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Arboviruses in polar areas

RNDr. thesis

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Annotation

Arthropode-borne viruses (arboviruses) are important human and veterinary pathogens. Due to their importance they ecology and epidemiology is intensively studied in mild and tropical climate zones. Nevertheless, there are only limited information about presence and prevalence of arboviruses in polar areas. From some areas such as Jan Mayen and Svalbard archipelago located in northern Atlantic are these information missing at all. In this thesis I tried to detect arboviruses from genera Alphavirus (family *Togaviridae*), Orthobunyavirus, Phlebovirus (both family *Bunyaviridae*), Flavivirus (family *Flaviviridae*), and Orbivirus (family *Reoviridae*) in sea-bird ticks Ixodes uriae located on Jan Mayen and Svalbard archipelago. No arboviruses were detected in tested samples showing that these viruses are either not present in tested areas or their prevalence is under detection limit of our screen.

Declaration [in Czech]

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Jiří Černý selected and designed experiments which were used to detect arboviruses in tested samples, he interpreted the results, wrote the manuscript and as a corresponding author he supervised the manuscript through whole review process.

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RESEARCH/REVIEW ARTICLE

Search for tick-borne pathogens in the Svalbard Archipelago and Jan Mayen

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Keywords
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Abstract

The tick species *Ixodes uriae*, parasitizing seabirds in the Arctic, may transmit many pathogens including various arboviruses, *Borrelia* spirochetes and *Babesia* apicomplexans. These pathogens may pose an important additional stress to seabirds, which are already stressed by environmental changes such as pollutants and decreased food availability. Here, we present the results of the first screening for arboviruses of the genera Flavivirus, Alphavirus, Orthobunyavirus, Phlebovirus and Orbivirus, as well as *Borrelia* spirochetes and *Babesia* apicomplexans from Svalbard and Jan Mayen. Using polymerase chain reaction technology with genus-specific primers, we tested 89 ticks collected on Jan Mayen, Bjørnøya and Spitsbergen between 2008 and 2012. We did not detect any of the screened tick-borne pathogens. Nevertheless, these pathogens may be introduced to Svalbard and Jan Mayen by migratory birds in the near future. The increasing numbers of ticks appearing in the studied areas make this introduction even more likely. Such an introduction would have serious impact on seabird ecology as well as on human public health. Therefore, continuous careful surveillance and monitoring of possible tick-borne pathogen introductions is important.

To access the supplementary material for this article, please see supplementary files under Article Tools online.

During the few months of cold Arctic summer, many species of seabirds migrate to the Svalbard Archipelago and Jan Mayen to breed. They often display high nest fidelity in crowded colonies with nests in close proximity to one another. Such a dense presence of vertebrate hosts enables the development of high densities of blood-sucking arthropods. The only tick species living in Svalbard is the seabird tick *Ixodes uriae* (Coulson & Refseth 2004; Dietrich et al. 2014). It can parasitize a wide range of seabirds as common guillemot (*Uria aalge*; Barton et al. 1996), Brünnich's guillemot (*Uria lomvia*; Coulson et al. 2009), Atlantic puffin (*Fratercula arctica*; Muzaffar & Jones 2007) and black-legged kittiwake (*Rissa tridactyla*; Smith et al. 2006). *Ixodes uriae* is not common in Svalbard (McCoy 2001) but the numbers of observations are increasing (Coulson et al. 2009). Descamps (2013) identified a relationship between recent warmer winters and tick infestation of Brünnich's guillemot, suggesting that climate change plays a role in the apparent increase in tick infestation. Ongoing climate change might have an impact on the distribution of ticks as vectors for many diseases.

Ticks such as *I. uriae* may serve as vectors for many tick-borne diseases of viral and bacterial origin.

