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Harnessing field investigations and high-resolution genomic analyses to gain insights into the emergence and spatiotemporal spread of a bacterial pathogen associated with widespread muskox mortalities in the Canadian Arctic

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Harnessing field investigations and high-resolution genomic analyses to gain insights into the emergence and spatiotemporal spread of a bacterial pathogen associated with widespread muskox mortalities in the Canadian Arctic

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Abstract

Populations of muskoxen (*Ovibos moschatus*), an important food and cultural resource for Inuit communities, have substantially declined in the Canadian Arctic Archipelago (CAA) over the last two decades. This has coincided with the detection of the 'Arctic clone' (AC) of the bacterium *Erysipelothrix rhusiopathiae* in association with mass mortality events since 2010. We conducted multi-year field investigations during mortality events on Ellesmere and Axel Heiberg Islands (2021 – 2025), sampling carcass sites, potential wildlife hosts, and environmental sources. We analysed 181 newly sequenced *Er* genomes to reassess the clone's geographic distribution and reconstruct its spatiotemporal spread. The AC became established on southwestern Victoria and Banks Islands as early as 2006, subsequently seeding at least two independent introductions to nearby Prince Patrick Island and spreading over 1000 km to Ellesmere and Axel Heiberg Islands within a decade. We found strong support that wolves and Arctic foxes contribute to pathogen dispersal, with possible roles for birds and marine mammals in its epidemiology. We also established that the AC can persist for up to seven years in insect larvae/pupae at carcass sites. Our findings provide new insights into the ecology, persistence and spread of this emerging Arctic pathogen.

Keywords: Erysipelothrix rhusiopathiae, muskox, Arctic, whole genome sequencing, single nucleotide polymorphism, phylodynamics

Introduction

Muskoxen (*Ovibos moschatus*) are large-bodied ungulates that play a key role in Arctic ecosystems, acting as one of the most significant herbivores[1–3]. They are also a critical food and economic resource for Arctic Indigenous communities, particularly the Inuit[4]. In recent decades, muskox populations have faced several significant challenges, including declining numbers associated with emerging infectious diseases (EIDs)[5], which pose a particular threat to Arctic wildlife that have evolved in relative isolation and with limited exposure to new pathogens[6]. Recent studies have indicated that muskox populations have very low genetic diversity, which may decrease their resilience to EIDs[7,8]. Moreover, the accelerated rate of warming in the Arctic – as much as four times higher than the global average[9,10] – is exacerbating these problems.

Erysipelothrix rhusiopathiae (*Er*) is an example of a newly emerging bacterium associated with widespread mortality in muskoxen. The occurrence of *Er* in Arctic muskox populations likely dates back at least several decades. Seroprevalence studies in muskoxen in Alaska and the Canadian Arctic from 1976 to 2017 suggested that the bacterium was present in the region as early as 1976[11]. However, it wasn't until 2012 that *Er* -associated mortality in muskoxen was detected, when approximately 150 dead muskoxen were found on Banks Island, Northwest Territories (NWT)[12]. The isolation of *Er* from tissue samples of multiple carcasses, alongside necropsy and histology results, suggested septicaemia with this bacterium as the cause of death. Subsequent analyses of archived samples from Banks Island and Victoria Island, Nunavut (NU) confirmed the presence of *Er* in muskox carcasses dating back to 2010-11. Mobilisation of Indigenous Knowledge held by local Inuit harvesters also indicated that die-offs happened in several consecutive summers on Victoria Island between 2009 and 2014, affecting more muskoxen than originally thought[13]. Moreover, one community member of Ulukhaktok – on the western part of the island – reported sighting four dead muskoxen in the summer of 2007[14].

Erysipelothrix rhusiopathiae is a zoonotic Gram-positive, rod-shaped bacterium known to opportunistically cause erysipelas in domestic animals, particularly pigs, and erysipeloid in humans[13,15]. In pigs, *Er* is commonly found in the tonsils, Peyer's patches, and other lymphoid tissues, with a high proportion of healthy pigs acting as carriers[16]. Transmission typically occurs through ingestion of contaminated feed, water, or faeces, or through skin wounds. Although best known for opportunistically causing disease in domestic livestock, *Er* is a generalist bacterium

that has been associated with carriage and/or disease in a wide range of domestic and wild host species around the world[17]. This bacterium has been detected in several North American wildlife species, including caribou (*Rangifer tarandus*), moose (*Alces alces*), and Pribilof Arctic foxes (*Vulpes lagopus pribilofensis*)[18], although how frequently it is a causative agent of disease is unclear.

Isolates of *Er* fall into three broad phylogenetic clades, with most isolates from North America falling within clade 3[19]. Phylogenetic analyses have revealed extensive *Er* diversity in wild ungulates from Alaska and mainland Canada, whereas most muskox mortality events across the Canadian Arctic Archipelago (CAA) have been associated with a single clonal lineage of *Er* within clade 3[17,20] known as the 'Arctic clone' (AC). This strain – with individual isolates differing from each other by only tens of single nucleotide polymorphisms (SNPs) – has been associated with multiple mass mortality events in muskox populations across the Arctic, with reports spanning over a decade and covering distances of more than 1,000 kilometres across the CAA. Namely, the AC has been detected on Banks Island (2012–2013), Victoria Island (2010–2011, 2015), and Prince Patrick Island (2017), as well as in recent mass mortality events on central Ellesmere Island starting in 2021, and on Axel Heiberg Island since 2022[12,17,20,21].

The rapid and widespread decline of muskox populations in regions such as Banks and Victoria Islands in the Canadian Arctic has raised concerns about the impact of *Er* on Arctic ecosystems. Between 2001 and 2019, the muskox population on Banks Island, once one of the largest in the world, plummeted from approximately 69,000 adult individuals[22] to just under 11,000[23], and is continuing to decline (Davison et al., unpublished data). Similar declines have been observed in the southeast and northwest regions of Victoria Island[24,25], with counts on the last survey of the Nunavut side of the island being so low that population size estimates could not be calculated. These declines have coincided with the spread of the *Er* AC and highlight the need to better understand the ecology and impacts of this emerging pathogen, including the factors driving its spread across the CAA. Furthermore, the high apparent rates of mortality observed in muskoxen also raise concern for the potential impact of this generalist pathogen in conspecific Arctic species that may not be as visible as these large-bodied ruminants[21]. The involvement of other host species in the disease ecology of this pathogen through its maintenance and/or transmission in the Arctic has been repeatedly raised as a point for further investigation[12,17,21]. Recent

mechanistic modelling work also provided theoretical support for the likely involvement of carnivore species in the perpetuation of infection cycles in this host-pathogen system[26].

