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No indication of arthropod-vectored viruses in mosquitoes (Diptera: Culicidae) collected on Greenland and Svalbard

RNDr. Thesis

Jana Müllerová

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Annotation:

Arboviruses are a large polyphyletic group of viruses being transmitted from arthropod vectors to vertebrate hosts. They are important human and animal pathogens. Among the most famous representatives of arboviruses include the Tick-borne encephalitis virus (TBEV), Dengue virus (DV), Zika virus (ZIKV) or Rift Valley fever virus (RVFV) etc. They are studied in tropical and temperate zones abundantly. However, in polar regions there are no traces of such viruses, despite climate change and the migration of vectors to higher latitudes. Therefore, the knowledge of potential worldwide occurrences of arboviruses is important due to their potential to be pathogenic for humans. Arboviruses were detected in many countries such as Alaska, Norway, Canada and in North America, but never in Svalbard nor in Greenland. This research study was focused on the monitoring of arboviruses in north Atlantic areas. Thousands of samples from the mosquito species *Aedes nigripes* were examined, both from Greenland and from Svalbard (collected during the years 2012-2016). I tested these samples for the presence of different arboviral genera; *Orthobunyavirus, Orbivirus, Flavivirus, Alphavirus, Phlebovirus.* No presence of arboviruses in examined mosquitoes were detected. These results may reflect an absence of arboviruses, or their prevalence is under the detection limit of our screening in areas studied.

Declaration [in Czech]

Prohlašuji, že svoji rigorózní práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své rigorózní práce, a to v nezkrácené podobě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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Jana Müllerová je prvním autorem této publikace, která je založená na výsledcích její magisterské práce. Jana se podílela na sběru vzorků komárů na Svalbardu. Dále vyšetřovala významný podíl materiálu pomocí molekulárních metod. Podílela se tedy na získání výsledků a také na přípravě předloženého rukopisu.

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ORIGINAL PAPER

No indication of arthropod-vectored viruses in mosquitoes (Diptera: Culicidae) collected on Greenland and Svalbard

Jana Müllerová[1,2] · Jana Elsterová[1,2,3] · Jiří Černý[1,3] · Oleg Ditrich[2] · Jakub Žárský[4] · Lauren E. Culler[5,6] · Helge Kampen[7] · Doreen Walther[8] · Stephen J. Coulson[9,10] · Daniel Růžek[1,3] · Libor Grubhoffer[1,2]

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Abstract

Viruses transmitted to vertebrates via arthropod vectors (so-called arboviruses) include many important pathogens such as dengue virus, Zika virus, and Sindbis virus. Mosquitoes represent the major vectors of many of these arboviruses and occur in all climatic zones, including the Arctic. The focal species, *Aedes nigripes* (*Diptera*: *Culicidae*), is the most widely distributed mosquito species in the Arctic. We screened over 11,000 specimens collected between 2012 and 2016 on Greenland (Kangerlussuaq) and Svalbard (Petuniabukta) for the presence of arboviruses which have previously been reported in latitudes up to 70°N. Assays for arbovirus detection using RT-PCR with primers specific for the genera *Alphavirus* (family *Togaviridae*), *Orthobunyavirus*, *Phlebovirus* (*Bunyaviridae*), *Flavivirus* (*Flaviviridae*), and *Orbivirus* (*Reoviridae*) were negative for all specimens. Similar results were recently obtained in a screening focused on tick-borne pathogens on Svalbard. The findings suggest that the circulation of arboviruses at studied localities is currently negligible or nonexistent, possibly due to dispersal, climate, or biotic restrictions. However, global climate change could enhance vector abundance and activity, introduction of invasive host species, and increase in tourism which then could lead to emerging arbovirus outbreaks in the future, with considerable impact on local ecosystems.

