome is comprised of commensal and symbiotic microorganisms that play essential role in tick physiology, and pathogens responsible for tick-borne diseases (TBDs). Characterization of the tick microbiome through metagenomic analysis can help in understanding tick symbiont interactions, identifying undiscovered pathogens, and designing effective control of TBDs. The tropical cattle tick *Rhipicephalus microplus* is a widespread ectoparasite of cattle in the Philippines, responsible for economic losses due to direct effects on animal productivity and the transmission of diseases of cattle. This study characterized the bacterial community of *R. microplus* from nine provinces in Luzon, Philippines through next-generation sequencing of 16S rRNA. Total DNA was extracted from a total of 36 partially engorged female ticks from nine provinces using a commercial extraction kit. The DNA samples were pooled per province, and then sequenced and analysed using an open-source bioinformatics platform. Overall, 498 bacterial taxa were identified with the majority detected in the provinces of Laguna, Batangas, and Cagayan. Bacteria identified in all nine provinces included *Coxiella*, *Corynebacterium*, *Staphylococcus*, *Acinetobacter*, Enterobacteriaceae, and Rhizobiales. Difference in bacterial diversity was noted among the nine provinces and two groups of provinces relatively in close proximity with each other shared similar taxa that were unique only to them. Five taxa initially considered as potential pathogens were further classified using BLAST. *Coxiella* was identified as an endosymbiont, whereas *Ehrlichia*, *Bartonella*, and *Dermatophilus* were confirmed pathogens. The characterization of the bacterial composition of *R. microplus* in this report is useful for future researches on cattle tick and TBD control strategy.

P17 Wildlife as sentinels of TBEV circulation and tick infestation in north western Italy

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Wild and domestic animals can be usefully employed as sentinels for the surveillance of diseases with impact on public health. In the case of tick-borne encephalitis virus (TBEV), the detection of antibodies in animals can be more effective than screening *Ixodes ricinus* ticks for detecting TBEV foci, due to the patchy distribution of the virus. In Piedmont region, north western Italy, TBEV is considered absent, but an increase in ticks – *I. ricinus* in particular – is being observed, and TBEV is spreading in bordering countries, e.g., Switzerland. Therefore, we have collected sera from wild ungulates during the hunting season (October-December) from 2017 to 2019 in the Susa Valley, Western Alps, and screened them for TBEV antibodies by a commercial competitive ELISA test. Moreover, we monitored tick infestation on the same animals, to evaluate tick presence and abundance in altitude. We collected 267 serum samples by endocranial venous sinuses puncture and inspected the skin of 373 carcasses, belonging to red deer, roe deer and Northern chamois. Animals were hunted in 13 different municipalities, at altitudes ranging between 750 and 2800 m a.s.l. Two tick species were collected, *Ixodes ricinus* and *Dermacentor marginatus*, with the first species being by far the most frequently detected (93.7%). Ticks infested 27.1% of the animals; in particular, red deer and roe deer culled at lower altitudes (< 1400 m a.s.l.) were significantly more parasitized than those from higher sites. However, ticks also infested 13.1% of ungulates from higher altitudes (1800-2000 m a.s.l). Serological survey on TBEV yielded negative results. Borderline results for 5 serum samples were further confirmed to be negative for TBEV by Plaque Reduction Neutralisation test. So far, our results indicate that the TBEV is not circulating in western Piedmont. However, monitoring on TBEV should continue, since TBEV and its vector are spreading in Europe. Data on tick infestation served as complementary data source to dragging for studying the current distribution of ticks at high altitudes. The wide-range distribution of wild ungulates and their role as feeding hosts, make them useful indicators of the health threats posed by Ixodid ticks.

P18 Membrane feeding as a tool for production of "bacteria-free" *Ixodes ricinus* ticks

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Ticks are obligatory ectoparasites that transmit a large number of human and animal pathogens. Apart from pathogens, ticks harbour also other bacteria of a possibly mutualistic relationship. The role of this bacterial microbiota as integral entity of ticks is still not completely understood. Various techniques have been described in the literature to achieve microbiota-free ticks with various degrees of success. To address the lack of experimental opportunities, we designed and validated a novel approach, utilising tick membrane feeding system, to produce bacteria-free *Ixodes ricinus* ticks. We can clearly see that Tetracycline-mediated clearance of bacteria profoundly inhibits proteosynthesis from tick mitochondrial genomes. To overcome this off-target effect, we screened other antibiotics and established a trans-stadial membrane-feeding protocol that leads to a production of axenic (bacteria-free) *I. ricinus* ticks (bacterial-cleared in a previous developmental stage). Our approach effectively reduces tick internal bacterial microbiota in all developmental stages, including *Midichloria mitochondrii*, an intra-mitochondrial bacterium known to bloom in the ovaries of fully engorged *I. ricinus* females. We can clearly see that axenic *I. ricinus* ticks display compromised blood-feeding physiology at various developmental stages. Our structural interaction Array Tomography analysis, in control vs. axenic ticks, nicely recapitulates the 3D model of the ovarian tissue architecture with clear intra-mitochondrial occupancy by *M. mitochondrii*. The development of the experimental procedure for production of axenic *I. ricinus* ticks, feasible in most molecular laboratory labs, opens up a whole plethora of novel experimental evaluations, including comparative metabolomics, RNA-seq, proteomics, vectorial competence, etc., which all hold high promises for a swift progress in our understanding of microbiota-tick interaction. Specifically, these will lead to description of the putative molecular systemic integration of bacterial products in the metabolism, physiology, and immunity of *I. ricinus* ticks. Acknowledgement: Supported by GACR Grant No. 22-18424M to J.P.; 19-04301S to L.Z. and P.K.

P19 The *Hyalomma marginatum* holobiont as a part of the Holis-Tiques project aiming at establishing the invasion history, the spread and associated risks

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Hyalomma marginatum is an endemic tick species in Mediterranean countries. Its area of distribution keeps expanding and has reached the South of France (region Occitanie) in 2015. *H. marginatum* is known as a vector of pathogens of interest in veterinary and human health, the most concerning *H. marginatum*-borne pathogen being the Crimean-Congo fever virus. Bacteria from genera *Rickettsia* and *Anaplasma* as well as the *Theileria equi* parasites are other pathogens *H. marginatum* is known to carry. It is within this context that reconstructing *H. marginatum* invasion history and assess associated risks has become a necessity for the tick control. The Holis-Tiques project founded by "Défi clé RIVOC région Occitanie" was built to handle this challenge. The scientific questions are spread into four work packages managed by several partner experts. Massive ticks sampling campaigns following different conditions are planned in order to provide the biological material necessary to cover the entire project. To get into the bottom of the subject, *H. marginatum* spread in Occitanie will be evaluated and mapped in time (2016-2022), and space. Key factors such as development and survival determinants -potentially explaining its invasion capacity- will be investigated according to experimental cages on the field assigned to different conditions (light, shadow, temperature, hygrometry) and will allow to feed distribution models. Moreover, potential genetic signatures associated to *H. marginatum* expansion will be highlighted by population phylogeographic and genetic studies. Since the tick is expanding, pathogen dissemination issues also raise a concern. About 40 pathogens (bacteria, virus, parasites) will be screened by the Fluidigm® technology in order to monitor pathogens distribution in space and time. On the other hand, *H. marginatum* microbiota have rarely been examined. The Holis-Tiques project will therefore assess the spatio-temporal dynamics of the *H. marginatum* microbiota. Combining all these microbial data, network analyses will be performed and studied to identify potential interactions between microbiota and pathogens on the same individual tick. Potentially positive or negative correlation patterns will help identifying how the microbiota can interfere with a given pathogen. To sum up, the Holis-Tiques projet will bring key information and knowledge about how *H. marginatum* settled in the south of France, what factors are implicated in its spread, will allow raising the sanitary risks and bring new information about the micro-organisms inhabiting the tick, potentially interfering with pathogens.

P20 Distribution and ecology of soft ticks in Southern Africa: historical review and recent data analysis

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Afrotropical ticks from the genus *Ornithodoros* are vectors of the virus of African swine fever, a hemorrhagic fever with high mortality for which no vaccine nor treatment are available. Since 2007, this disease has spread over Europe, Asia, Pacific and more recently to the Caribbean. In Southern Africa, ticks take part in the sylvatic cycle of the disease: they transmit the virus to wild suids and act as a natural reservoir for the virus. Over a century of research of *Ornithodoros* ticks in Southern Africa brought valuable information about their ecology and distribution. We started an extensive review of the literature as well as a statistical analysis of field data recently collected in Mozambique. Review of the historical literature show a large distribution of *Ornithodoros* ticks, in bush and savannah environments from the Equator Line (Gabon, Congo, Kenya) to South Africa. Those soft ticks can live in high altitude (up to 3000 meters above sea level). As endophilous ticks, they hide in caves and cracks. In the past, soft ticks of the *O. moubata* complex were frequently found in human buildings (rustic huts and pigsties). Sometimes, they were even found attached to their hosts: waterbuck, elephant, pangolin, lion, bushpig and small rodents. Many authors mentioned their presence in warthog, porcupine and antbear burrows where they can still easily be found. In Mozambique, field surveys were carried out in 2020 and 2021 in the game reserve of Coutada 9. As a result, 88 sites were investigated for the presence of soft ticks using a standardized protocol based on manual sampling. Among the 88 sites, 24 were free of ticks, whereas in the other sites tick density varies greatly (from one to more than 500 ticks sampled in a single warthog burrow). In each site, environmental parameters were registered: altitude, habitat type, vegetation, distance to water, proximity to human buildings and soil characteristics. Statistical analyses indicate that habitat type is the only variable that explains variation in tick density. Surprisingly, we found more ticks in rocky areas used by warthogs for resting than inside warthog burrows. We believe that more field work is needed to widen our dataset and register other relevant parameters such as temperature and humidity inside tick habitats. Other approaches such as population genetics may also bring valuable complementary information to understand soft tick distribution.