Understanding evolutionary rates and timeframes can provide useful insights into pathogen evolution and spread. Phylodynamic approaches, wherein high-resolution whole genome sequences (WGS) associated with key epidemiological metadata – particularly sampling dates and locations – are widely used to estimate molecular clock rates for a range of pathogens[27], providing invaluable information on spatiotemporal patterns of spread.

This study aimed to use WGS data from *Er* isolates collected from across the CAA and more widely in northern Canada over more than a decade to:

1. elucidate the distribution and likely transmission routes of the AC;
2. explore evidence for a molecular clock in the *Er* AC to inform the likely timing of introduction events; and
3. determine the range of host species carrying the AC and which may thus be contributing to its epidemiology.

Results

Erysipelothrix rhusiopathiae isolation from muskoxen and alternative host species

A total of 122 *Er* isolates were newly sequenced from samples collected from muskox carcasses (n = 60) on Ellesmere and Axel Heiberg Islands in 2022 and 2023, including bone marrow, organs, faeces, and soil collected directly under carcasses and up to 5 m away (for full sample collection and testing details, see Wooten et al.[21]). Faecal samples (n = 338) collected from muskox herds never yielded any live *Er*, although some samples were PCR-positive (36/316, 30 of which were AC; all positives from 2023 collections).

Five hundred sixteen samples were also collected from non-muskox alternative hosts on Ellesmere and Axel Heiberg Islands (e.g., faeces, whole animals, tissues and insects) representing at least 13 species (Table 1; https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02179; https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02275). Sixty-seven of these were *Er* culture positive, 36 of which were submitted for WGS (from samples collected in 2022 and 2023). The highest proportions of sample types yielding positive test results (by culture or qPCR) were larvae/pupae retrieved from muskox carcasses (66/72, 92%); carnivore (Arctic fox or wolf) faeces or tissues (66/112, 60%); and castings or faeces of unknown bird species (19/46, 41%). Two of five faecal samples from Peary caribou were PCR positive. Despite collecting a large number of faecal samples from Northern Collared lemmings (n = 115), only one tested positive for the *Er* AC by culture, with no further PCR positive samples. Relatively small numbers of samples from Arctic hares (n = 6), Lapland longspurs (n = 7), Red Knot (n = 1), Snow Buntings (n = 2), Snow Geese (n = 3) and mosquitoes (n = 12) were all negative by culture or PCR.

Table 1. Summary of non-muskox species from Ellesmere and Axel Heiberg Islands assessed in this study (2021 – 2025). Most samples underwent selective culture for *Erysipelothrix* spp. unless stored in a solution that was bactericidal (e.g., mosquitoes, some insects). Samples that did not yield bacterial growth, and a few that did, underwent follow-up PCR to identify *Erysipelothrix* spp. DNA.

Species	Sample Type	n (individual/pooled)	Erysipelothrix rhusiopathiae Testing Results		
			Culture + / n Tested (% AC)	PCR + / n Tested (% AC)	Any Test + / n Tested (% AC)
Aedes spp.	Adult Mosquitoes	12 (0/12)	NT	0/12 (0)	0/12 (0)
Arctic Fox (*Vulpes lagopus*)	Faeces	41 (41/0)	2/41 (100)	11/39 (81.8)	13/41 (84.6)
	Tissue	1 (1/0)	1/1 (100)	NT	1/1 (100)
Arctic Hare (*Lepus arcticus*)	Faeces	2 (2/0)	0/2 (0)	0/2 (0)	0/2 (0)
	Tissue	4 (4/0)	0/1 (0)	0/4 (0)	0/4 (0)
Arctic Wolf (*Canis lupus arctos*)	Faeces	68 (68/0)	11/68 (100)	41/57 (87.8)	51/68 (90.2)
	Tissue	2 (2/0)	0/2 (0)	1/2 (100)	1/2 (100)
Northern Collared Lemming (*Dicrostonyx groenlandicus*)	Faeces	115 (1/114)	1/115 (100)	0/114 (0)	1/115 (100)
	Tissue	35 (35*/0)	0/14 (0)	0/35 (0)	0/35 (0)
Lapland Longspur (*Calcarius lapponicus*)	Faeces	1 (1/0)	0/1 (0)	0/1 (0)	0/1 (0)
	Tissue	6 (6*/0)	0/6 (0)	0/6 (0)	0/6 (0)

Table 1. Summary of non-muskox species from Ellesmere and Axel Heiberg Islands assessed in this study (2021 – 2025). Most samples underwent selective culture for *Erysipelothrix* spp. unless stored in a solution that was bactericidal (e.g., mosquitoes, some insects). Samples that did not yield bacterial growth, and a few that did, underwent follow-up PCR to identify *Erysipelothrix* spp. DNA.

Species	Sample Type	n (individual/pooled)	Erysipelothrix rhusiopathiae Testing Results		
			Culture + / n Tested (% AC)	PCR + / n Tested (% AC)	Any Test + / n Tested (% AC)
Peary Caribou (*Rangifer tarandus pearyi*)	Faeces	5 (5/0)	0/5 (0)	2/5 (100)	2/5 (100)
Red Knot (*Calidris canutus*)	Tissue	1 (1/0)	0/1 (0)	0/1 (0)	0/1 (0)
Snow Bunting (*Plectrophenax nivalis*)	Tissue	2 (1/1)	NT	0/2 (0)	0/2 (0)
Snow Goose (*Anser caerulescens*)	Faeces	3 (3/0)	0/3 (0)	0/3 (0)	0/3 (0)
Unknown Bird	Casting	34 (34/0)	0/34 (0)	10/34 (90)	10/34 (90)
	Faeces	12 (12/0)	4/12 (100)	5/8 (100)	9/12 (100)
Unknown Insect	Larvae/Pupae	72 (0/72)	48/68 (93.8)	27/36 (88.9)	66/72 (92.4)
	Adult Insects	81 (74/7)	NT	20/81 (100)	20/81 (100)

*Some of the "individual" samples were individual organs from the same animal. The 35 lemming samples are representative of 19 total animals. The 6 Lapland longspur samples are representative of 3 animals.
Abbreviations/Terminology:
% AC – percentage of the positive samples for a given cell that were of the *E. rhusiopathiae* Arctic clone

Table 1. Summary of non-muskox species from Ellesmere and Axel Heiberg Islands assessed in this study (2021 – 2025). Most samples underwent selective culture for *Erysipelothrix* spp. unless stored in a solution that was bactericidal (e.g., mosquitoes, some insects). Samples that did not yield bacterial growth, and a few that did, underwent follow-up PCR to identify *Erysipelothrix* spp. DNA.