Keywords *Aedes nigripes* · Mosquitoes · Arbovirus · Arctic · Svalbard · Greenland

Introduction

Arthropod-borne viruses (arboviruses) are a group of evolutionarily unrelated viruses sharing a similar ecological niche by being transmitted by arthropod vectors to vertebrate hosts. Numerous arboviruses are important human

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✉ Jiří Černý
cerny@paru.cas.cz

[1] Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, 37005 České Budějovice, Czech Republic

[2] Centre for Polar Ecology, Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31, 37005 České Budějovice, Czech Republic

[3] Veterinary Research Institute, Hudcova 296/70, 62100 Brno, Czech Republic

[4] Department of Ecology, Charles University in Prague, Viničná 7 Prague 2, 128 42 Prague, Czech Republic

[5] Institute of Arctic Studies, Dartmouth College, Hanover, NH, USA

[6] Environmental Studies Program, Dartmouth College, Hanover, NH, USA

[7] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Uppsala, Germany

[8] Leibniz-Center for Agricultural Landscape Research (ZALF) e. V, Eberswalder Straße 84, 15374 Müncheberg, Germany

[9] Department of Arctic Biology, University Centre in Svalbard, P.O. Box 156, 9171 Longyearbyen, Norway

[10] ArtDatabanken, Swedish Species Information Centre, Swedish University of Agricultural Sciences, P.O.Box 7007, 750 07 Uppsala, Sweden

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and veterinary pathogens such as dengue virus, Zika virus, chikungunya virus, tick-borne encephalitis virus, and bluetongue virus (Weaver and Reisen 2010; Hubálek et al. 2014).

Arboviruses have been reported from all continents except Antarctica, including all climatic zones up to 70 °N (McLean 1975) and down to 54 °S (La Linn et al. 2001; Major et al. 2009). Arboviruses from polar regions have been found in numerous hosts with infections remaining asymptomatic or showing a wide range of clinical signs including lethal disease. Antibodies against mosquito-borne viruses have been described in a wide variety of mammals such as ruminants, rodents, lagomorphs, carnivores, and humans, as well as birds (Zarnke et al. 1983; Descamps 2013).

In polar areas, arboviruses are transmitted mainly by ticks and mosquitoes (McLean et al. 1978; Traavik et al. 1978; Deardorff et al. 2013). In the southern hemisphere, arboviruses have been repeatedly detected in ticks from Subantarctic Macquarie Island (Doherty et al. 1975; St George et al. 1985; La Linn et al. 2001; Major et al. 2009) but never in samples from continental Antarctica. In the northern hemisphere, mosquito-borne arboviruses, transmitted mainly by mosquitoes of the genera *Culex* and *Aedes* (McLean 1975), have been reported repeatedly. Among these, Snowshoe Hare virus and Jamestown Canyon virus (both California encephalitis serogroup, genus *Orthobunyavirus*, family *Bunyaviridae*) have been found in Alaska, Canada, and Arctic Russia (McLean et al. 1977; McLean 1983; Mitchell et al. 1993; Carson et al. 2017). Other bunyaviruses from the California encephalitis serogroup circulating in the Eurasian Arctic are Chatanga virus and Inkoo virus (Vanlandingham et al. 2002; Putkuri et al. 2014, 2016; Tingström et al. 2016). Bunyaviruses from the Bunyamwera serogroup are represented by Northway virus in Arctic North America (Stansfield et al. 1988; Sahu et al. 2002). Various alphaviruses (family *Togaviridae*), such as Sindbis virus and Getah virus, have also been described in the Arctic and sub-Arctic regions (Walters et al. 1999). Furthermore, antibodies against mosquito-borne flaviviruses such as St. Louis encephalitis virus and West Nile virus have been reported in various vertebrate hosts from Alaska and Canada (Pedersen et al. 2016).

While arboviruses have been documented in many continental polar locations, information about the presence of arboviruses is lacking from Arctic Ocean islands such as Greenland, Iceland, or the Svalbard Archipelago. In addition, the northernmost isolations of arboviruses were made at almost 70°N in Norway (Traavik et al. 1978) and Canada (McLean 1975, 1983) despite the fact that vectors able to transmit arboviruses even occur further north (Coulson and Refseth 2004). Previously, we were not able to detect any tick-borne pathogens in *I. uriae* samples from Svalbard and Jan Mayen (Elsterova et al. 2015), although these results were based on a limited number of specimens. Given the abundance of mosquitoes in Greenland's and Svalbard's tundra ecosystems and that climate change rapidly alters the distributions of vectors and pathogens (Descamps 2013; Culler et al. 2015), building a baseline dataset of arbovirus occurrence can serve as a reference for future studies and help anticipate any impacts to important natural resources as the Arctic continues to warm up (Overland et al. 2015).