P21 Molecular survey of *Babesia* spp. in *Ixodes ricinus* ticks collected from humans in Romania

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Babesiosis is a world-wide disease of animals and humans being maintained in the environment by a complex transmission cycle involving different species of mammals and ticks. *Ixodes ricinus* are medically important vectors for various *Babesia* species. In Europe, most cases of human babesiosis have been associated with only few species, mainly *B. microti* and *B. divergens*. The aim of our study was to provide epidemiologic data regarding the presence of *Babesia* spp. in ticks feeding on humans in Romania. *Ixodes ricinus* ticks were collected from humans during 2013-2015 in the north-western part of Romania. Nested-PCR was used to detect 18S rDNA of *Babesia* spp., respectively sequencing for species identification. The overall infection prevalence with *Babesia* spp. in ticks was 2.9%. Two *Babesia* species were identified, *B. microti* (2.1%) and *B. venatorum* (0.8%), the last species being previously recognized as an agent of human babesiosis. Infection with these species was identified in each year with significant statistically differences during the study period. None of the larvae were *Babesia*-positive, whereas nymphs (3.4%) and females (1.2%) were infected. Our study is the first report of *Babesia* spp. infections in ticks attached to humans in Romania. The present data evinces endemic occurrence of potentially zoonotic *Babesia* spp. in Romania. Due to the lack of information on human infections with *Babesia* spp. in Romania, the implementation of molecular diagnosis methods as a routine diagnosis assay is needed. The potential improvement of screening the distribution of *Babesia* spp. in ticks collected from humans may enlighten about the infection prevalence at local, regional and national level.

P22 Emergence of *Babesia bovis* in Kenya: Implications on national strategy for tick-borne diseases control

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Among protozoan parasites in the genus *Babesia*, *B. bigemina* is endemic and widespread in the East African region, while the status of the more pathogenic *B. bovis* remains unclear despite the presence of the tick vector, *Rhipicephalus microplus* which transmits both species. Recent studies have confirmed the occurrence of *R. microplus* in Kenya, and *B. bovis* DNA has previously been detected in cattle blood in Kenya. However, no surveillance has been done to establish its prevalence. A molecular survey of *Babesia* parasites was carried out using species-specific multiplex Taqman Real Time PCR (Real Time-PCR) assay targeting two *B. bovis* genes: 18S and cytochrome b; and *B. bigemina* cytochrome b gene, on cattle blood samples collected from Kwale County, Kenya. DNA samples were further amplified using a *B. bovis* specific spherical body protein-4 (SBP-4) nested PCR and the resulting products sequenced to confirm the presence of *B. bovis*. The overall babesiosis prevalence was 25.8% (131/506). Of 131 infected animals, 87 (17.2%) were positive for *B. bovis*, while 70 (13.8%) had *B. bigemina* and 26 (5.1%) were observed to be co-infected with both *Babesia* species. There was a significant association between infection prevalence and collection site (sub-county) $p<0.0001$. Sequences of isolates identified matched to previously reported Kenyan isolates, with a similarity of 96-100%, while some shared 99-100% similarity with isolates from Benin, South Africa and Indonesia. These findings reveal high prevalence of pathogenic *B. bovis* in Kenya and a potential threat of fatal babesiosis.

P23 Detection of tick-borne pathogens in ticks feeding on dogs and cats in south-western Slovakia

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Domestic dogs and cats are frequently infested with ticks and are good sentinels for surveys of the occurrence and prevalence of tick-borne pathogens. In addition, they can carry infected ticks to human dwellings. The aim of this study was to investigate the presence of tick-borne pathogens in ticks collected from dogs and cats and the role of pets in the epidemiology of tick-borne diseases in Slovakia. Ticks were collected from dogs and cats in different areas of south-western Slovakia during 2011-2021. Ticks (1873 and 498 from dogs and cats, respectively) of four species, *Ixodes ricinus*, *Dermacentor reticulatus*, *Ixodes hexagonus* and *Haemaphysalis concinna* were collected. Part of them were examined for the presence of *Borrelia burgdorferi* s.l. (Bbsl), *Anaplasma phagocytophilum* (AP), *Babesia* spp., *Rickettsia* spp. and *Neoehrlichia mikurensis* (NM) by molecular methods. Genomic DNA was extracted from ticks using commercial isolation kits. *Borrelia*, *Rickettsia* and *Babesia* were detected by conventional PCR followed by sequencing, NM and AP by real-time PCR. Genotyping of Bbsl was done by RFLP of 5S-23S rRNA. *Ixodes ricinus* dominated and were infected with AP (16.7% and 17.5% from dogs and cats, resp.), Bbsl (7.8% and 7% from dogs and cats, respectively). *Borrelia afzelii* was the dominant genospecies, followed by *B. valaisiana*, *B. spielmanii*, *B. lusitaniae* and *B. garinii*), *Rickettsia* spp. (5.4% and 26.5%, resp. - *R. helvetica* and *R. monacensis* were identified), piroplasmids (1.7% and 1.6%, resp. - *B. microti*, *B. venatorum*, *B. canis* and *Hepatozoon canis* were identified) and NM (1.2% and 5.8%, resp.). In *D. reticulatus* from dogs, AP (6.5%), *Rickettsia* spp. (15.9%, with *R. raoultii* dominating), and *Babesia* spp. (23.8%) were detected. *Babesia canis* was identified in part of the positive ticks. *Ixodes hexagonus* from dogs and cats, resp., were infected with Bbsl (4.3% and 0%), AP (4.3% and 0.2%) and *Rickettsia* spp. (2.1% and 0.6%). In *H. concinna* from dogs, *Babesia* sp. were detected (7.4%; one identified as *B. crassa*). Results of this study confirmed the presence of several human disease agents in ticks infesting pets and show that pets can serve as sentinels in expressing the epidemiological risk for humans and domestic animals of being bitten by infected ticks, especially in urban areas of south-western Slovakia including the agglomeration of Bratislava. This study was supported by the Scientific Grant Agency of Ministry of Education and Slovak Academy of Sciences (projects VEGA 2/0137/21 and 2/0021/21).

P24 Tick infestation of free-living ungulates and their infection with Piroplasmida in south-western Slovakia

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Piroplasmida are protozoan blood parasites transmitted by ticks and include *Babesia* spp., *Cytauxzoon* spp. and *Theileria* spp. These parasites cause diseases in humans and animals with major economic consequences over the world. Ticks and wildlife are important reservoirs of Piroplasmids. In Slovakia, data on occurrence of *Babesia* and *Theileria* species in ticks and wildlife and the reservoir role of free-living ungulates for zoonotic *Babesia* spp. is limited. In this study, spleen samples from wild ruminants (Cervidae, mouflons) and wild boar, and ticks feeding on them were screened for the presence of piroplasmids by molecular methods. The samples were obtained from hunters from south-western Slovakia (regions of Bratislava, Trnava, Záhorie and Levice) between 2018 and 2021. *Babesia* and *Theileria* species were detected by polymerase chain reaction targeting a 450 bp fragment of the 18S rRNA gene. Positive amplicons were sequenced. In regions of Bratislava, Trnava and Záhorie, the ungulates were infested with *Ixodes ricinus*, *Haemaphysalis concinna* and *Dermacentor reticulatus*. None of the 186 wild boar and four mouflon spleen samples were positive for piroplasmids, while 44% (15/34) fallow deer, 100% (4/4) red deer and 52% (15/29) roe deer were positive for *Theileria capreoli*. 47.8% of the examined ticks were positive for *Babesia*/*Theileria* spp. In *I. ricinus* and *H. concinna* from cervids, *T. capreoli* prevailed, in one *I. ricinus* from roe deer *B. venatorum* was detected. In 42% of ticks (*D. reticulatus*, *I. ricinus*) feeding on wild boar *Babesia* spp. were detected and are currently analysed. Levice region differs from the other study areas by sympatric occurrence of five tick species (*I. ricinus*, *D. reticulatus*, *D. marginatus*, *H. concinna*, *H. inermis*). Red deer were infested with *I. ricinus*, *H. concinna* and *H. inermis*. From wild boar, depending on the season, all five tick species were collected. In 15 examined wild boar, no piroplasmids were detected, but in 80% (8/10) of red deer *T. capreoli* was identified. Ticks from the Levice region are being currently screened. The obtained results suggest that populations of cervids in south-western Slovakia are highly infected with *T. capreoli*. However, the vector of the parasite in the study areas remains unknown as no *T. capreoli* has been detected in questing ticks. In contrast, prevalence of the zoonotic *B. venatorum* species in *I. ricinus* is very low and its absence in ungulates raises the question if this parasite poses a risk to humans in the studied regions.

P25 Molecular prevalence, characterization and associated risks factors of anaplasmosis and theileriosis in small ruminants of northern Pakistan

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Anaplasmosis and theileriosis are important tick-borne diseases and causes considerable economic loss to livestock production in tropical and sub-tropical parts of the world. The present study was conducted in four districts of northern Pakistan to investigated the prevalence, associated risk factor and phylogenetic analysis of *Theileria* and *Anaplasma* in small ruminants. A total of 800 blood samples (200 from each district) were collected from goat (n=401) and sheep (n=399). Information regarding animals was noted in a designed questioner for the analysis of risk factors. The samples were analysed by microscopy and PCR. A PCR assay was performed by using generic primers as well as species specific primers for *A. ovis* and *T. ovis*. High prevalence of both pathogens was noted by PCR (45.12%) as compared to microscopy (23.37%). Comparatively *Theileria* (23.37%) was more prevalent than *Anaplasma* (21.75%) in infected animals. Prevalence for both pathogens were higher in sheep (28.37%) as compared to goats (16.7%). In our study *A.a ovis* (21.7%) was the only specie found, while *T. ovis* (14.4%) followed by other *Theileria* spp. (9 %) were identified, by sequencing the 18 SSU rRNA gene was confirm that *T. ovis*, *T. annulata* and *T. lestoquardi* were among the *Theileria* species infecting the animals. Univariable analysis of risk factors showed that host, age, grazing system and acaricides treatment were significant determinants ($P < 0.05$). Multivariable analysis of risk factors revealed that host, gender, age, tick infestation and grazing system were significant elements ($P < 0.005$) for both pathogens. Phylogenetic analysis revealed variants between the *A. ovis* and *T. annulata* samples analysed indicating that different genotypes are circulating in the field while *T. ovis* presented the same genotype on the samples analysed. The results in this study highlights the need for a more detail study to characterize the regional genetic diversity of *A. ovis*, *T. annulata* and *T. lestoquardi* in Pakistan to estimate the influence of commercial activities and mobility of animals, additional studies with larger sample universe are required to elucidate new variants of these pathogens in the fields of Pakistan and neighbour countries.