Species	Sample Type	n (individual/pooled)	Erysipelothrix rhusiopathiae Testing Results		
			Culture + / n Tested (% AC)	PCR + / n Tested (% AC)	Any Test + / n Tested (% AC)

Aedes spp. – including *Aedes nigripes* and *Aedes impiger*
Any Test + – samples were positive by either culture or PCR
NT – Not Tested
Tissue – including samples such as whole organs, bone marrow, and swabs (typically of animals in advanced states of decay)
Unknown Bird – while taxonomic information was not available for these samples, it was presumed these fecal and casting samples were from predatory and/or scavenging species such as common ravens *(Corvus corax principalis*), long-tailed jaegers (*Stercorarius longicaudus*), gyrfalcons *(Falco rusticolus),* or snowy owls *(Bubo scandiacus*)
Unknown Insect – taxonomic information unavailable. Larvae/Pupae samples were pooled collections of carrion-feeding insects collected directly from carcasses (64 muskox and 1 Arctic wolf). Adult insects were collected directly off muskox carcasses, in Malaise traps (0/8 *Er* positive, including one pooled sample), or in BG-Pro traps (0/21 *Er* positive, including six pooled samples).

Phylogenetic relationship of newly-sequenced *E. rhusiopathiae* isolates with those previously characterised

A total of 189 *Er* isolates were sequenced as part of this study, of which 181 had not been previously sequenced (Table 2). All newly sequenced isolates from mainland Canada were genetically distinct from the AC (Figure 1); these comprised two tissue isolates (spleen and tonsil; these were identical so only one sequence is shown on the tree) from a wood bison (*Bison bison athabascae*) from the Dehcho region (NWT) and a bone marrow isolate from a caribou from Bathurst Inlet (NU) (all belonging to clade 2); a muskox bone marrow isolate from the Sahtu region, and a polar bear bone isolate from Sentry Island, NU (both belonging to clade 3) (Figure 2A). Nearly all (167/176, 95%) newly-sequenced *Er* isolates from the CAA belonged to the *Er* AC. The nine non-AC isolates were found from across three clades of *Er*. Clade 1 isolates (n = 6) were from i) a muskox bone marrow sample and two soil samples from Axel Heiberg Island; and ii) two muskox bone marrow samples and a sample of carrion fly larvae/pupae from Ellesmere Island. A clade 2 isolate was obtained from a lymph node of an Arctic fox harvested on Victoria Island in 2023. Non-AC Clade 3 isolates were obtained from a bone marrow sample from a muskox each on Bathurst Island (2022) and Ellesmere Island (2023) (Figure 2A, 2B).

Table 2 *Erysipelothrix rhusiopathiae* isolates (n = 189) newly sequenced (or re-sequenced) in this study. Those previously-published isolates that were re-sequenced were done so to obtain a higher depth of coverage to improve phylogenetic accuracy. For complete metadata associated with all newly sequenced isolates, along with accession numbers, see Supplementary Table S1 and https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02164

Location	Years of isolation	Host species (number of unique individuals)	Sample types**	# isolates (# previously sequenced)	# isolates confirmed AC (# included in molecular clock analyses)
Ellesmere Island	2021-2023	Arctic fox (2), Arctic wolf (5), Muskox (35), Unidentified bird (2)	Spleen, Kidney, BM, Gut, Soil, Faeces, Skin, Nerve, CFL	88 (2)	84 (43)

Location	Years of isolation	Host species (number of unique individuals)	Sample types**	# isolates (# previously sequenced)	# isolates confirmed AC (# included in molecular clock analyses)
Axel Heiberg Island	2022-2023	Arctic fox (1), Arctic wolf (5), Collared lemming (1), Unidentified bird (2), Muskox (10)	Blood, BM, Faeces, Heart, Kidney, Liver, Lung, Gut, Soil, Spleen, CFL, Joints, LN, UB	70 (0)	67 (31)
Banks Island*	2013	Muskox (1)	BM	1 (1)	1 (1)
Prince Patrick Island	2017	Muskox (6), Peary caribou (3), Arctic fox (1)	BM, Bone, Soil, Heart, Lung, Gut	16 (5)	16 (14)
Victoria Island	2015, 2023	Muskox (2), Phocid seal^{+} (1), Arctic fox (1)	Heart, Lung, Blood, LN	8 (0)	7 (3)
Bathurst Island	2022	Muskox (1)	BM	1 (0)	0 (0)
Mainland Canada	2022-2023	Bison (1), Caribou (1), Polar bear (1), Muskox (1)	BM, Bone swab, LN, Spleen, Tonsil	5 (0)	0 (0)

Note. *Sequence AMX7 used as the reference sequence for single nucleotide polymorphism (SNP) analysis **BM – Bone marrow, CFL – Carrion fly larvae, LN – Lymph node, UB – Urinary Bladder; $^{+}$ unverified taxon