We collected mosquito samples on Svalbard and Greenland to test for the presence and prevalence of arboviruses among the most abundant and widely distributed mosquito in the Arctic, *Aedes nigripes* (Zetterstedt, 1838). *Ae. nigripes* is the only mosquito species present in Svalbard and the most prevalent species in Greenland. It is a species with circumpolar distribution and the northernmost occurrence (up to 80°N) of all mosquito species (Coulson and Refseth 2004; Becker et al. 2010). Vector competence of *Ae. nigripes* for arboviruses is largely unknown. The species has been suggested to transmit Getah virus in Siberia by L'vov et al. (1995). Hubálek et al. (2014) also list *Ae. nigripes* among the possible vectors of Getah virus in the review of pathogenic arboviruses, but the reference for the primary literature source supporting this statement is lacking. Finally, there is evidence of natural infection of *Ae. nigripes* by Anadyr virus and Chatanga viruses (unpublished results, from GenBank accession numbers EU616894, FJ956756, FJ956750, GQ355970, GQ340744, HQ734817, HQ734821, HQ734824, GQ330482, FJ913881, KT288281-KT288283, KT313747-KT313752, KT313743).

Methods

Sample collection and storage

Mosquitoes were collected during the summer seasons (July and August) from 2012 to 2016. On Svalbard, mosquitoes were collected at several localities in Petuniabukta (78°41′N 16°32′E), while in Greenland sampling was done around Kangerlussuaq (67°01′N 50°41′W) (Fig. 1). Mosquito identification was performed morphologically using the identification key by Becker et al. (2010).

Mosquitoes were pooled in samples of up to 30 individuals according to developmental stage, locality of collection, and day of collection. The collected samples were stored in RNAlater (QIAGEN, Germany) or in a solution of 25 mM sodium citrate, 10 mM EDTA, and 70 g ammonium sulfate/100 ml solution (pH 5.2) which corresponds to the RNAlater composition. The samples were stored on melting ice in the field and frozen to − 80 °C as soon as possible (not later than two weeks after collection).