P26 Detection of phleboviruses in ticks in Slovenia

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Phleboviruses represent a large and heterogeneous genus within the family Phenuiviridae, order Bunyavirales. The genus *Phlebovirus* is divided into two antigenic complexes that also correspond to the main vector, either sandflies or mosquitoes and ticks. Sandfly/mosquito-borne phleboviruses include well-known human pathogens such as Rift Valley fever virus, Toscana virus, Sicilian and Naples Sandfly fever viruses, and many others, but until recently, tick-borne phleboviruses were not considered important human pathogens. After the discovery of severe fever with thrombocytopenia syndrome in China and Heartland virus in the United States, interest in tick-borne phleboviruses has greatly increased. In addition, with the development of molecular and sequencing methods, new tick-borne phleboviruses have been discovered worldwide, indicating their global distribution and great genetic diversity. Phylogenetically, tick-borne phleboviruses are divided into at least 4 groups: Uukuniemi, Bhanja, SFTS, and Kaisodi. Both Bhanja and Uukuniemi were discovered in ticks in Europe as early as the 1960s. In Slovenia, little is known about phleboviruses in ticks, thus a pilot study was conducted in 2019. We included about 8000 ticks, mainly *Ixodes ricinus*, but also some *Haemaphysalis punctata*, *H. concinna* and *Dermacentor reticulatus* ticks collected from 8 different locations in Slovenia. Depending on species, sex, developmental status and location, ticks were grouped into pools from which nucleic acid was isolated. We used the pan-phlebovirus RT-PCR assay based on degenerate primers targeting the polymerase gene fragment. Fourteen specific amplicons were generated. Phleboviruses were detected only in *I. ricinus* ticks, at 6 of 8 sites. Direct sequencing of the amplicons and subsequent phylogenetic analysis revealed a divergent phlebovirus group. Phylogenetic analysis revealed that Slovenian ticks harbor phleboviruses that cluster in two distinct groups: the Uukuniemi group and unclassified phleboviruses.

P27 Viral load and inflammatory immune response in the pathogenesis of tick-borne encephalitis

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Tick-borne encephalitis (TBE) is the most important viral infection of the human central nervous system in Slovenia. This disease is caused by TBE virus (TBEV) (family Flaviviridae, genus *Flavivirus*). Humans acquire TBEV infection mainly through tick bites and rarely through consumption of unpasteurized milk and milk products from infected livestock. The clinical manifestations of TBE are most likely the result of the virus subtype and the host immune response to infection, but knowledge is still incomplete. Phylogeographic characterization of TBEV in Slovenia revealed clear geographic clustering of the virus and a high degree of variability among patients, ticks, and rodents. We investigated TBEV viremia in patients in the initial phase and its impact on disease severity. In most samples, levels were in the range of 3-6 log copies RNA/ml. Viremia was higher in female compared to male patients, but we found no association between viral load and laboratory and clinical parameters, including disease severity. However, a weak humoral immune response was associated with a more severe disease course, suggesting that inefficient clearance of virus results in a more severe disease. In addition, we characterized the innate and adaptive inflammatory immune response in matched serum and cerebrospinal fluid samples. We have established that the inflammatory immune responses were generally site-specific. Cytokines and chemokines associated with innate and adaptive Th1 immune responses were significantly higher in CSF, whereas mediators associated with Th17 and B-cell responses were generally higher in serum. Moreover, mediators associated with innate and adaptive Th1 immune responses were positively associated with disease severity.

P28 Possible role of NS1 viral protein during tick-borne encephalitis

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Tick-borne encephalitis virus, family *Flaviviridae*, belongs to important human pathogens that infect the central nervous system. Even though there is an effective vaccine to prevent the disease, so far there is no specific therapy to treat already infected patients. Flavivirus nonstructural protein 1 (NS1) is a highly conserved protein that might serve as a therapy target. NS1 is associated with several roles during flavivirus infection. Intracellularly it acts as a cofactor in virus replication and is exported to the surface of the infected cell. In addition, it is secreted as a soluble hexamer from the infected cells into the bloodstream, where it serves as a marker of the infection. The hexameric NS1 has been shown to play important role in the pathogenesis of several medically relevant mosquito-borne flaviviruses such as the dengue virus, West Nile virus, Zika virus, and others. Dengue virus NS1 increases the permeability of endothelial cells both *in vitro* and *in vivo*, which leads to increased vascular permeability and damage to blood vessels. The damage to endothelial cells was shown to be tissue-specific for various viruses and thus potentially correlating with flavivirus pathogenesis. Neurotropic viruses, such as the West Nile virus and Japanese encephalitis virus, induced increased permeability of the blood-brain barrier by similar mechanisms to the dengue virus. Moreover, hexameric NS1 was shown to induce the production of pro-inflammatory cytokines and chemokines from macrophages, and contribute to evasion of the host immune system by assisting the degradation of complement protein C4. Although the role of NS1 in pathogenesis is characterized in more detail for mosquito-borne flaviviruses, there is no information so far about the role of NS1 in the pathogenesis of tick-borne encephalitis. Based on the results obtained for other neurotropic viruses, we hypothesize that NS1 could play a crucial role in the pathogenesis of tick-borne encephalitis, especially in the process which the virus uses to cross the blood-brain barrier as the mechanism remains not yet fully understood. As described previously for mosquito-borne flaviviruses, NS1 is an interesting target for therapeutic monoclonal antibodies without the risk of antibody-dependent enhancement, therefore the clarification of the role of NS1 in the pathogenesis of tick-borne encephalitis is an important step on the journey to targeted antiviral therapy.

P29 Development of the aptamer selection protocol against *Anaplasma phagocytophilum*

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Anaplasma phagocytophilum is a strict intracellular bacterium, difficult to isolate and study in the laboratory. As a result, many questions remain concerning the variability of strains and the mechanisms of infection and interaction with its vertebrate and invertebrate hosts. Aptamers are innovative and powerful tools in infectious diseases, both for the detection of pathogens and for the identification of innovative therapeutic and vaccine targets. The selection and application of aptamers against *A. phagocytophilum* is a real opportunity to develop a capture method for genomic studies and better understand the molecular mechanisms involved in the infection and interactions with its hosts. Currently, few studies have succeeded in selecting aptamers against strict intracellular bacteria due to the difficulty to isolate the target and also due to the complexity and time-consuming nature of the aptamer selection method (SELEX). Therefore, our first objective was to develop successful selection protocol applied to *A. phagocytophilum*. In this context, three main steps were optimized: target purification, PCR conditions and single-stranded DNA generation. This last step is crucial, as it directly affects the enrichment and the selection of potentially binding sequences. Several ssDNA generation methods have been reported for aptamer selection but very few studies have compared them, making the advised selection of a ssDNA generation method difficult. To overcome these gaps of knowledge, the quality and the quantity of the ssDNA generated were compared by the two most used methods, capture on streptavidin-coated beads and lambda exonuclease digestion, before and after purification steps. In addition, the ssDNA yields were determined by three quantification techniques (Qubit, Gel quantification and Nanodrop), allowing the comparison of their performances. The results, obtained on a rigorous basis, demonstrated both the accuracy of the gel-based quantification and the superiority of lambda exonuclease digestion compared to the capture on streptavidin-coated beads, in terms of quantity and quality of ssDNA. Our in-depth study provides solid ground for implementing essential but often overlooked steps for successful aptamers selection. Our first results are promising as a rapid and specific enrichment of potential sequences against *A. phagocytophilum* was observed by qPCR and sequencing. Our study will also help to improve the selection of specific aptamers against other intracellular vector-borne bacteria or pathogens, with their broad applications.

P30 Aptamers, an innovative tool for *Anaplasma phagocytophilum*

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Anaplasma phagocytophilum is a zoonotic and tick-borne bacterium of great interest in animal and human health. Many questions persist, particularly concerning the variability of strains, epidemiological cycles and the reservoir hosts of strains infecting farm animals, pets and humans in Europe. Furthermore, the mechanisms governing the difference in host tropism(s), including the zoonotic potential, the difference in virulence of the strains as well as those involved in the interactions with invertebrate hosts remain largely to be explored. These gaps can be explained by the difficulty to cultivate and study *A. phagocytophilum* because of its strict intracellular location, its short life span in samples and the lack of specific tools, in particular monoclonal antibodies, currently unavailable. Aptamers are innovative tools and represent a good alternative to antibodies, as these oligonucleotides are able to bind with high affinity and specificity to a wide range of targets. In this context, our team is working on the selection of DNA aptamers targeting specifically *A. phagocytophilum* and/or proteins specifically expressed by infected cells. The first step was to select aptamers with a method of enrichment by contact with the target, named SELEX (Systematic Evolution of Ligands by EXponential Enrichment). The selection process starts with the incubation of a random ssDNA library with the target, followed by PCR amplification of the binding sequences and single-stranded DNA generation. These steps have been implemented and optimised to select aptamers specific to *A. phagocytophilum*. After 12 cycles of selection, the bound sequences at each round were determined by qPCR and sequencing. Four main families were particularly interesting, as they were characterised by a rapid and specific enrichment, and are currently under study. The first binding and affinity results are promising: some sequences appear to be specific either to proteins expressed by infected cells or to *A. phagocytophilum*, and will help to better understand the interactions between *A. phagocytophilum* and its host cells. In addition, aptamers specific to *A. phagocytophilum* are a real opportunity to develop a capture method, which will allow purifying bacteria directly from blood samples for in-depth genomic studies and for diagnostic purposes in animals and humans. In this context, several objectives will be pursued: to better explore epidemiological cycles, to understand the mechanisms involved in virulence and species barriers and to identify new potential vaccine and therapeutic targets in the longer term.