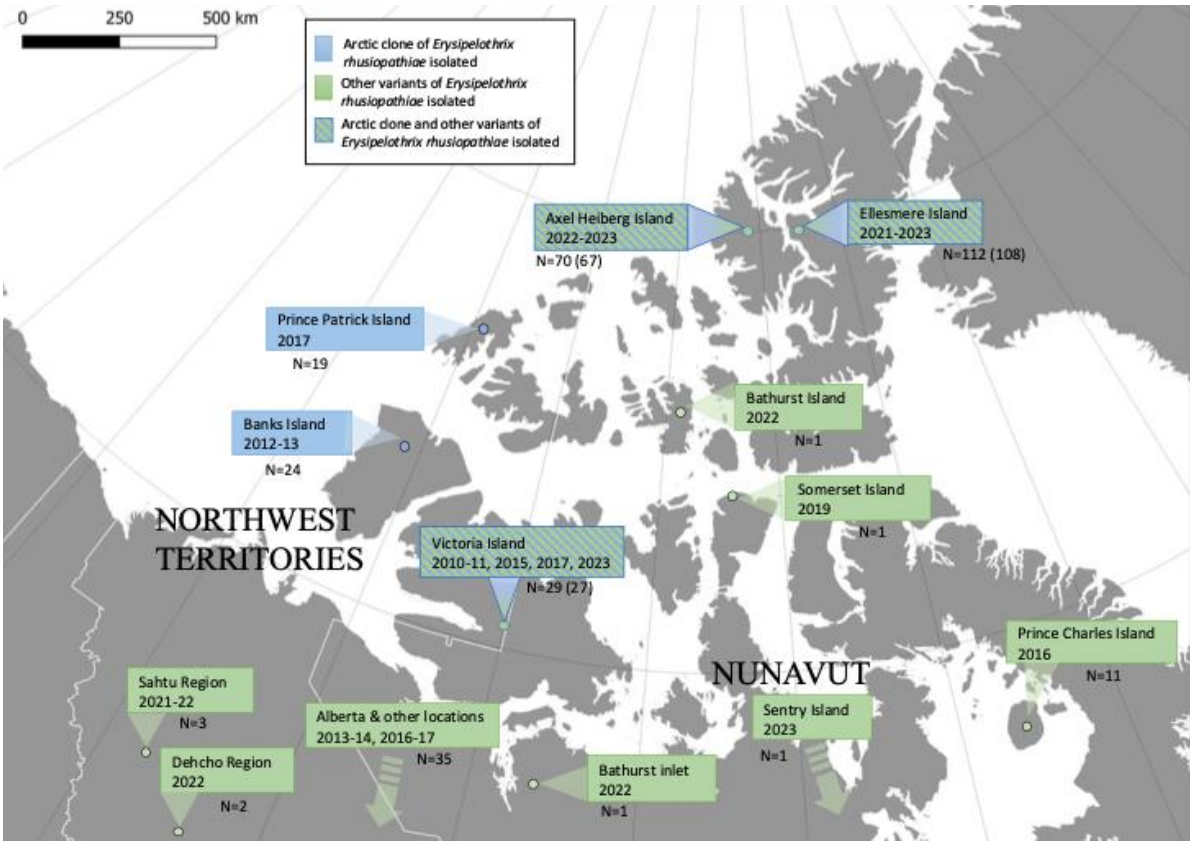

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Figure 1. Map summarizing the origins of *Erysipelothrix rhusiopathiae* isolated from wildlife from various locations across the Canadian Arctic Archipelago and Canadian mainland. White lines indicate territorial boundaries. Colours indicate the strains of *E. rhusiopathiae* isolated, according to the legend. Number of isolates sequenced per location are indicated below boxes; for locations with mixed variants, numbers in parentheses indicate number of Arctic clone isolates.

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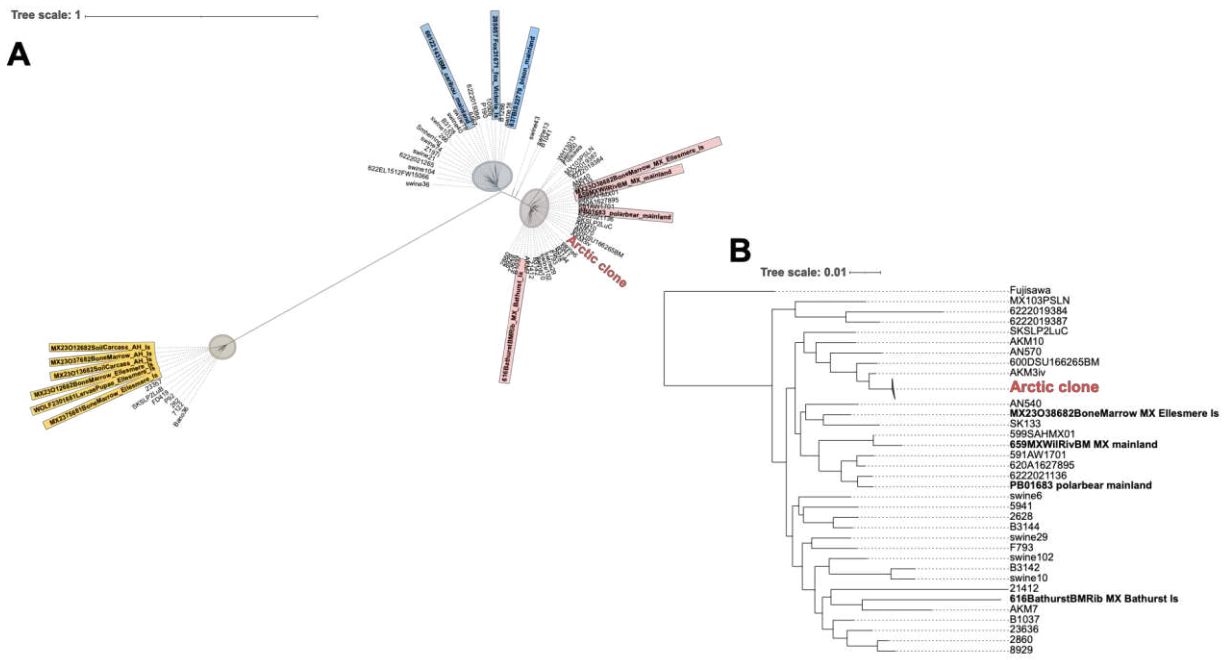

Figure 2. Diversity of newly sequenced *Erysipelothrix rhusiopathiae* (*Er*) isolates shown within the broader global phylogeny. This maximum likelihood phylogeny was estimated in Parsnp v1.2. A) Full *Er* diversity shown as an unrooted tree. Clade 1 isolates are shown circled in yellow, Clade 2 in blue, and Clade 3 in pink, with intermediate isolates un-circled between clades 2 and 3. Newly sequenced isolates appear with taxa labels highlighted and in bold text. Where not included in the isolate name, species and location of newly-sequenced isolates are given at the end of the name, where MX = muskox; AH = Axel Heiberg; Is = island. Representative Arctic clone (AC) sequences are included, but with the clade collapsed. For the full AC phylogeny, see Figure 3. B) Zoom in to Clade 3 to show within-clade diversity, highlighting that the AC forms a monophyletic clade amidst the wider diversity within Clade 3. The most closely related non-AC isolate (AKM3iv, obtained from a muskox from Alaska) differs by >600 SNPs. Tree is rooted to the Fujisawa strain (intermediate).

Diversity of *E. rhusiopathiae* AC isolates within and between CAA islands

Sequences of 228 AC isolates – including the newly-sequenced AC reference genome AMX7 – were included in the SNP pipeline. These isolates were obtained from 105 individual animals at 90 unique carcass sites between 2010 and 2023 on five CAA islands and had a minimum average depth of coverage of 20x. SNPs were identified at 350 unique base positions when compared to

the reference AMX7 sequence. For individual sequences, the mean number of SNP differences from the reference genome was 32.03 (median = 33, range = 17-46). Of these base positions with identified SNPs, the majority (212/350, 61%) were present in only single genomes, while there were nine sites where all 224 sequences had a SNP (i.e. where the reference genome differed from all others). The mean number of SNP differences between a pair of sequences was 13.95 (median = 12, range = 0-48). The largest SNP difference of 48 SNPs was observed between a 2023 muskox bone marrow isolate from Axel Heiberg Island and one isolate each from two different carcasses from 2010 on Victoria Island. The mean pairwise SNP difference between islands (16.88, 95% CI: 16.75, 17.01) was significantly higher than within islands (7.78, 95% CI: 7.67, 7.89, $p < 0.005$). Indeed, isolates from past outbreaks on Victoria (2010-2011), Banks (2012) and Prince Patrick (2017) Islands, as well as those from individual mortality events from Victoria Island (2015) were closely related by geographic origin and clustered together phylogenetically (see phylogeographic analyses).