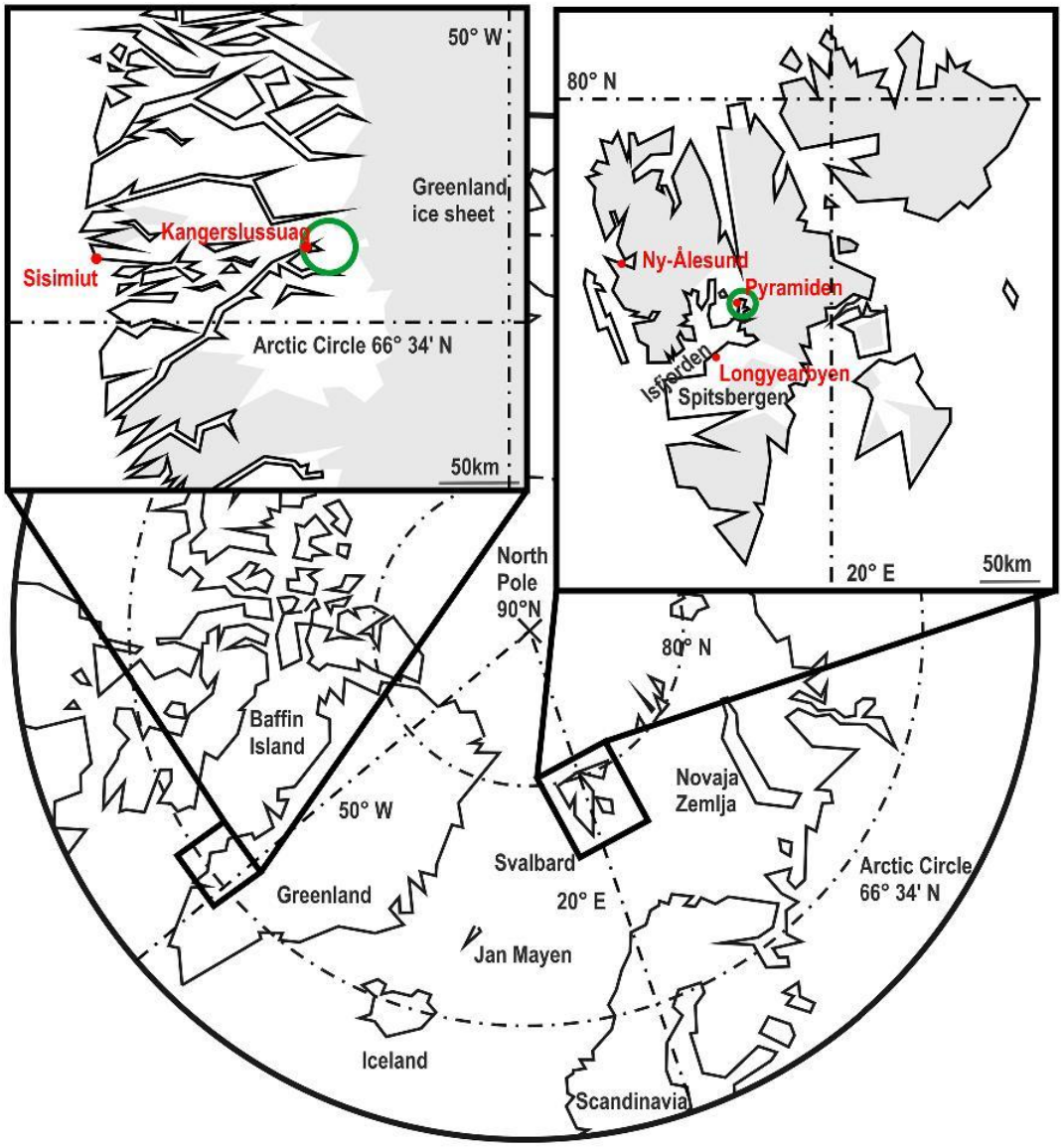

Fig. 1 Sampling localities Samples were collected in Greenland close to Kangerlussuaq (67°01′N 50°41′W) and on Svalbard in Petuniabukta (78°41′N 16°32′E). The sampling localities are marked by green circles on the map. Glaciated areas are marked in gray and important settlements by red dots. Polar circle is indicated by dashed line

Nucleic acid isolation

Samples were thawed at 4 °C. Whole mosquito bodies were removed from RNA stabilizing solution, transferred to 1 ml of cooled PBS solution, and homogenized using a TissueLyser II (QIAGEN, Germany). RNA was isolated from 280 µl of homogenate by RNAGem (Zygem, New Zealand) according to the manufacturer's protocol.

RT-PCR detection of mosquito-borne arboviruses

Arbovirus detection was performed by RT-PCR. The RT-PCR reaction was prepared using the KAPA SYBR FAST One-Step qRT-PCR Kit (Kappa Biosystems, USA) according to the manufacturer's protocol. Arbovirus genus-specific primers were selected according to Kuno et al. (1996) for the genus *Orthobunyavirus*, Sánchez-Seco et al. (2001) for the

genus *Alphavirus*, Scaramozzino et al. (2001) for the genus *Flavivirus*, Sánchez-Seco et al. (2003) for the genus *Phlebovirus,* and Palacios et al. (2011) for the genus *Orbivirus*. Primer sequences and length of expected RT-PCR amplicons are listed in Online Resource 1. Tahyna virus, Semliki Forest virus, tick-borne encephalitis virus, Uukuniemi virus, and Tribec virus were used as positive controls for the various genera screened. For the detection of viruses from the genera *Orthobunyavirus, Alphavirus, Flavivirus,* and *Orbivirus*, the amplification program was as follows: 5 min at 42 °C for reverse transcription, 3 min at 95 °C for enzyme activation, 3 s at 95 °C for DNA denaturation, and 1 min at 60 °C for primer annealing and extension. The last two steps were repeated forty times. The protocol for the detection of genomic RNA of viruses of the genus *Phlebovirus* was slightly different: 5 min at 42 °C for reverse transcription, 3 min at 95 °C for enzyme activation, 3 s at 95 °C for DNA denaturation, 20 s at 45 °C for primer annealing, and 1 min at 60 °C for primer annealing and extension. PCR products were analyzed by a SYBR green-stained 2% agarose gel and visualized by UV light. All tested specimens and pools are listed in Table 1.

Sequencing

PCR amplicons from positive control and potentially positive samples were purified by the QIAquick PCR Purification Kit (QIAGEN, Germany) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA) by Biogen PRAHA (Czech Republic).

Results and discussion

We collected over 11,000 mosquito specimens to test for the presence of arboviruses. The collection included 8852 mosquitoes from Svalbard (953 larvae, 1108 pupae, and 6791 adult females) collected during 2012–2016 and 2166 adult mosquito females from Greenland collected in 2013 and 2014 (Table 1). In all cases, they were determined as *Ae. nigripes* (Diptera: Culicidae). This is in concordance with the previously described geographical distribution of mosquito species on Svalbard (Coulson and Refseth 2004). Similarly, all collected individuals from Greenland were determined to be *Ae. nigripes*. *Aedes impiger* is a second species occurring in Greenland but it does not occur in Kangerlussuaq (Jenkins 1956).