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Arthropod-borne viruses (arboviruses), *Borrelia* spirochetes and *Rickettsiales* species are commonly transmitted pathogens in ticks (Chastel et al. 1993; Olsén et al. 1993; Major et al. 2009). Moreover, *Babesia* apicomplexans were found in common murres (*U. aalge*), which are common hosts of *I. uriae* (Yabsley et al. 2009). Five genera of tick-borne arboviruses occur in polar areas and the North Atlantic Ocean region: Flavivirus (family *Flaviviridae*), Orthobunyavirus and Phlebovirus (*Bunyaviridae*), Orbivirus (*Reoviridae*) and Alphavirus (*Togaviridae*), as reviewed by Labuda & Nuttall (2004). Moreover, *Borrelia garinii*, the most neurotropic species from the *Borrelia burgdorferi* sensu lato complex, has been repeatedly detected in *I. uriae* and various seabirds (Olsén et al. 1993; Gylfe et al. 1999; Smith et al. 2006; Larsson et al. 2007). A high diversity among *B. garinii* isolated from *I. uriae* in Arctic Norway, the Faroe Islands and northern Sweden was found, signalling the epidemiological importance of the marine enzoonotic cycle of *B. garinii* (Comstedt et al. 2009). Clinical symptoms of seabirds infected by arboviruses are mostly unknown. There is only evidence from experimentally infected birds showing clinical symptoms, such as encephalitis with paresis after inoculation of Tyluiney virus, a member of the flavivirus family (Berezina et al. 1974).

It has been suggested that birds migrating to the Arctic trade off the energetic demands of the journey against a lesser requirement for immunocompetence in the parasite-poor Arctic environment (Piersma 1997). Species utilizing such a strategy may therefore be particularly susceptible to parasites and pathogens at their breeding sites. Some bird populations in the Arctic are already stressed by various industrial toxins, and often a decrease in food availability (Letcher et al. 2010; Routti et al. 2013; Schultner et al. 2013). Tick-borne pathogens present a significant medical and veterinary threat in most tropical and temperate areas by infecting humans, domestic and wild animals and causing epidemics with high morbidity and mortality, significantly affecting public health (Stricker & Johnson 2014), veterinary health (Costard et al. 2013) as well as local ecological relationships (Eidson et al. 2001; Komar et al. 2003). In polar areas, tick-borne pathogens are mostly neglected. The introduction of a new environmental stress, a viral load, may therefore have significant implications for bird population dynamics in the Arctic. To our knowledge, there is no information about the presence or absence of tick-borne pathogens in the Svalbard Archipelago and Jan Mayen. Here we present data collected on Spitsbergen, Bjørnøya and Jan Mayen islands.

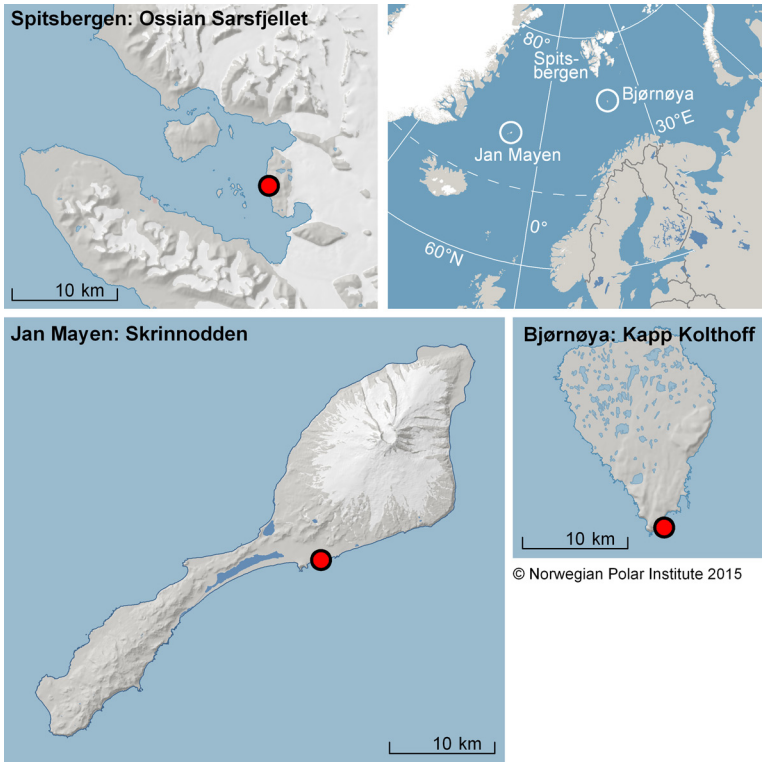

Fig. 1 Localities (red dots) where the samples were collected at Skrinnodden (Jan Mayen, 70.99°N, −8.24°E), Kapp Kolthoff (Bjørnøya, 74.35°N, 19.13°E) and Ossian Sarsfellet (Spitsbergen, 78.93°N, 12.5°E).