Phylogeographic analyses to elucidate the spatiotemporal spread of the AC

The combined posterior set of phylogenies estimated using BEAST v1.10.4 is summarized as a maximum clade credibility tree in Figure 3A. The mean clock rate for the AC of *Er* was estimated to be 6.55×10^{-7} substitutions/site/year (95% highest posterior density [HPD]: 5.19×10^{-7} - 8.06×10^{-7}), translating to approximately 1.18 SNPs/genome/year when assuming a mean genome size of 1.8 Mb for *Er*. The most recent common ancestor (MRCA) for all included cases of the *Er* AC (n = 131) was dated to 2006 (HPD: 2004 – 2008; node support posterior probability [p_{node}] = 1); node a in Figure 3A, four years before the first confirmed (by bacterial isolation) outbreak in 2010 on Victoria Island. Although the origin of this outbreak remains uncertain, with roughly equal location assignment posterior probabilities ($p_{location}$) for Banks Island (0.51) and Victoria Island (0.49), there was clear evidence of independent transmission (monophyletic clusters with $p_{node} = 1$ and $p_{location} > 0.98$) on Victoria Island by 2008 (HPD: 2007 – 2009; node b) and Banks Island by 2010 (HPD: 2009 – 2010; node c). This relative independence was also reflected in low estimated rates of exchange between these two islands (posterior medians ~ 1 transition/year in either direction; Figure 3B), with limited support that either rate is > 0 (in the variable selection underlying discrete state phylogeography, rates with Bayes factors [BF] > 3 are generally considered well supported [28]; here BF = 0.94 and 0.76 for Banks to Victoria and

Victoria to Banks, respectively). The majority of cases observed on Prince Patrick Island in 2017 – involving muskoxen, Peary caribou and an Arctic fox – could be confidently linked to a single origin on Banks Island, with an MRCA dated to 2014 (HPD: 2012 – 2015; $p_{node} = 1$; node d) shared by all but one sequence from this island ($BF_{[rate > 0]} = 7.81$). Similarly, all cases on Ellesmere and Axel Heiberg Islands appear to derive from a single introduction ultimately linked to Victoria Island ($BF_{[rate > 0]} = 1.56$), which was estimated to have arrived at Ellesmere Island around 2016 (HPD: 2015 – 2017; $p_{node} = 1$; node e). The location of this introduction is uncertain, however, with Axel Heiberg receiving a non-trivial amount of support as an alternative introduction point ($p_{location} = 0.25$, vs. 0.74 for Ellesmere). This uncertainty may be driven by frequent observations of exchange between these two islands (Figure 3A). Rates of exchange were similar in both directions, with an estimated 1.25 transitions/year from Axel Heiberg Island to Ellesmere Island (HPD: 0.02 – 3.29; $BF_{[rate > 0]} = 29.39$) and 1.20 transitions/year in the opposite direction (HPD: 0.14 – 3.06; $BF_{[rate > 0]} = 10766$). However, while largely independent transmission is evident from at least 2017 on Ellesmere Island (HPD: 2016 – 2018; $p_{node} = 1$; node f) many of the ancestral nodes grouping clusters of sequences derived from Axel Heiberg Island received relatively low posterior support, including the first – and largest – of these, dated to 2019 (HPD: 2018 – 2020; $p_{node} = 0.65$; node g), leaving it unclear whether the *Er* AC has been circulating independently on this island, or if cases have been primarily due to recurring transmission from Ellesmere Island (Figure 3A).

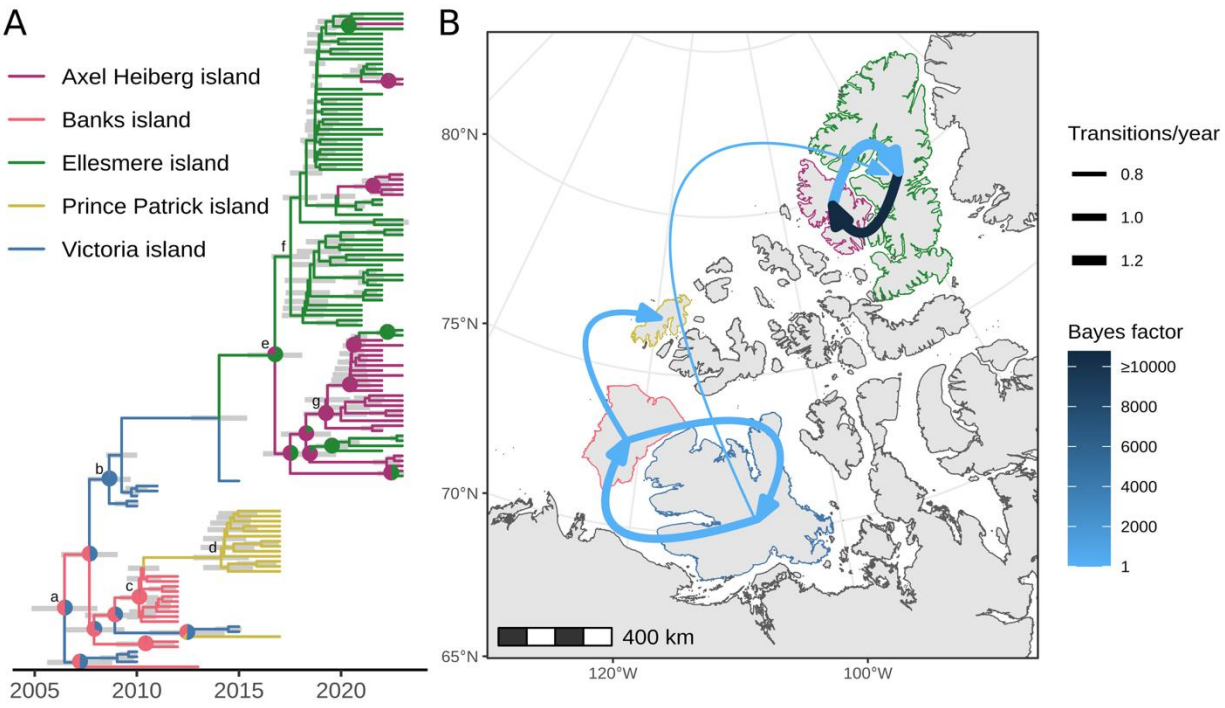

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Figure 3. Discrete phylogeographic reconstruction of inter-island movements from a subset of 131 Arctic clone sequences. (A) Maximum clade credibility tree. Grey bars indicate credible intervals for estimated dates, and pie charts show probabilities assigned to each location at that node, when the probability assigned to the most likely location (i.e. the one used to colour branches) is < 0.99. Lowercase letters (a-g) mark nodes referred to in the main text. (B) Statistically supported transition rates between islands. Line widths represent posterior median transition rates, while colours indicate statistical support for that rate being > 0. For clarity, only rates supported by a Bayes factor ≥ 0.5 are displayed.