We did not detect any of the arboviral genera in our mosquito specimens. This was despite the fact that DNA molecules of the expected amplicon size were produced by RT-PCR in all positive controls, which underwent the same isolation and amplification process as the samples demonstrating that our screening methodology was able to detect the searched viruses.

According to the best of our knowledge and in agreement with our results, there have been no reports of arboviruses from Greenland or Svalbard so far. Our previous search for tick-borne pathogens on tick samples from Svalbard also did not reveal the presence of arboviruses (Elsterova et al. 2015). In general, arbovirus presence in the high Arctic is limited, with the northernmost latitude where arboviruses have been detected being 70°N (McLean 1975; Traavik et al. 1978), which is far south from the collection localities on Svalbard. On the other hand, Kangerlussuaq is located just north of the Arctic Circle, where, according to previous studies, the presence of arboviruses might be expected.

There are five main reasons why arboviruses may be absent from Greenland and Svalbard. First, given that both are islands, it could be that the viruses have just not established due to a lack of mosquito infection sources (infectious hosts). Second, it is possible that the harsh climates in the studied locations do not allow arbovirus circulation due to physiological constraints. Replication of arboviruses in their mosquito hosts is very sensitive to temperature, although

Table 1 List of collected *Aedes nigripes* specimens

Locality	Year	Larvae		Pupae		Adult females		Total	
		Individuals	Pools	Individuals	Pools	Individuals	Pools	Individuals	Pools
Greenland, Kangerslussuaq	2013	0	0	0	0	337	15	337	15
	2014	0	0	0	0	1829	65	1829	65
Subtotal—Greenland		0	0	0	0	2166	80	2166	80
Svalbard, Petuniabukta	2012	953	36	718	28	378	15	2049	79
	2013	0	0	0	0	522	19	522	19
	2014	0	0	0	0	2310	81	2310	81
	2015	0	0	390	16	1710	60	2100	76
	2016	0	0	0	0	1871	67	1871	67
Subtotal—Svalbard		953	36	1108	44	6791	242	8852	322
Total		953	36	1108	44	8957	322	11,018	402

Northway virus was shown to be able to replicate in Arctic *Aedes* mosquitoes even at 4 °C (McLean et al. 1979). Third, arboviruses tend to occur in northern localities within boreal forests, such as in the Yukon Valley and in northern Norway (McLean 1975; Traavik et al. 1978), while the studied localities are within the tundra biome. Fourth, suitable vertebrate hosts may not be present at our studied locations. Although the Arctic hare (*Lepus arcticus*), a suitable vertebrate host of Northway virus, is present in Greenland (Bennike et al. 1989), it might not belong to the blood host spectrum of *Ae. nigripes* mosquitoes (Corbet and Downe 1966). More information is needed about the adult feeding preferences of *Ae. nigripes* mosquitoes in Greenland and Svalbard, which would support testing hypotheses about arbovirus transmission by this species. In addition, the vector competence of *Ae. nigripes* for the Northway virus is not known. It would be beneficial to evaluate this in the laboratory in the future, as vector incompetence would be equivalent to no transmission risk. Lastly, the prevalence of arboviruses in Arctic mosquitoes can be extremely low. For example, Batai and Getah virus prevalence in Siberia was shown to be as low as 1:24,000 (L'vov et al. 1995). Such rare viruses could be missed in our geographically limited sampling.

In summary, data from the literature as well as the results of our current mosquito and recent tick screening studies show that arboviruses either do not circulate or do at below-detection limits on Greenland and Svalbard. Nevertheless, global climate change is very apparent in the Arctic and could well support an increase in vector abundance and activity due to easier overwintering (Descamps 2013) and faster development (Culler et al. 2015) as well as the invasion of novel blood host species (Yoccoz and Ims 1999; Markova et al. 2016). Growing tourism could also bring about novel mosquito species through human-mediated displacement. Monitoring and surveillance of vectors and associated arboviruses should be a research priority because the introduction of arboviruses into the Arctic could pose an important stressor for local animal populations and significant medical threat for indigenous human populations.

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