P31 Occurrence of tick-borne pathogens in Carpathian National Nature Park in Ukraine

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Tick-borne diseases affect animals and humans and are spreading worldwide, including in Ukraine. Climate changes, the expansion of ixodid ticks, and anthropogenic influence contribute to the spread of pathogens to previously non-endemic areas. However, data on ticks and tick-borne pathogens in Ukraine are rather scarce. This study thus aims to investigate and compare the prevalence of *Neoehrlichia mikurensis*, *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Borrelia burgdorferi* sensu lato, and *Rickettsia* spp. in ticks collected from non-studied Alpine meadows and forests in the Carpathian National Nature Park in Ukraine. Ticks were collected from the vegetation and a total of 647 ticks were subjected to pathogen-specific PCR analyses. The most abundant among identified tick species are represented by *Dermacentor reticulatus* (78%) and *Ixodes ricinus* (22%). Ticks of the genus *Dermacentor* were rare in the high mountain areas, mostly brought there from low lands by migrating farm animals. The largest populations of ticks were observed in the foothills of the south-eastern slopes of the Carpathians, in the Transcarpathian lowlands, and in the eastern part of the Precarpathians. The average prevalence in *I. ricinus* was the highest for *Rickettsia* spp. – 31%, 27% for *Anaplasma* spp., 17% for *Neoehrlichia mikurensis*, 11% for *B. burgdorferi* s.l., 10% for *Bartonella* spp., and 2% for *Babesia* spp. For *D. reticulatus* ticks the highest prevalence was also for *Rickettsia* spp. – 48%. The prevalence of *Anaplasma* spp. was higher than in *I. ricinus* – 39%, the same as for *Babesia* spp. – 5%, but for *Neoehrlichia mikurensis* and *Bartonella* spp. the occurrence was lower – 9% and 3% respectively. Overall, there was no significant difference in the prevalence of any of the pathogens for the respective ticks among the studied locations. This study is the first to provide a prevalence of *Neoehrlichia mikurensis*, *Anaplasma* spp., *Babesia* spp., *Bartonella* spp. in natural ecosystems of the Carpathian National Nature Park and, except *Babesia* spp., demonstrated an unexpectedly high prevalence of these tick-borne pathogens among collected ixodid ticks.
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P32 Prevalence of *Babesia* sp. and *Borrelia burgdorferi* sensu lato co-infections in *Ixodes* ticks from urban environment of the city of Poznań

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The co-occurrence of infections with different pathogens can facilitate the maintenance of co-infecting pathogens in enzootic cycles. Such mechanisms have been demonstrated for bacterial co-infections of *Coxiella burnetii* and *Rickettsia phytoseiuli* as well as for *Borrelia burgdorferi* sensu lato (s. l.) and protozoan *Babesia microti*. These associations may cause diagnostic difficulties and more severe pathogenic symptoms than those induced by a single infection. Although *B. microti* as a human pathogen is a frequent research object, data regarding relationships of other *Babesia* species with bacterial agents are scarce. The aim of the study was to assess the prevalence of *B. burgdorferi* s.l. and *Babesia* species coinfections among ticks inhabiting urban areas. Two groups of ticks were collected and analysed including: (i) 1059 host-seeking *Ixodes ricinus* (30 larvae, 460 nymphs, 289 females and 280 males) collected from vegetation in seven recreational areas of Poznań, west-central Poland, and (ii) 837 engorged *Ixodes* sp. (831 females and 6 nymphs) removed from companion animals (567 dogs and 113 cats) examined in 17 veterinary clinics. The presence of *Babesia* DNA was detected by Sanger sequencing of the 18S rDNA fragment, while *Borrelia* spirochetes were identified by the V4 16S rDNA-profiling using the Ion Torrent PGM system and confirmed by Sanger sequencing of the flaB gene fragment. *Babesia* DNA was found in 14.7% (123/837) of the engorged ticks where three species were identified: B. canis (5.9%), B. microti (4.7%), *B. venatorum* (1.9%) and unidentified *Babesia* sp. (2.2%). Co-occurrence of *Babesia* and *Borrelia* DNA was found in 2.9% of the engorged ticks. The most common co-infection was *B. microti* with *B. afzelii* (1.7%). *Babesia* DNA was found in 6.7% of the host-seeking ticks including *B. canis* (3.2%), *B. microti* (2.2%), *B. venatorum* (0.6%) and *Babesia* sp. (0.75%). In host-seeking ticks 3.4% (36) were identified in co-infections, with predominance of *B. canis* and *Borrelia* sp. (1.2%). Our data indicate that the co-occurrence of *Babesia* spp. and *Borrelia* spp. in *Ixodes* ticks is relatively common which could have an impact on public health.

P33 Using spatial statistics to assess the relative risk of ticks and tick-borne disease on livestock farms in Great Britain

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Ixodes ricinus, the most widespread tick species in Great Britain, is responsible for the transmission of a range of pathogens that cause disease in livestock. Empirical data on risk is required to inform farm management strategies, but these data need to be collected consistently across time and space to allow comparison of relative risk between regions. A retrospective questionnaire survey of sheep and cattle farmers was used to assess the reported prevalence of ticks on livestock across Great Britain. A total of 7200 questionnaires were sent to farmers asking about the presence or absence of ticks on their livestock and cases of tick-borne disease in the previous year. Spatial scan statistics and kernel density maps were used to assess relative risk and spatial clustering of cases to identify areas of significantly high risk, independent of the underlying distribution of respondents. Logistic regression models were used to identify factors associated with tick presence. Tick infection risk to livestock is shown to be spatially aggregated, with areas of significantly high risk in north Wales, northwest England and western Scotland. Overall prevalence was 13% for sheep farms and 6% for cattle farms, but the prevalence of ticks on farms in 'hot spot' clusters ranged between 48–100%. The reported farm prevalence of tick-borne disease was 6% for sheep and 2% for cattle, but of farms reporting ticks, prevalence was 44% and 33% for sheep and cattle farms, respectively. Upland farming, larger flock sizes, region and the presence of sheep on cattle farms were all significant risk factors for tick presence. These data have important implications for assessing both the risk of tick-borne disease in livestock and optimising approaches to disease management. In particular, the study highlights the need for effective livestock tick control in upland regions and the southwest, and provides evidence for the importance of sheep as tick maintenance hosts in Britain.

P34 Morphological analysis of ixodid tick species collected in five provinces, South Africa

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Ticks appear to be a constraint in livestock production in tropical and subtropical regions of the world. They are causative agents of tick-borne diseases (TBDs) and directly impact animals through heavy infestations and weight loss. Different tick species have unique host preferences for the completion of their developmental stages. Moreover, biotic and abiotic factors play a crucial role in the epidemiology of ticks and TBDs. Tick identification down to the species level is beneficial as it assists in (1) Tick population studies for tick control strategies, (2) Understanding of a disease's epidemiological situation (3) Disease diagnosis for surveillance and monitoring, (4) Minimize the chances of new diseases being spread by ticks through transportation, and (5) Improving personnel's confidence and skill in dealing with tick-borne infections. This study aimed at evaluating ticks infesting bovines using morphological and molecular characteristics as key for identification. ±1893 hard ticks were collected from livestock in 23 locations throughout six provinces (Limpopo, KwaZulu Natal, Eastern Cape, Mpumalanga, Gauteng, and Free State). Initially, morphological features were used to classify these ticks into species. Using the BLAST algorithm, we were able to confirm the species' identity. After 16S rDNA amplification, all samples were positive with a size of 610 bp and had clear bands on a 2% agarose gel electrophoresis, and 250 PCR positive products were sent to the CAF DNA Sequencing Unit in Stellenbosch (South Africa) for forward and reverse sequencing using primer 16S rDNA gene. To clarify the genetic identity of hard ticks, a clustering analysis based on 16S rDNA sequences was performed using MEGA 11 to construct a Neighbour-joining (NJ). The phylogenetic study revealed the genera *Amblyomma*, *Hyalomma*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus*. Ten ixodid tick species were identified from five sample sites and these were *Amblyomma hebraeum*, *Haemaphysalis silacea*, *Hyalomma rufipes*, *H. truncatum*, *Rhipicephalus decoloratus*, *R. evertsi evertsi*, *R. exophthalmos*, *R. microplus*, *R. appendiculatus*, *R. sanguineus*. *Amblyomma hebraeum* (56%) was more prevalent followed by *R. evertsi evertsi* (13%), *Rhipicephalus decoloratus* (12%), *Hyalomma truncatum* (7.1%) and all other species were less collected (7.9%). The genus *Hyalomma* was more dominant in the Mpumalanga collection site whereas *R. evertsi evertsi* was more dominant in the Gauteng region. When analysing intra- and interspecies K3P distances, most samples revealed that intraspecific distances ranged between 0 to 0.5. Ticks were classified into species using morphological features such as tick's capitulum, the idiosoma, presence of festoons or not, palps, hypostome, short-medium or long mouth parts and presence or absence of eyes, anal groove, scutum ornate or inornate and so forth and were further confirmed through amplification of 16SrDNA. This study provides evidence that a morphological analysis alone can be so challenging and molecular confirmation is key and an accurate tool for tick identification.

P35 Synopsis of ticks of Algeria with new hosts and localities records and the first report of *Ixodes inopinatus*

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Ticks are hematophagous arthropods with great relevance to human and animal health. There are over 900 species worldwide, divided into three families: Ixodidae (hard ticks), Argasidae (soft ticks), and Nuttalliellidae. Ticks feed on various vertebrate hosts, passing through three active developmental stages (larva, nymph, adult). Depending on the behaviour of each species, they may parasitize one, two, or three hosts during a life cycle. Ticks have a worldwide geographic distribution conditioned by biotic (temperature and humidity) and abiotic (host) factors. As a result, ticks are predisposed to harbouring several types of microorganisms: bacteria, viruses, and parasites and have become incriminated in the emergence of vector-borne diseases. They play an important role from a medical, veterinary, and economic point of view. This paper presents a study conducted from January 2018 to December 2019. Based on previously published data, a synopsis was established. Results: 35 valid tick species in Algeria belonging to two families: Argasidae (3 *Argas*, 9 *Ornithodoros*, and Ixodidae (1 *Dermacentor*, 3 *Haemaphysalis*, 10 *Hyalomma*, 4 *Ixodes*, 5 *Rhipicephalus*). The geographical distribution of each species is provided; 7 new tick-host associations were recorded: 4 for *Ixodes inopinatus*, 1 for *Rhipicephalus bursa*, and, 1 for *Hyalomma marginatum* and 1 for *H. lusitanicum*. To our knowledge, this paper is the first one to report *Ixodes inopinatus* in Algeria. Moreover, this paper is the first to report all tick species (Argasidae and Ixodidae) present in Algeria. This work emphasizes the ticks-host associations and highlights the distribution of tick species across Algeria.