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Methods

Sample collection and storage

Eighty-nine *Ixodes uriae* ticks were collected during the summers of 2008–2012. Ticks were collected at *Uria lomvia* and *U. aalge* bird colonies on Jan Mayen (Skrinnodden), Bjørnøya (Kapp Kolthoff) and Spitsbergen (Ossian Sarsfjellet) (Coulson et al. 2009; Fig. 1). Ticks from Jan Mayen and Spitsbergen were collected directly from birds, whereas ticks from Bjørnøya were found on the breeding ledges or on the clothes of people working close to the bird colonies. The ticks were pooled in one sample if collected from one bird or from one nest. All tested samples are listed in Supplementary Table S1.

Nucleic acid isolation

Ticks were pooled in one tube from each bird or nest—one to four ticks per sample in samples from Jan Mayen, nine ticks in the sample from Spitsbergen and nine and two ticks in the two samples from Bjørnøya (see Supplementary Table S1)—and homogenized using a TissueLyser II (Qiagen, Hilden, Germany) in cooled, phosphate-buffered saline and kept at −80°C. The RNA was isolated using RNAGem (Zygem, Hamilton, New Zealand) for arbovirus detection and DNA was isolated with a NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany) for screening of *Borrelia* ssp. and *Babesia* spp. Both kits were used according to the manufacturers' protocols.

Reverse transcription polymerase chain reaction and polymerase chain reaction detection of tick-borne pathogens

Detection of arboviruses was performed by reverse transcription polymerase chain reaction (RT-PCR) as described by Pabbaraju et al. (2009). The RT-PCR reaction was prepared using a Titan One Tube RT-PCR System (Roche, Basel, Switzerland). Arbovirus, genus-specific primers selected according to Kuno et al. (1996), Sánchez-Seco et al. (2001), Scaramozzino et al. (2001), Charrel et al. (2006) and Palacios et al. (2011) and length of expected RT-PCR resulting amplicons is listed in Supplementary Table S2. Tick-borne encephalitis virus, Semliki forest virus, Tahynya virus, Uukuniemi virus and Great Island virus served as positive controls for genera Flavivirus, Alphavirus, Orthobunyavirus, Phlebovirus and Orbivirus, respectively. The amplification programme consisted of these steps: denaturation at 96°C for 10 min and then 40 cycles of denaturation at 96°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for one minute. The programme was completed with a 72°C incubation for 10 min.

Detection of *Borrelia* spp. was performed by polymerase chain reaction (PCR) using primers amplifying a 154-bp fragment of the flagellin gene (Schwaiger et al. 2001). *B. garinii* was used as a positive control. The reaction conditions were: 12.5 µl of FastStart PCR Master (Roche Diagnostics, Mannheim, Germany), 10 pmol of primers FlaF1A and FlaR1 (Supplementary Table S2), template DNA (100 and 300 ng) and distilled water up to 25 µl. The amplification programme consisted of denaturation at 95°C for 10 min and then 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 40 s. The programme was finished by a seven-minute incubation at 72°C.

Detection of *Babesia* spp. was performed by nested PCR amplifying a fragment of the 18S rRNA gene (Malandrin et al. 2010). *Babesia canis* was used as a positive control. The reaction conditions and amplification programme were the same as for *Borrelia* spp. Primers CRYPTOF and CRYPTOR were used in the first round and primers BABGF2 and BABGR2 (Supplementary Table S2) in the second round of amplification. A 1.5 µl aliquot of the first PCR product was used as a template in the second round of PCR.

As a control of RNA or DNA degradation, two specific primers for actin and elongation factor-1 alpha from *Ixodes ricinus* were used for each sample (kindly provided by Dr Pavlína Věchtová from the Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic). The amplification programme consisted of these steps: denaturation at 96°C for 10 min and then 40 cycles of denaturation at 96°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for one minute. The programme was finished by a 72°C incubation for 10 min.

PCR products were analysed by a SYBR Green stained 2% agarose gel and visualized by UV light.

Sequencing

DNA of potentially positive samples was isolated from agarose gel using a Qiagen QIAquick Gel Extraction Kit. Amplified DNA was cloned to pCR4-TOPO plasmids (Life Technologies, Carlsbad, CA). Individual clones were sequenced using a Life Technologies BigDye Terminator v3.1 Cycle Sequencing Kit by the sequencing company SeqMe Ltd. (Dobris, Czech Republic).

Results

Arbovirus screening

DNA molecules of the expected size were produced after PCR amplification in all positive controls which under-

went the same isolation and amplification process as the samples (see Supplementary Table S2 for exact molecular sizes) confirming the screening methodology. In all samples, PCR amplification using primers complementary to tick actin and elongation factor-1 alpha resulted in production of DNA molecules of the expected sizes of 91 and 166 bp, respectively, showing that RNA molecules in samples were not degraded.

