Screening for multiple tick-borne pathogens in Ixodes ricinus ticks from birds in Denmark during spring and autumn migration seasons

Presently, it is uncertain to what extent seasonal migrating birds contribute to the introduction of ticks and tick-associated pathogens in Denmark. To quantify this phenomenon, we captured birds during the spring and autumn migration at three field sites in Denmark and screened them for ticks. Bird-derived ticks were identified to tick species and screened for 37 tick-borne pathogens using real-time PCR. Overall, 807 birds, representing 44 bird species, were captured and examined for ticks during the spring (292 birds) and autumn migrations (515 birds). 10.7% of the birds harboured a total of 179 Ixodes ricinus ticks (38 ticks in spring and 141 in the autumn) with a mean infestation intensity of 2.1 ticks per bird. The European robin (Erithacus rubecula), the common blackbird (Turdus merula), and the common redstart (Phoenicurus phoenicurus) had the highest infestation intensities. 60.9% of the ticks were PCR-positive for at least one tick-borne pathogen. Borrelia DNA was found in 36.9% of the ticks. The Borrelia species detected were B. spielmanii (15.1%), B. valaisiana (13.4%), B. garinii (12.3%), B. burgdorferi s.s. (2.2%), B. miyamotoi (1.1%), and B. afzelii (0.6%). In addition, 10.6% and 1.7% of the samples were PCR-positive for spotted fever group rickettsiae and Candidatus Neoehrlichia mikurensis. All of the tick-borne pathogens that we found in the present study are known to occur in Danish forest populations of I. ricinus. Our study indicates that migrating birds can transport ticks and their pathogens from neighboring countries to Denmark including sites in Denmark without a sustainable tick population. Thus, a tick-borne pathogen affecting human or animal health emerging at one location in Europe can rapidly be introduced to other countries by migrating birds. These movements are beyond national veterinary control. The current globalization, climatic and environmental changes affect the potential for introduction and establishment of ticks and tick-borne pathogens in Northern Europe. It is therefore important to quantify the risk for rapid spread and long distance exchange of tick-borne pathogens in Europe.
Migrating birds and carnivores introduce ticks and tick-borne pathogens to Denmark – but are they also a public health risk?

Since the end of the ice age, spring migrating birds from Africa and Europe and autumn migrating birds from Northern Scandinavia have entered Denmark, and recently a small wave of long migrating carnivores have started arriving in Denmark from Central Europe. Theoretically, migrating birds could introduce new tick species as well as tick-associated pathogens to Denmark. These migrating animals may also carry ticks and pathogens which already exist in native tick populations in Denmark. The potential supplement of native ticks and existing pathogens to the established high density tick populations in Danish forest and nature areas can be expected to be of little practical importance. However, some of the infected ticks, introduced by migrating birds, may be deposited in private gardens and public parks that are otherwise not able to sustain a viable tick population. Migrating birds may therefore introduce a low level risk of tick-borne infections to urban areas. Also, the recent unexpected wave of long migrating golden jackals (Canis aureus) and grey wolves (Canis lupus), arriving at the Danish peninsula of Jutland, constitutes an emerging risk of introduction of especially Dermacentor spp ticks and their associated pathogens from Germany and Central Europe. Here, we present the results of screening migrating birds and a golden jackal for ticks as well as ticks collected by flagging in selected urban areas in Denmark. The collected ticks were screened for exotic tick species and 38 different tick-borne pathogens. We show that the risk is not just theoretical and we suggest that these introductions may have a practical public health impact.

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Identification of Dermacentor reticulatus Ticks Carrying Rickettsia raoultii on Migrating Jackal, Denmark

From a migrating golden jackal (Canis aureus), we retrieved 21 live male Dermacentor reticulatus ticks, a species not previously reported from wildlife in Denmark. We identified Rickettsia raoultii from 18 (86%) of the ticks. This bacterium is associated with scalp eschar and neck lymphadenopathy after tick bite syndrome among humans.

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Microbiota analysis of environmental slurry and its potential role as a reservoir of bovine digital dermatitis pathogens

At present, very little information exists regarding what role the environmental slurry may play as an infection reservoir and/or route of transmission for bovine digital dermatitis (DD), a disease which is a global problem in dairy herds. To investigate, if DD-related bacteria belong to the indigenous microbiota of the dairy herd environment, we used deep amplicon sequencing of the 16S rRNA gene in 135 slurry samples collected from different sites in 22 dairy farms, with and without DD-infected cows. Both the general bacterial populations as well as digital dermatitis-associated Treponema were targeted in this study. The results revealed significant differences in the bacterial communities between the herds, with only 12 bacterial taxa shared across at least 80% of all the individual samples. These differences in the herd microbiota appeared to reflect mainly between-herd variation. Not surprisingly, the slurry was dominated by ubiquitous gastrointestinal bacteria, such as Ruminococcaceae and Lachnospiraceae. Despite the low relative abundance of spirochetes, which ranged from 0 to 0.6%, we were able to detect small amounts of bacterial DNA from DD-associated treponemes in the slurry. However, the DD-associated Treponema spp. were only detected in samples from herds with reported problems of DD. These data indicate that treponemes involved in the pathogenesis of DD are not part of the normal environmental microflora in dairy herds without clinical DD and, consequently, that slurry is not a primary reservoir of infection. Importance Bovine digital dermatitis (DD), a dermal disease which causes lameness in dairy cattle, is a serious problem worldwide. To control this disease, the infection reservoirs and transmission routes of DD pathogens need to be clarified. The dairy herd slurry may be a possible pathogen reservoir of DD-associated bacteria. The rationale for the present study was, therefore, to examine whether DD-associated bacteria are always present in slurry or if they are only found in DD-afflicted herds. The results strongly indicated that DD Treponema are not part of the indigenous slurry and, therefore, do not comprise an infection reservoir in healthy herds. This study applied next-generation sequencing technology to decipher the microbial compositions of environmental slurry of dairy herds with and without digital dermatitis.

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Potential bacterial core species associated with digital dermatitis in cattle herds identified by molecular profiling of interdigital skin samples

Although treponemes are consistently identified in tissue from bovine digital dermatitis (DD) lesions, the definitive etiology of this debilitating polymicrobial disease is still unresolved. To study the microbiomes of 27 DD-infected and 10 healthy interdigital skin samples, we used a combination of different molecular methods. Deep sequencing of the 16S rRNA gene variable regions V1–V2 showed that Treponema, Mycoplasma, Fusobacterium and Porphyromonas were the genera best differentiating the DD samples from the controls. Additional deep sequencing analysis of the most abundant genus, Treponema, targeting another variable region of the 16S rRNA gene, V3–V4, identified 15 different phylotypes, among which Treponema phagedenis-like and Treponema refringens-like species were the most abundant. Although the presence of Treponema spp., Fusobacterium necrophorum and Porphyromonas levii was confirmed by fluorescence in situ hybridization (FISH), the results for Mycoplasma spp. were inconclusive. Extensive treponemal epidermal infiltration, constituting more than 90% of the total bacterial population, was observed in 24 of the 27 DD samples. F. necrophorum and P. levii were superficially located in the epidermal lesions and were present in only a subset of samples. RT-qPCR analysis showed that treponemes were also actively expressing a panel of virulence factors at the site of infection. Our results further support the hypothesis that species belonging to the genus Treponema are major pathogens of DD and also provide sufficient clues to motivate additional research into the role of M. fermentans, F. necrophorum and P. levii in the etiology of DD.