228 ## Genetic diversity of *E. rhusiopathiae* within and between carcass sites

229 Between 2010 and 2023, there were 23 unique carcasses across all CAA islands with at least two
230 *Er* 'carcass isolates' (i.e. isolates obtained from samples collected within a carcass such as
231 tissues, blood or faeces) obtained in a single year. The mean pairwise SNP difference between
232 isolates obtained from within a single carcass in the same year was 2.93 SNPs (95% CI: 2.49-
233 3.37; median = 2; range = 0 to 24 SNPs). While most carcasses (n = 14, 61%) had mean pairwise

SNP differences of ≤ 2 SNPs between their various isolates, seven had mean SNP differences of ≥ 3 SNPs, with some isolates differing by 12, 13 or 24 SNPs. Between-carcass diversity of isolates from the same island (mean pairwise SNP difference of 5.75 (95% CI: 5.21- 6.29; median = 4; range = 0-28 SNPs) was significantly higher than within-carcass diversity ($p < 0.001$). Results from the GLM focusing on the diversity from the recent outbreak on Ellesmere and Axel Heiberg Islands also showed that the pairwise comparisons of SNP differences between different carcasses (predicted SNP difference of 8.35, 95% CI: 8.03-8.68) was significantly higher than SNP differences within carcasses (predicted SNP difference of 2.87. 95% CI: 1.97-4.41, $p<0.001$), Carcass vs Soil (predicted SNP difference of 3.41, 95% CI: 2.13-5.45, $p = 0.002$), and Carcass vs Larvae/Pupae (predicted SNP difference of 3.48, 95% CI: 1.91-6.35, $p = 0.04$), but similar to Carcass vs Faeces (predicted SNP difference of 4.47, 95% CI: 2.06-9.68, $p = 0.51$) (Figure 4). When focusing on faecal samples only, pairwise comparison of SNP differences did not significantly vary depending on the species (predicted SNP differences for: muskox vs arctic wolf: 6.35, 95% CI: 3.12-12.93; muskox vs arctic fox: 1.86, 95% CI: 0.29-12.07; muskox vs bird: 0.11, 95% CI: 0-21.82; muskox vs lemming: 1.87, 95% CI: 0.22-16.18, all p-values >0.6) (Figure 4).

The model investigating differences in SNPs for pairwise comparisons between samples collected during the initial sampling of a muskox carcass and samples collected from the same carcass site the subsequent year (bone marrow, larvae/pupae or soil) did not show any significant differences. Predicted SNP differences from isolates collected in year one only (Within carcass: 2.82, 95% CI: 1.53-5.22; Carcass vs Larvae/Pupae: 3.66, 95% CI: 0.95-14.09; Carcass vs Soil: 1.59, 95% CI: 0.55-4.57) were not significantly different from SNP differences from isolates collected in year one compared to isolates collected the subsequent year (Within carcass: 2.93, 95% CI: 1.53-5.60; Carcass vs Larvae/Pupae: 3.80, 95% CI: 1.38-10.42; Carcass vs Soil: 3.21, 95% CI: 2.10-4.91, all p-values >0.7; Figure 4).

Figure 4. Pairwise single nucleotide polymorphism (SNP) distances of *Erysipelothrix rhusiopathiae* Arctic clone isolates compared across different sample types. (A) Visualisation of density estimates for pairwise SNP distances, comparing those for isolates obtained within

muskox carcasses, between muskox samples and larvae/pupae, between muskox samples and soil, between muskox samples and faeces and between different muskox carcasses; (B) pairwise SNP distance comparisons between muskox samples and faecal samples from other hosts, i.e., lemmings, birds, Arctic foxes, and Arctic wolves; and (C) pairwise SNP distance comparisons of isolates within muskox carcasses, between muskox samples and larvae/pupae and between muskox samples and soil for samples collected in year one only versus collected in year one for the muskox samples and in the subsequent year for the bone marrow, larvae/pupae and soil. N represents the number of pairwise comparisons, with the number of carcasses from which these comparisons derive shown in parentheses.

Despite the relatively high within-host diversity observed in some instances, we found evidence of identical isolates being shared across carcass sites. After excluding non-carcass isolates, across the CAA there were 11 unique 'epidemiological clusters', i.e., where at least one identical sequence (having a pairwise SNP difference of 0) was shared by two or more carcasses (Supplementary Table S2). The largest epidemiological cluster was on Ellesmere Island, involving eight carcasses sampled between 2021 and 2023 over an area of 163 km^2. Additionally, isolates from the sole epidemiological cluster identified on Axel Heiberg Island were genetically identical to an Ellesmere Island isolate from a site ~92 km away, providing strong evidence for inter-island movement of bacteria (Figure 5). One of the clusters on Victoria Island involved identical carcass isolates obtained from fresh mortalities (animals that were sampled a few hours to a few days postmortem) in 2010 (two carcasses) and 2011 (one carcass), suggesting ongoing circulation of the same AC genotype for at least one year. In contrast, in some instances, spatiotemporally related carcasses had isolates with larger SNP differences; for example, in one case, isolates obtained in the same year from carcasses situated only 333 m apart, differed by 4-7 SNPs.