P36 Hard ticks of Danube Delta Biosphere Reserve and the surroundings

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The presence of hard ticks in the Danube Delta Biosphere Reserve (DDBR) and the surroundings has been studied by various authors under different approaches: their abundance, seasonal dynamics, host associations, and their importance for the public health. To date, several tick-borne pathogens, including *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and *Coxiella burnetii* have been identified from different hard tick species collected in the DDBR. The purpose of this study was to perform a retrospective analysis of tick species identified in DDBR and the surroundings, and to report new data on tick species identified during an ongoing survey. According to literature data, 20 species of ticks (out of the 28 species of hard ticks reported in Romania) were identified in the study area: *Ixodes ricinus*, *I. hexagonus*, *I. apronophorus*, *I. redikorzevi*, *I. crenulatus*, *I. arboricola*, *Dermacentor marginatus*, *D. reticulatus*, *Haemaphysalis punctata*, *H. concinna*, *H. sulcata*, *H. inermis*, *H. parva*, *Hyalomma marginatum*, *H. aegyptium*, *H. scupense*, *Rhipicephalus bursa*, *R. rossicus*, *R. sanguineus* s.l. and *R. annulatus*. Concerning the new data, 572 specimens (331 from 2018 and 241 from 2019) were collected from the vegetation and from various hosts (including, wild birds, hedgehogs, dogs, cattle, sheep, goats, wild boars, dogs), from different collection points located in the study area. During 2018-2019, eleven species of ticks were identified as follows: *H. sulcata* (n=136), *H. punctata* (n=7), *D. reticulatus* (n=63), *D. marginatus* (n=4), *R. bursa* (n=5), *R. rossicus* (n=118), *R. sanguineus* s.l. (n=83), *I. ricinus* (n=20), *I. hexagonus* (n=70), *Hyalomma* spp. (n=70) and *H. marginatum* (n=63). The majority of the ticks were collected from vegetation and wild birds. Our data adds new distribution points and new hosts associations regarding the hard tick fauna of DDBR and the surroundings.

P37 Contribution of geometric morphometry in the discrimination of tick species in Europe

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Ticks are vectors of infectious diseases of major importance in human and veterinary medicine. For epidemiological studies, accurate identification of ticks is crucial to define their potential role as vectors and to develop control and prevention strategies. Modern morphometric has enabled the quantification of size and shape variations to investigate species in anatomical structures. We therefore applied landmark-based and outline-based geometric morphometric approaches to study 250 field-collected adult males and females in France and Slovakia. This research includes four species: *Ixodes hexagonus* (n=39), *I. ricinus* (n=44), *Dermacentor marginatus* (n=71) and *D. reticulatus* (n=96). Coordinates of 16 landmarks, the contour of the external boundary of the dorsal face and the contour of the coxa1, were selected and digitized. The best scores of correct assignment, in both sexes, were obtained by the contour of the first coxa 1 (91% of correct attribution in females, 76% in males), slightly higher than for landmark technique (72% and 80%) or the contour of the body (72% and 75%) techniques. The average level of correct species recognition by the coxa1 was satisfactory, and slightly higher for females than for males. Our results show that the outlines were very effective for identifying *Dermacentor* and *Ixodes* to genus and species level as well as for separating sexes within species. Moreover, shape differences were found between *D. reticulatus* population of France and Slovakia. A molecular approach was used to validate the morphological and morphometric identification. Our results suggest that landmark-based and outline-based geomorphometric approaches could be a useful tool to allow an accurate identification of ticks at the genus and species level and deserve to be expanded.

P38 The bacterial microbiome of *Rhipicephalus sanguineus* ticks in the Mnisi community, South Africa

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Many emerging communicable diseases amongst humans can be ascribed to zoonotic pathogens arising from wildlife or domestic animals. *Rhipicephalus sanguineus* ticks are ideal vectors of various zoonotic pathogens. It is capable of parasitizing most vertebrates and is one of the most prevalent tick species on dogs, especially in rural resource poor communities (Bryson et al., 2000) such as Mnisi, a rural community in Bushbuckridge, Mpumalanga, South Africa. The community lies at the wildlife-livestock-human interface where humans are at risk of infection with various tick-borne zoonotic diseases. The aim of this study was to characterize bacterial microbiome of *Rhipicephalus sanguineus* ticks collected from dogs in the Mnisi community, South Africa, using a next-generation sequencing approach. To achieve this, we analysed the bacterial microbiome of ticks sampled from community dogs over three non-consecutive years. Ticks were collected in the Mnisi community and were kept in a humidity and temperature-controlled chamber for two days to allow them to digest their blood meal. Ten, male *R. sanguineus* ticks from each dog were surface sterilized, and dissected to remove their midguts and salivary glands and then pooled. In 2016 and 2017 27 tick pools in total were processed, while in 2019 10 tick pools were processed. Genomic DNA was extracted and PCR amplified, using universal 16S rDNA barcoded primers. Sequencing was done at Washington State University using Pacific Bioscience's circular consensus sequencing strategy. Our study detected the presence of *Anaplasma centrale*, *Anaplasma platys* as well as *Coxiella*-like endosymbionts. This highlights the role that *R. sanguineus* ticks play as a reservoir of important bacterial pathogen and symbionts. Furthermore, this study upholds the theory that *R. sanguineus* ticks harbour a *Coxiella*-like endosymbiont, that is specific to *R. sanguineus* ticks. This study also provides insight into a possible correlation between *Coxiella* and *Anaplasma*, which warrants further research.

P39 Identification and characterization of two non-canonical members of the *CCp* gene (CCp5 and FNPA) family in *Babesia bovis*

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Babesia bovis, which are transmitted by *Rhipicephalus* ticks, is the most important causative agent of bovine babesiosis, a disease that causes enormous economic losses to the livestock industry. Current methods to control bovine babesiosis have severe limitations and novel approaches, including transmission-blocking vaccines, are needed. Members of the widely conserved CCp family are multi-domain adhesion proteins containing LCCL motifs, which are differentially expressed on gametocytes of apicomplexans, including *Babesia* spp. and *Plasmodium* spp. While *Plasmodium* parasites contain 6 distinct *CCp* genes, only 3 genes (*CCp 1-3*) were previously identified in *B. bovis*. Importantly, knocking out *P. falciparum CCp* genes blocks the development of parasite stages inside the mosquito vector. In this study, we describe the identification and characterization of two novel non-canonical members of the *CCp* gene family in *B. bovis*, named CCp5 and FNPA. The genes were identified in silico by TBLASTN using *P. falciparum* CCp family domains as queries. Unlike CCp1-3, the *B. bovis* CCp5 and FNPA proteins lack the LCCL canonical domain but do contain other typical multidomain adhesion motifs which are present in classical CCp proteins. In addition, the *B. bovis* CCp5 and FNPA are in synteny with known *CCp* genes in related apicomplexans. Sequence analysis of these two proteins demonstrated high sequence conservation among *B. bovis* different isolates. Transcription, immunoblot, and immunofluorescence analyses demonstrated expression of CCp5 and FNPA in blood and *in vitro* induced sexual stages of *B. bovis*. The FNPA, in contrast to CCp5, has a predicted transmembrane domain, suggesting that it might be expressed in the surface of sexual stage parasites. Altogether, finding of this study support FNPA as a possible target of a transmission-blocking vaccine against *B. bovis*. Future studies will be focused on evaluating the functions of the CCp5 and FNPA proteins using gene knockouts. The role of CCp5 and FNPA in the formation of gametocytes, ookinetes, and sporozoites at the tick stage will also be investigated using tick artificial feeding systems.

P40 High prevalence of *Babesia aktas* n. sp. in goat in Turkiye

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Babesia species transmitted by ixodid ticks are common in domestic and wild animals in tropical and subtropical region of the world, including Türkiye, and cause clinical infections with high mortality. More than 100 species of *Babesia* have been described so far, and the discovery of new species in vertebrates continues in various regions of the world. In sheep and goats, babesiosis is associated with *Babesia ovis*, *Babesia motasi*, and *Babesia crassa*, the most important of which is *B. ovis*, which has been reported from Europe, Africa, Asia, South America, and the Far East. This study was carried out to reveal the prevalence of *Babesia aktas* n. sp., which is infective for goats and newly defined, in sheep and goats. A total of 640 blood samples collected from sheep (n=137) and goats (n=503) were examined in the study. Genomic DNA extraction was performed and nested PCR-RLB were accomplished for all samples. According to the nested PCR-RLB showed positivity in 220/640 (34.4%) of the sampled apparently healthy sheep and goats and revealed the presence of three *Theileria* and two *Babesia* species. The most abundant species identified was *Babesia aktas* n. sp. with 22.5%, followed by *B. ovis* 4%, *T. ovis* 2.8%, *T. annulata* 2%, *Theileria* sp. OT1 0.8% in goat. It was determined that sheep were infected only *T. ovis* 51.8%. In conclusion, in this study, it was determined that although *Babesia aktas* n. sp. showed a high prevalence in goats, it was not found in sheep. In the future studies, whether *Babesia aktas* n. sp. is infective for sheep, its pathogenicity in sheep and goat will be determined by experimental infection.

P41 Establishment of co-infection models in ticks and mice with two bacteria and one virus

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Nowadays, ticks and tick-borne pathogens (TBPs) are an increasing One Health problem. The main tick-borne diseases in Europe are Lyme borreliosis, granulocytic anaplasmosis and tick-borne encephalitis. Their vector is *Ixodes ricinus*, which has a wide geographical distribution and can feed on many different vertebrate hosts. It can acquire and/or transmit more than one pathogen to animals including humans at each blood-feeding stage. Tick co-infections and co-transmissions of pathogens by ticks have been demonstrated in diverse studies. For example, co-infections in ticks are common in the wild. In humans, a study of patients with chronic Lyme disease (confirmed by medical diagnosis) has demonstrated that among them 23.5 % of the patients show at least one co-infection with other tick borne pathogens like *Babesia*, *Bartonella*, *Ehrlichia*, and *Anaplasma* and 30% report two or more co-infections with those pathogens by laboratory diagnosis. The outcome of these co-infections and co-transmissions deserves to be considered with great interest because they are likely to lead to synergy and/or competition between pathogens. Those interactions could have consequences for individual pathogen fitness in mammalian hosts. In particular, individual infection rates can be reduced if pathogens directly compete for resources or via toxin production. Conversely, the down-regulation of the host immune response can result in an increased pathogen burden and facilitate transmission from host to vector. Thus, these interactions can potentially have a major impact on public health, both clinically and in terms of therapeutic applications. The small number of co-infection or multiple infection models to study the complex interactions between TBPs in co-infected ticks, and co-infected vertebrate hosts prompted us to establish co-infection models using two bacteria (*Borrelia afzelii* and *Anaplasma phagocytophilum*) and a virus (Tick-Borne Encephalitis Virus (TBEV)) in ticks (*I. ricinus*) and mammals (mice). Ticks were co-infected using different techniques (artificial feeding system, capillary feeding and micro-injection), whereas the mice were co-infected by inoculation of the different pathogens (intraperitoneal/subcutaneous/intradermal). Our preliminary results obtained: (i) in mice, single and co-infection with TBEV and *B. afzelii* were established using different concentrations/amounts of each pathogen; (ii) in ticks, single infections and co-infection were set up using an artificial feeding system with *B. afzelii* and TBEV; (iii) in ticks, single infections and co-infections were set up by capillary feeding and microinjection by *B. afzelii*, TBEV and *A. phagocytophilum*. These preliminary results are the first steps to studying the transmission success of these pathogens from co-infected ticks to non-infected mice and from co-infected mice to non-infected ticks.