Eight samples from Jan Mayen (4, 5, 9, 11, 14, 15, 21 and 22), collected from *Uria lomvia* and *U. aalge*, yielded 251-bp-long DNA molecules after PCR amplification using the Orthobunyavirus genus-specific primers, with the expected molecular size. Subsequent sequencing of amplified DNA showed a mixture of DNA molecules. Amplified DNA from four of these samples (9, 11, 14 and 15) were selected and cloned into the sequencing plasmid pCR4. Ten of the resulting clones from each sample were randomly selected and sequenced. Obtained sequences had no homology to any bunyaviral or any nucleic acid deposited in GenBank. Sequencing of the DNA from positive control containing Tahynya virus RNA yielded a molecule of the expected length and sequence. All samples were negative after PCR amplification using other genus-specific primers.

Screening for *Borrelia* spirochetes and *Babesia* apicomplexans

No samples positive for *Borrelia* or *Babesia* spp. were found.

Discussion

Our screening for tick-borne diseases on two islands in Svalbard and on Jan Mayen revealed no tick-borne pathogens in tick samples. These pathogens have been found in ticks in similar biotopes but at lower latitudes (Olsén et al. 1993; Gylfe et al. 1999; Smith et al. 2006; Larsson et al. 2007). Careful handling of the samples and controls over the process excluded massive degradation of arboviral RNA or *Borrelia* and *Babesia* DNA during sample storage and processing. Degradation of a minor portion of the nucleic acids cannot be ruled out but it should have only a marginal effect on screening results as viral RNA is present in quite large amounts in infected cells plus some portion of it is already packed in virions and therefore well protected against nuclease action.

The number of ticks screened in this study is low in comparison with other studies but is nonetheless the most extensive collection of Arctic ticks screened for tick-borne pathogens to date. Tick collection in the High Arctic is hampered for several reasons: (1) the vast majority of sampling locations are managed as nature reserves, which have restrictions on disturbing wildlife; (2) tick collecting can be quite stressful for the birds and only a small number of individuals can be sampled; (3) birds often nest on frost-fractured cliffs that are dangerous to climb; (4) random sampling from within a colony is precluded as only readily accessible birds can be caught; and (5) the overall number of birds infested by ticks in Svalbard is low in comparison with other localities in the Arctic. There are even colonies from which ticks have not been observed. Nevertheless, it seems that the tick population in Svalbard and Jan Mayen is growing (Coulson et al. 2009; Léger et al. 2013). Having tested only a small number of ticks, we cannot claim that Svalbard and Jan Mayen are free of tick-borne pathogens, but rather that these pathogens are either absent or rare.

Theoretically, tick-borne pathogens may be easily introduced to Svalbard and Jan Mayen by infected ticks or infected seabirds coming from lower latitudes. The current trend of global warming causes geographical shift of many infectious agents to higher latitudes and altitudes (Parkinson et al. 2014). Among tick-borne pathogens, the best studied example of such a shift is the tick-borne encephalitis virus, which has spread northwards (Ytrehus et al. 2013; Pettersson et al. 2014; Hjetland et al. 2015).

In Svalbard, several seabird populations are declining (MOSJ 2014). This can be caused by novel stressors, such as environmental pollution, as well as decreased food availability (Letcher et al. 2010; Routti et al. 2013; Schultner et al. 2013). The appearance of novel arthropod parasites may also be an important stressor (Coulson et al. 2009; Gwiazdowicz et al. 2012; Descamps 2013) and arthropod-borne pathogens would pose an additional significant stress.

Climate change, which is particularly marked in the Svalbard region on account of Arctic amplification (Symon et al. 2005; Dicks 2011), influences the structure and function of ecosystems. Climate projections indicate mean temperatures increases for this region of between 3 and 7°C by 2100 over the 1986–2005 baseline and the Svalbard region has already experienced a long-term normal annual air temperature rise from −6.7°C (period 1960–1991) to −4.6°C (period 1981–2010) (Førland et al. 2011). Higher temperatures bring the emergence of new parasites and their pathogens to higher latitudes (Kutz et al. 2005; Staszewski et al. 2008; Descamps 2013). This may influence zoonotic diseases including arthropod-transmitted diseases (Parkinson et al. 2014). Monitoring and understanding the responses of high-latitude host–parasite–pathogen systems could assist in predicting the impact of temperature changes on seabird populations in the Arctic.

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