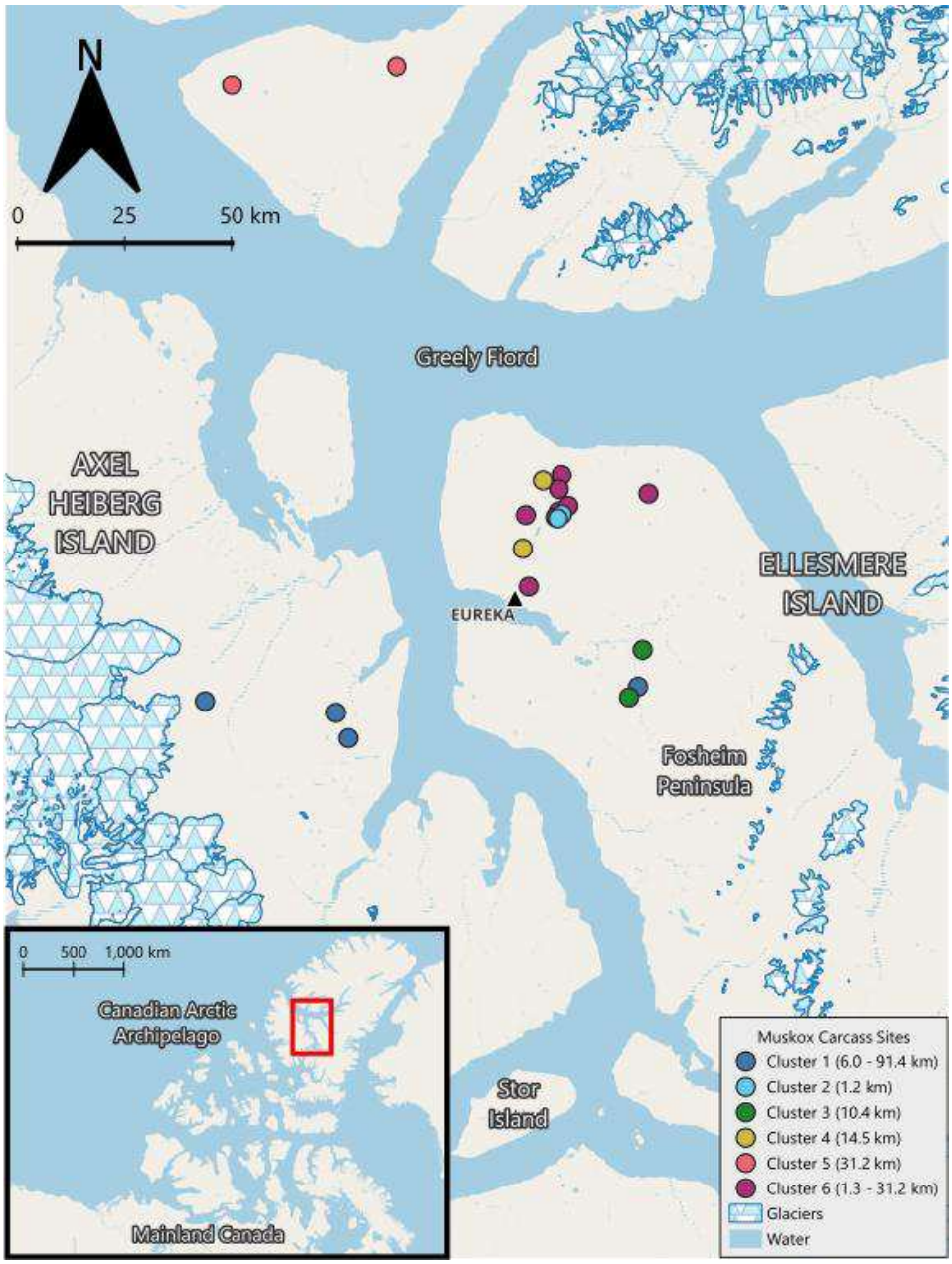

Figure 5. Epidemiological clusters identified on and between Axel Heiberg and Ellesmere Islands. Each point represents a unique carcass site, with colours indicating linked carcass sites where at least one pair of genetically identical (having a pairwise SNP difference of 0) *Erysipelothrix rhusiopathiae* Arctic clone isolates were obtained from those carcasses. Distances between carcasses ranged from 1.2 to 91.4 km, which are denoted in parentheses by cluster. Map developed using QGIS version 4.0.0 ("Norrköping"), available at: https://www.qgis.org.

Discussion

In this study, we report on multi-year field investigations instigated by muskox mortalities in the Canadian high Arctic associated with the Arctic clone (AC) of *Erysipelothrix rhusiopathiae* (*Er*). The combined results of in-depth, multi-host sampling over five years on Ellesmere and Axel Heiberg Islands, along with phylodynamic analyses based on whole genome sequences collected from across the Canadian Arctic Archipelago (CAA) over 14 years, provide novel insights into the epidemiology of this bacterial clone. Specifically, molecular clock estimates shed light onto the spatiotemporal dynamics of the AC's spread across the CAA. Moreover, we uncover multiple factors that could be contributing to the spread and maintenance of this bacterium, with the AC isolated from the faeces of multiple species, and viable bacteria persisting at carcass sites up to seven years, including in insect larvae/pupae.

Phylogenomic analyses suggest the AC has spread across the CAA within 20 years

The availability of AC genomic data from over a 14-year period allowed us to estimate a molecular clock for this clone, and to use this to infer likely timings of introduction events to the different islands of the CAA. Our analyses using BEAST suggest that these events tended to have occurred within only a few years before cases were observed and sampling took place, indicating only limited periods of time wherein the bacterium went undetected at each location, despite their remoteness. The genomically-inferred spatiotemporal spread of the AC thus mirrors the reported outbreaks, with data supporting an initial emergence on Banks or Victoria Island around 2006. While the role of *Er* in muskox mortalities on Victoria Island was confirmed microbiologically in cases only from 2010 onwards, local Inuit harvesters had observed mortalities as early as 2007[13,14], which is consistent with this earlier estimated date of introduction.

Significantly, our analyses suggest that the AC spread north-eastward from Victoria Island to Ellesmere or Axel Heiberg Island by around 2016, covering over 1000 km in approximately a decade. How the bacteria may have spread over such vast distances is discussed below. On the islands where the AC has been isolated, this clone appears to dominate; indeed, 94% of newly sequenced CAA isolates in this study were of the AC, although non-AC isolates were also identified on Ellesmere, Axel Heiberg and Victoria Islands (2023) representing major clades 1, 2, and 3, suggesting the dominance of AC over the wider background of *Er* species diversity. The

limited SNP diversity observed within the AC compared with the largely genetically unrelated strains isolated from other locations is consistent with what would be expected in an outbreak. To the authors' knowledge, such clonal outbreaks of *Er* in wildlife have only otherwise been described in association with mortalities of hundreds of harbour porpoises (*Phocoena phocoena*) in the Netherlands in 2021[29]. This – and its association with the high levels of muskox mortality – has previously led us to hypothesize that this clone may have heightened virulence characteristics[20], although this has not yet been established experimentally. Opportunistic sampling and sequencing of *Er* from animal carcasses on the northern Canadian mainland and from islands in the southeastern part of the CAA in this and previous studies[20] have to date yielded highly diverse *Er* of strains other than the AC (Figures 1 and 2). Expanding the monitoring of muskox and other wildlife populations in neighbouring areas to islands where the AC has been detected would be valuable for understanding the ongoing spread and population-level impacts of this clone; several islands between Banks/Victoria Islands and Ellesmere/Axel Heiberg Islands have been largely unsampled and unmonitored to date.