P42 Interactions between tick-borne encephalitis virus and host cytoskeleton differs in human and tick cells

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Tick-borne encephalitis virus (TBEV) is a flavivirus causing one of the most important tick-transmitted neurological diseases in human. Due to the absence of specific antiviral treatment, a detailed knowledge of host molecular factors that take part in complex virus-host interactions is crucial. The cytoskeleton has been repeatedly mentioned in the context of flaviviral infections, however, its role seems to vary significantly, depending on the virus, virus strain, and/or cell type being used for the experiment. Since most of the cytoskeleton-related research has been focused on mosquito-borne flaviviruses in mammalian host cells, we investigated molecular interactions between TBEV and cytoskeleton of human neuroblastoma cells as well as tick cells to address potential differences between these two important host/vector environments. Using specific pharmacological inhibitors, we showed that in both cell lines, TBEV efficient replication relies on intact integrity and dynamics of both microtubules and actin filaments. Moreover, inhibition of motor proteins cytoplasmic dynein and myosin II led to a significant decrease in virus titre, suggesting their potential involvement in TBEV intracellular transport. Using immunocytochemistry, we revealed certain structural changes during a later stage of the infection in human neuroblastoma cells, but not in tick cells. Given the character of the changes, including disruption of actin filaments in neurites, they most probably indicate cell injury that could be related to neurological symptoms in TBE patients. In addition, we also examined expression of various cytoskeletal and cytoskeleton-related genes upon the infection. TBEV infection in human neuroblastoma cells induced possibly compensatory up-regulation of genes for actin and spectrin, both essential for neurite structure. Interestingly, TBEV-infected tick cells showed the opposite trend in down-regulation of actin and talin genes. Our results offer a systematic comparison of TBEV-cytoskeleton interactions in human and tick cells, and suggest protein targets for future research of this important human pathogen.

P43 Detection of *Hepatozoon* parasites in wild rodents of Central Europe

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Canine hepatozoonosis caused by *Hepatozoon canis* is an emerging disease in Europe. Clinical signs are usually non-specific and vary from subclinical to life-threatening manifestations. Ticks, fleas and other arthropods, such as mites and lice, act as definitive hosts, and dogs become infected by ingesting these parasite-bearing ectoparasites. The present study was aimed at analyzing the *Hepatozoon* species found in wild rodents of Hungary and Slovakia, acting as potential parasite reservoirs. DNA was extracted from spleen and lung samples of rodents and infesting parasites and a region of the18S rRNA gene was PCR-amplified and sequenced. Two closely related genetic variants, named *Hepatozoon* sp. BV1 (AY600626) and *Hepatozoon* sp. BV2 (AY600625) were detected in *Myodes glareolus* (bank vole). Furthermore, *Hepatozoon* sp. BV1 was also detected in the flea species *Ctenophthalmus agyrtes* and *Megabothris turbidus*, collected from these rodents. Phylogenetic analysis demonstrated that 18S rRNA variants BV1 and BV2 correspond to previously described genotypes UR1 and UR2 of *H. erhardovae*, respectively. In addition, a different 18S rRNA sequence was identified in lung and spleen samples of the rodent *Apodemus flavicollis* (yellow-necked mouse), which showed an identity of only 99.32% with a sequence of *Hepatozoon* sp. (KX4536361) from an Arabian snake. This finding strongly suggested that it is a new, hitherto unknown isolate, here designated SF1. Phylogenetic analysis confirmed this assumption, since the SF1 sequence fell into an independent, strongly supported branch, found to be associated with *Hepatozoon* species infecting rodents. In summary, analysis of 18S rRNA sequences derived from fleas and rodents and their comparison with the GenBank database revealed the presence of the two genetic variants, *Hepatozoon* sp. BV1 and BV2, which coexist and which were previously detected in isolates from Spain, Slovakia, Hungary and Poland. The BV1 variant was also detected in fleas parasitizing the sampled rodents, indicating that they represent the definitive host. Finally, in the rodent *A. flavicollis*, a putative novel species has been detected as evidenced by molecular phylogenetic analysis. *H. canis* was not detected in this study suggesting that wild rodents are not reservoirs for this parasite (Financed by the INTA project I103 and I109).

P44 Can passive "Citizen Science" be used to monitor tick-human interaction?

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Monitoring tick-human interactions is fundamental for assessing potential transmission rate of tick-borne infections and their associated public health impact. This is however associated with considerable challenges, as individuals often are unaware that they have been bitten by ticks, and even when they are aware of this, they would be unlikely to report a frequently occurring 'insignificant' event. Citizen Science has increasingly been suggested as a solution to such monitoring-challenges. A number of mobile phone based tick monitoring solutions have been launched. The precision and spatial bias of the information will however depend on the size of the user-base and its distribution. Maintaining the base of informants such that the monitoring continues, may be a challenge and the long-term viability of these monitoring schemes remain uncertain. Using records of web-search-term frequencies represents an alternative form of information, which may be classified as passive "Citizen Science". The strength of this approach lies in the sizable base of informants, which are recruited continuously. We here looked at web search trends in the period 2007-2019 using Google Trends. Specifically, the credibility of Internet search records by investigating the temporal and geographic characteristics for Danish search terms synonymous with "tick(s)" and also assessed how search-frequencies varied with temperature and precipitation month-by-month across the European continent by looking at search trends in nine different European countries. Our findings point to significant limitations in the records due to changes in search-term preferences over the given years. However, the seasonal dynamics are comparable among search terms. Moreover, the seasonal pattern in search terms vary across Europe in tune with changes in temperature and precipitation. We conclude that, the within-year variation for given search-terms provide credible information, which systematically vary with local weather patterns such as we would expect when searches results from tick encounters. Thus using records of web-search term frequencies data might be useful as proxy for tick–human interactions.

P45 Isolation and characterization of novel relapsing fever borreliae from world-wide distributing *Ornithodoros capensis*

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Borrelia are arthropod-borne spirochetes that are classified into Lyme disease borreliae, reptile-associated borreliae, and relapsing fever borreliae. Some relapsing fever borreliae cause human diseases, which are characterized by recurrent febrile episodes and spirochetemia. Relapsing fever borreliae were divided into two groups: soft tick-borne relapsing fever borreliae and hard tick-borne relapsing fever borreliae. From the 1950s, soft tick-borne relapsing fever had not been reported in Japan. However, in 2006-2008, we detected relapsing fever *Borrelia* sp. from *Ornithodoros sawaii* collected from nest of streaked shearwater in Japan. In this study, we isolated two strains of *Borrelia* sp. from *Ornithodoros sawaii* and *Ornithodoros capensis* collected from nest of seabirds. By experimental mice infection, novel isolated *Borrelia* sp. showed bacteremia in BALB/c and C3H mice. Although, two strains were isolated from geographically distant area and different host birds, the genotypes were highly conserved by Multi locus sequencing typing analysis. From phylogenetic analysis of house-keeping genes, these strains were related with North American strains of soft tick-borne relapsing fever, *Borrelia turicatae*. In this study, *O. capensis* were mainly collected from an active volcanic island, where has been in eruption from 2013. In addition, genetic diversity of ticks collected from newly formed lava plateau were higher than *O. sawaii* or *O. capensis* collected from another island. The suspected blood feeding host of *O. capensis*, collected from volcanic island, were genus *Sula* spp. The *Sula* spp. and *O. capensis* was distributed worldwide, from Asia to Africa. Therefore, ticks and also bacteria are thought to have migrated with seabirds.

P46 Development of microsatellite markers for *Ornithodoros phacochoerus* (Acari: Argasidae) in the context of the international NifNaf project

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African swine fever (ASF) is a hemorrhagic fever with high mortality in domestic pigs, and for which no vaccine nor treatment are available. Afrotropical soft ticks from the genus *Ornithodoros* are vectors of the ASF virus in the sylvatic cycle of the disease in which they transmit the virus to wild suids (warthogs and bushpigs). Contrary to domestic pigs, wild suids do not present any clinical sign when infected. Understanding how ASF virus circulates between wild suids and domestic pigs, and how soft ticks are involved, is of primary importance to predict future ASF outbreaks in Southern African countries. This is the main objective of the international NifNaf project founded by the United States Department of Agriculture (USDA). In this poster, we will present how the NifNaf project stimulates research on the role of soft ticks in the transmission of African swine fever between the sylvatic and the domestic cycles. We will briefly present the research objectives of this project in South Africa, Mozambique and Madagascar as well as the international collaborators. Then, we will focus on one objective of the project: investigating the genetic structuration of *Ornithodoros* populations in Mozambique to characterize soft tick migration. During years 2020 and 2021, around 75 warthog burrows were sampled for *Ornithodoros* ticks in the Coutada 9 reserve in Macossa district, Mozambique. A total of 20 burrows were selected for population genetics analysis and the DNA of 30 ticks was extracted for each site selected. After species identification as *Ornithodoros phacochoerus*, microsatellite markers development started using the only available genomes, from *Ornithodoros moubata* and *Ornithodoros porcinus*. In this poster, we will present the tick samplings performed in Mozambique, the development of microsatellite markers in CIRAD, France, and the objectives of the population genetics study that will use them.

P47 Exploring the eukaryotic microbiome in ticks

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Ticks are important vectors of various pathogens, including viruses, bacteria, and protozoa, to humans and animals and also carry nonpathogenic microorganisms, some of which are in a symbiotic relationship with ticks or have an influence on the pathogenic microbial burden of ticks. Thus, knowledge of the tick microbiome is important for understanding the physiology of ticks and their interactions with pathogens. Recently, the bacterial and viral microbiomes of ticks have been explored using next-generation sequencing (NGS) technologies. However, the eukaryotic microbiome of ticks remains unclear even if transmitting eukaryotic pathogens such as apicomplexan parasites. To explore the eukaryotic microbiome of ticks, amplicon analysis using NGS can be a useful strategy but the universal PCR for eukaryotic 18S rDNA amplifies tick DNA at high abundance, resulting in lower resolution of the microbiome. Here, we developed new methods to selectively amplify microeukaryotic DNA by blocking the tick DNA amplification using blocker nucleic acids (PNA and LNA). In addition, another PCR specifically amplifying non-metazoa, reported as UNonMet-PCR, was also employed to assess its usefulness in tick eukaryotic microbiome study. Illumina sequencing libraries were prepared from amplicons of conventional universal PCR, PCRs using PNA or LNA blockers, and UNonMet-PCR using 17 DNA samples of field-collected ticks and run on Illumina Miseq. The relative abundances of the eukaryotic microbes and the alpha diversity were estimated subsequently, and the differences of detected taxa among the methods were determined by linear discriminant analysis effect size (LEfSe) analysis. As a result, almost all sequences obtained by conventional universal PCR were derived from ticks, whereas the relative abundance of non-tick reads and alpha diversity were rich upon PCR using blockers and UNonMet-PCR. The richness was the most pronounced in PNA-based blocking PCR, of which the abundance of the non-tick reads was 178-fold more than conventional universal PCR on average. Based on the LEfSe analysis, the detection rates of Alveolata and fungi were significantly abundant in both blocking PCR and UNonMet-PCR, while those of other eukaryotes and unclassified sequences were significantly abundant only in blocking PCR. Also, several sequences of Apicomplexan parasites including gregarines, which are common parasites of arthropods, were detected by both blocking PCR and UNonMet-PCR. In conclusion, the developed PNA-based blocking PCR enabled the pan-eukaryotic analysis, and UNonMet-PCR was suitable for the detection of apicomplexan parasites and fungal communities in ticks. We expect the application of these methods to improve our understanding of the tick microbiome.

P48 *Coxiella burnetii* in ticks in Çorum Province, Turkey

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Ticks are important vectors of various pathogenic protozoa, bacteria and viruses causing morbidity and mortality in humans and animals worldwide. Turkey is a suitable area in terms of circulation of tick-borne pathogens, and Çorum is endemic area for important tick-borne diseases such as Crimean Congo hemorrhagic fever virus. Q fever is a zoonotic disease caused by the rickettsial organism *Coxiella burnetii*. The aim of this study was to reveal the prevalence rate of *C. burnetii* in ticks in Çorum province of Turkey. A total of 144 ticks were collected from dogs, and from the field by flagging methods between 2017-2019. They were morphologically identified as *Ixodes kaiseri*, *Hyalomma* spp., *Rhipicephalus turanicus*, *Hyalomma aegyptium* and *Haemaphysalis parva*. *C. burnetii* was analyzed by using nested PCR with sets of primers targeting the gene encoding the OMP and sequence analysis. Only 3 (1♂, 2♀) *Hae. parva* ticks collected from dogs were found *C. burnetii* DNA. Based on this data, it is considered that *Hae. parva* ticks on dogs are likely higher risk in terms of Q fever compared to host-seeking individuals and other tick species in Çorum.

P49 Tick-borne encephalitis virus infection (TBEV) in milk and milk products from domestic ruminants in Europe: a systematic review

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Tick-borne encephalitis virus (TBEV) is a Flavivirus responsible for one of the most important zoonoses in Europe, whose incidence is increasing. Although tick-borne encephalitis (TBE) is a vector-borne disease and is mainly transmitted to humans through the bite of infected ticks, it can also be contracted through the consumption of raw milk and dairy products from viremic domestic ruminants. We conducted a systematic review to assess the prevalence of TBEV in milk and milk products in Europe, and to evaluate the usefulness of monitoring TBEV infection in dairy products for the early identification of the viral circulation. Following protocol registration (PROSPERO: CRD 42021279317), a comprehensive search was performed in three databases (Medline, Embase and CAB Abstracts) to identify relevant publications. Screening, data extraction and critical appraisal was conducted independently by two reviewers. TBEV prevalence was calculated using the number of milk or milk product samples tested for TBEV RNA or specific anti-TBEV antibodies, and number of samples testing positive. A narrative synthesis was performed.381 articles were identified from the searches, of which 52 were selected for full-text screening, and 11 articles were finally included in the review. 34 studies were extracted (28 on milk and 6 on cheese), of which the sample size ranged from a single sample (7 studies) to 1363 samples. In milk, studies with larger samples (N≥29; corresponding to the sample size needed to detect at least one positive sample, with an expected prevalence of 10%, and 95% confidence level) had a median prevalence of infection of 4.5% (range 0% to 20.7%). Overall, 19.2% of cheese specimens were positive (all studies combined). Epidemiological surveillance of TBEV in field ticks and wild vertebrate hosts can be challenging, due the focal nature of TBEV occurrence and to the specific expertise required and limits in laboratory tests. Our systematic review showed that surveillance on milk and milk products from grazing domestic ruminants could be a valuable tool for studying TBEV prevalence and assessing the epidemiological situation in a geographic area. Dairy products can be easily obtained and their testing can be helpful for risk assessment and for the epidemiological surveillance of TBE in a One Health perspective.

P50 Tick species characterization and *Anaplasma* spp. detection in different areas of a Sicilian Natural Park (South Italy)

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Ticks are obligate blood-sucking ectoparasites able to transmit several pathogens to animals and humans. Bacteria belonging to *Anaplasma* genus are among the most important tick-borne pathogens of domestic and wild animals. The study concerned Ixodidae ticks infesting cattle grazing in different areas of the Nebrodi protected area, in Sicily (South Italy), to investigate tick ecology and *Anaplasma* spp. prevalence. The park is the largest Sicilian protected area and it is characterized by different microclimates. Cattle breeding is one of the main economic activity in the area. Sampling was carried out from September 2017 to October 2018 in three different steps. In September 2017, a pilot herd was sampled and the encouraging results led to continue. In July and August 2018 (summer sampling), the grazing areas of 20 herds from different longitudes, latitudes and altitudes inside the Park were sampled. In October 2018 (autumn sampling), the sampling activity took place in the same herds, but with grazing areas differing from the previous ones, as the animals returned from summer transhumance. Ixodidae ticks collected from cattle were morphologically identified and submitted to different PCRs targeting 16S rDNA of *Anaplasma* spp., msp4 gene fragments of *Anaplasma marginale*, *A. ovis* and *A. phagocytophilum* and to a Real Time PCR targeting msp1β gene fragment of *A. marginale*. Totally, 505 ticks were collected from 163 cows. During the three sampling phases, *Rhipicephalus bursa* was the main species collected (35.4%), followed by *Ixodes ricinus* (27.9%), *Haemaphysalis punctata* (13.7%), *Hyalomma marginatum* (12.3%), *Hyalomma lusitanicum* (4.5%), *Rhipicephalus sanguineus* (3.8%), *Dermacentor marginatus* (1.6%), *Rhipicephalus annulatus* (0.8%). Molecular analysis has shown a prevalence of 11.1% of *Anaplasma* spp., 0.79% of *A. marginale*, 0.19% of *A. phagocytophilum*. Detection of *Anaplasma* spp. infection in *Haemaphysalis*, *Hyalomma* and *Dermacentor* ticks suggests that these genera can be involved in maintenance and/or transmission of the pathogen in Sicily. The survey expands the knowledge about the eco-epidemiology of *Anaplasma* spp., according to the distribution of tick species, habitats and seasons in an area characterized by a great diversity of tick species and representing one of the few habitats suitable for *I. ricinus* in Sicily. Moreover, all the identified tick species are potential or already recognized vectors of animal/human pathogens. Obtained results can support management and prevention actions by the park management bodies and local health authorities. Thanks to Dr. Massimo Geraci and Dr. Nicola Vaneria of the Nebrodi Park for their collaboration.

P51 *Coxiella burnetii* outbreaks in sheep farms and ticks in Sicily (Southern Italy)

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Q fever is a widespread disease caused by *Coxiella burnetii*, an obligate gram-negative intracellular bacterium infecting humans, wild and domesticated animals. It is considered a vector-borne disease, but the role of ticks in transmission has not fully been clarified yet. Inhalation of contaminated aerosols is considered the main transmission route. This study aimed at the investigation of a potential outbreak in two Sicilian herds holding some sheep with watery eyes. The farms were located in western Sicily at a distance of about 4 kilometers from each other. Conjunctival swabs were collected from two farms, (A and B), and subjected to differential diagnosis against different etiologic agents. Subsequently, blood samples, individual and bulk milk samples were collected from the two herds. Ticks (if present) were collected from the animals of the two herds and morphologically identified under a stereomicroscope. A Real-time PCR, targeting *C. burnetii* insertion sequence IS1111 gene, was carried out on conjunctival swabs, blood, ticks and milk samples. Anti-*C. burnetii*-antibodies were researched in serum and milk samples by ELISA. *Coxiella burnetii* DNA was detected in 2/3 and 8/8 conjunctival swabs from Farm A and B, respectively. In the Farm A, *C. burnetii* DNA was detected in 9/126 (7.1%) blood samples, 8/40 (20%) milk samples and in the bulk milk. Anti-*C. burnetii* antibodies were found in 97/126 (77.0%) sera and 37/40 (92.5%) milk samples and in the bulk milk. All the 40 collected ticks were *Rhipicephalus sanguineus* s.l. and tested positive for the pathogen. In the farm B, *C. burnetii* DNA was detected in 11/293 (3.8%) blood samples, 28/71 (39.4%) individual milk samples and in the bulk milk. Anti-*C. burnetii* antibodies were detected in 157/293 (53.6%) sera and 52/71 (73.2%) milk samples and in the bulk milk. The study identified two *C. burnetii* outbreaks in Sicilian sheep farms, allowing for timely treatment of the disease and preventing further infection spread.

P52 Genetic diversity of *Theileria orientalis* from cattle in Kyrgyzstan

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Bovine theileriosis caused by *Theileria orientalis* is generally benign, but some genotypes can cause fatal disease and economic losses to the cattle industry. Chitose (Type 1) and ikeda (Type 2) genotypes have been reported to cause significant morbidity and mortality. Genetic variables of the major piroplasm surface protein (MPSP) expressed on piroplasm surface inside *T. orientalis*-infected erythrocytes are considered to be associated with variation in the pathogenicity of T. orientalis. The aim of our study was to determine the different genotypes of *T. orientalis* from cattle in Kyrgyzstan. Blood samples were collected from eight villages, and *T. orientalis* positive 149 samples were analyzed by amplifying the *MPSP* gene region by PCR. As a result of the single-strand conformation polymorphism (SSCP) analysis, samples with different band profiles were subjected to sequence analysis and genotypes were determined. *T. orientalis* genotype-specific PCR was performed to determine the mix genotypes. Type 1 (chitose) and type 3 (buffeli) were found positive as a single infection in 2% and 26,2% of the samples, respectively. In addition, apart from single infections, type 1-3 mix infections (71.8%) were determined. Type 3 was the most prevalent *T. orientalis* genotype. Phylogenetic analysis of the *MPSP* gene demonstrated that multiple genotypes of *T. orientalis* were circulating in the local cattle population, with pathogenic *T. orientalis* genotypes being detected for the first time in Kyrgyzstan. The geographical distribution and different genotypes of *T. orientalis* in cattle could be further studied by increasing the number of samples.

P53 Ticks and wild small mammals in Zoological garden Brno, Czech Republic – reservoir of agents with zoonotic potential?

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In zoo, there is a diverse collection of exotic animals living in small area with high concentration of different infectious agents. Rodents are reservoirs of tick-borne pathogens causing zoonotic diseases and ticks play an important role spreading of zoonoses in nature but also in captive animals. The aim of this study was to monitor selected agents with zoonotic potential in reservoirs and vectors in zoo, and to draw attention on the risk of possible contact with these pathogens. In total, 117 wild rodents and 166 ticks were collected in area of zoo Brno. Heart rinses of rodents were examined by modified enzyme-linked immunosorbent assay to detect antibodies to *Coxiella burnetii*, *Francisella tularensis*, and *Borrelia burgdorferi* s.l. Antibodies to *Leptospira* spp. were detected by microscopic agglutination test in heart-printing of rodents. Antibodies to *C. burnetii*, *F. tularensis*, *B. burgdorferi*, and *Leptospira* spp. were detected in 17%, 4%, 15%, and 6% of rodents, respectively. Prevalence of *C. burnetii* statistically differed according to the years of trapping. Parasites *Toxoplasma gondii* and *Encephalitozoon* spp. were detected by PCR in brain tissue of 16% and 20% of rodents, respectively. Bacteria *B. burgdorferi* s.l., *Rickettsia* sp. and *A. phagocytophilum* were detected by PCR in 16%, 6% and 1% of ticks, respectively without coinfection. Sequences of four samples showed homology with *Rickettsia helvetica* and sequence of one sample shoved homology with *A. phagocytophilum*. The results of our study showed that wild small mammals and ticks in zoo are reservoirs of infectious agents that are circulating in zoo.

P54 Ticks in Brno (Czech Republic): 7 different pathogens proven!

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In this study, we monitor the activity of *Ixodes ricinus* ticks in three park localities in the city of Brno and at one locality 100 km from Brno in 2018-2019. Sampling was carried out by flagging at weekly intervals throughout the growing season, including measurement of meteorological conditions at the sites. The prevalence of selected pathogens in the collected ticks was also studied. For this purpose, PCR analysis involving up to 4 pathogens at a time was carried out. The presence of the following pathogens was analyzed: *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, *Coxiella burnetii*, *Ehrlichia chaffeensis*, *Francisella tularensis*, *Babesia* spp., and *Toxoplasma gondii*. Furthermore, Real Time PCR for *Toxoplasma gondii* and dark field microscopy method in selected samples were used. The following results were obtained: (1) Brno – near reservoir in Rakovec (704 ticks collected): *Borrelia burgdorferi* s.l. 10.23%, others weren´t tested; (2) Brno – park near the housing estate Líšeň (197 ticks collected): *Borrelia burgdorferi* s.l. 13.2%, *Toxoplasma gondii* 5.0%, Babesia spp. 3.0%; (3) Brno – park near housing estate Pisárky (236 ticks collected): *Borrelia burgdorferi* s.l. 6.6%, *Anaplasma phagocytophilum* 3.4%, *Coxiella burnetii* 0.4%; (4) natural locality near Uherský Brod, 100km from Brno (333 ticks collected): *Borrelia burgdorferi* s.l. 9.3%, *Anaplasma phagocytophilum* 1.8%, *Ehrlichia chaffeensis* 0.9%, *Francisella tularensis* 0.3%. For the population of the city of Brno, there is a possibility of infection by several pathogens causing zoonotic diseases. The presence of *Toxoplasma gondii* in the samples of the *Ixodes ricinus* vector was unexpectedly detected in this area.

P55 Does *Borrelia burgdorferi* sensu lato influence tick behavior?

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Numerous studies indicate that pathogens may modify the behavior of infected animal, which may have epidemiological consequences. Manipulating animal behavior by pathogens represents one of the most fascinating issues in zoology. For example, it was proven that animals infected by tick-borne encephalitis virus showed higher activity and tolerance to the DEET-containing repellents (N, N-diethyl-meta-toluamide, a frequently used active ingredient in arthropod repellents). In our experiments the moving-object-bioassay was used to study repellent efficiency on the *Ixodes ricinus* nymphs captured in the suburban park Pisárky Brno, Czech Republic. Five selected commercial repellents based on DEET (N, N-diethyl-3-thylbenzamide) showed statistically different effects on the non-repellent control group. After this and as well as other studies with repelents we hypothesized that the presence of *Borrelia burgdorferi* sensu lato in the nymphs of *Ixodes ricinus* ticks collected in the same locality Pisárky Brno affects their response to the essential oil extracted from *Curcuma xanthorrhiza*, a substance with potentially repellent properties. A sample of 50 nymphs, on which the repellent effect of essential oil (0.005 mg/cm^2) was tested, was subsequently analyzed for the presence of *Borrelia burgdorferi* sensu lato by the PCR method. Based on the statistical evaluation of the results (Chi-square, $p = 0.31844$; Fisher's exact test, $p = 0.28153$), it can be concluded that the link between the presence of *Borrelia burgdorferi* sensu lato in ticks and their response to the repellent formulation was not proven.

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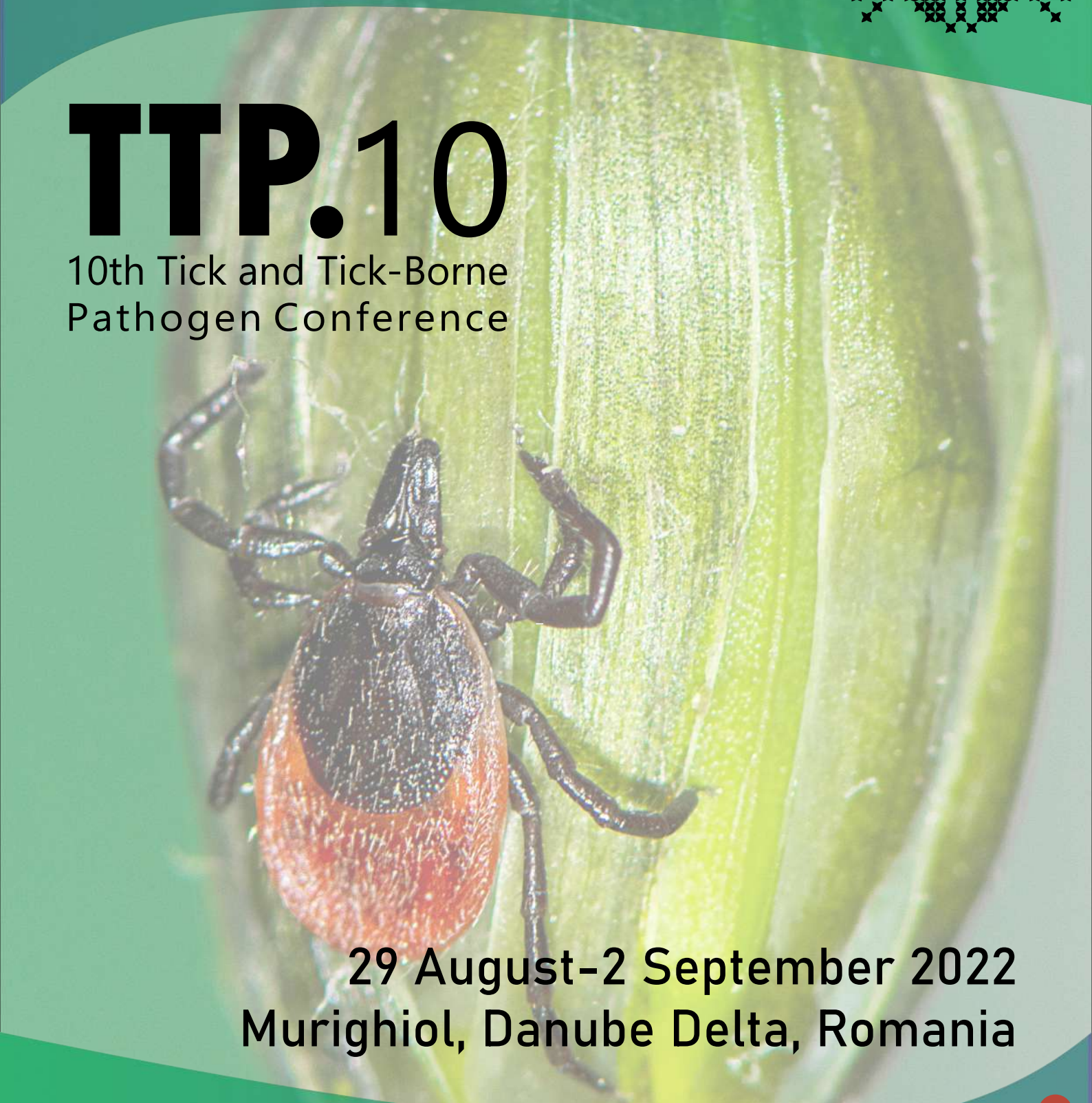

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