Carnivores, birds and insect larvae could play a key role in the spread and maintenance of the AC in the Arctic

This study highlights the likely contribution of carnivores and birds in the spread of the AC. Viable AC were isolated from a high proportion of faeces collected from Arctic wolves and Arctic foxes, as well as faeces and castings of birds of unknown species. While muskoxen do not range widely[30], carnivores – particularly Arctic foxes – are known for long-distance dispersals[31], covering an average of approximately 50 km daily, and potentially over 76 km[32,33]. Such movements could conceivably result in much wider spread of the AC than might occur based on between-muskox contacts, particularly since there appears to be a very short infectious period prior to death based on our lack of culture from healthy muskoxen, including during the outbreak on Victoria Island[17]. Indeed, recent epidemiological modelling exercises conducted by our research team suggest that faecal transmission by alternate hosts such as carnivores had a strong potential to influence the ability of *Er* to persist within an ecosystem over time[26]. The fact that highly similar (or in some cases identical) genomes were isolated from carnivores or birds and muskoxen on Ellesmere and Axel Heiberg Islands suggests that these species are likely picking up *Er* when scavenging at carcass sites. Comparisons made between carcass isolates and those obtained from nearby faeces showed high similarity (Figure 4A, B). While not significant due to

the low sample size and associated high confidence intervals, isolates from wolf samples near carcasses trended towards having higher differences, potentially suggesting that they may have picked up these strains from other carcass sites, and are thus contributing to wider strain circulation. Whether or not the bacteria are simply transiting the gastrointestinal tract or lead to a carrier state or persistent infection is unclear; however, either scenario would conceivably allow for bacterial spread through carnivore or bird faeces. Non-exclusive alternate routes of transmission could be via marine mammals, or human-mediated transmission. Marine mammals are well-known to harbour *Er* as carriers, and also regularly display clinical disease[29,34,35]. Long-distance movements between islands could be conceivable[36,37], and given that these are common prey for carnivores in the Arctic, transmission between sea and land is plausible. Interestingly, the AC was isolated from a seal carcass sampled on Victoria Island in 2015, and that isolate was the most closely related to the MRCA of Ellesmere and Axel Heiberg genomes within our dataset (as few as 3 SNP differences). Human-mediated transmission can also not be excluded, including anecdotal reports of a muskox carcass being transported from Victoria Island to Ellesmere Island in 2018, just after the time period (2015-2017) the AC was inferred to have arrived on the latter.

It remains unknown whether the *Er* AC is causing disease in these alternate hosts. Clinical erysipelas has been previously described in Pribilof Island Arctic foxes, where clinical signs of incomplete shedding and polyarthritis led the authors to describe the disease in these animals as 'shaggy lame fox syndrome'[38]. Researchers working to the east of our main study site – on the East coast of Ellesmere Island in the summer of 2022 – mentioned having seen a lame Arctic fox with scruffy pelage that could be consistent with these clinical signs. Moreover, *Er* was isolated from the tail of an Arctic fox found on the tundra in our main study site at Eastwind lake on Fosheim peninsula. Sporadic reports have also described clinical erysipelas in a wide range of bird species[39]. Whereas several bird faecal samples cultured positive for *Er,* no dead birds tested positive for *Er* during our study; however, cause of mortality for the majority was incidental trapping or accidentally confined in a storage shed.

Interestingly, insect larvae/pupae samples collected at carcass sites up to seven years after muskox deaths were regularly culture-positive (Table 1; https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02179; https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02275). Many of these larvae/pupae were alive at the time of sampling (as opposed to being old castings), and were often found in the intervertebral spaces.

Pairwise SNP comparisons and associated GLMs show that larvae/pupae harboured AC strains with the same range of differences to isolates from muskox carcass isolates as those found within the carcass (Figure 4A). Previous investigations of adult invertebrates as putative vectors for *Er* in domestic species have shown a variety of species to be competent transporters of bacteria that can ultimately result in clinical disease in the vectors' hosts[40–42]. As such, the role of adult carrion flies as potential mechanical vectors of *Er* in Arctic systems is an important epidemiological factor for consideration given that at least some of the adult flies we assessed were PCR-positive for *Er* (25%). Whether *Er* infection in larval and pupal stages of carrion insects perpetuates through to adulthood is unknown, so the significance of our high rates of live bacterial recovery from these samples from the perspective of the developing invertebrates serving as disease vectors in adulthood is unclear. However, consumption of carrion larvae by scavengers and insectivorous birds is highly probable and an important consideration as a point of continued exposure for the alternate host species we have identified. The use of scavengers and detritovores by the host-adapted *Er* to extend its life cycle beyond the death of a host, and even be transmitted to new primary hosts, is an open area for further inquiry. Extensive evaluations of the interactions between *Er* and the floral and faunal inhabitants of freshwater ecosystems in Ukraine would suggest that the bacterium is capable of inter-trophic transit and may even undergo selection for enhanced virulence in some microscopic relationships[43].

This research complements our recent work describing the multi-year persistence of viable *Er* in tissues (particularly bone marrow) and in the soil directly beneath muskox carcass sites, as well as the 'greening' around such sites that may attract grazers to *Er* -contaminated zones (Wooten et al., in review). However, our modelling work suggested that carcass sites on their own would unlikely be sufficient for sustaining ongoing *Er* infection within a system[26].

In domestic swine, *Er* is primarily transmitted via the faecal-oral route[44]. However, despite widespread sampling of faeces from live muskoxen during the time of the outbreak on Ellesmere Island (2022-2025), we failed to culture viable *Er* in any of these samples, and only detected *Er* DNA in low abundance (i.e., high Ct values) in a small proportion of samples tested (11%). Further, we found limited evidence to support our previous hypothesis that lemmings could play an important role in the epidemiology of *Er* in the Arctic[17,26]; despite fairly extensive sampling efforts in years following the outbreak, *Er* was isolated from one faecal sample of over 100 samples tested from this species. However, had sampling occurred during the main outbreak

years (2021-2022), we may have had different results, and we cannot discount the possible role of lemmings in the initiation of the outbreak.

Transmission within and between islands

While our phylogeographic analyses establish that the AC has been spread between islands within the CAA, limited subsequent intermixing has been observed between Victoria, Banks and Prince Patrick Islands. We acknowledge that this is likely influenced by the fact that comparatively few carcasses were opportunistically sampled during the outbreaks on these islands, and none afterwards. Despite this limited sampling, it appears that the AC has likely been introduced independently to Prince Patrick Island on at least two occasions (Figure 3A). In contrast, intensive sampling on Ellesmere and Axel Heiberg from 2021 to 2023 established that the *Er* AC has been frequently transmitted between these islands (Figure 3A). Indeed, one epidemiological cluster we observed (Figure 5) involved four carcasses spanning sites as far as 92 km apart between Ellesmere and Axel Heiberg Islands. It is unlikely that muskoxen regularly move between Ellesmere and Axel Heiberg Islands, but long-distance carnivore movements during the winter are common. While most of the epidemiological clusters we observed were more spatiotemporally linked, one further cluster involved eight carcasses detected over three years (2021-2023) on Ellesmere Island. Thus, while we showed that the AC accumulates 1.18 SNPs/genome/year on average, it can go unchanged across multiple years. This could reflect multiple cases being seeded from a common source (e.g., a carcass). We were interested in assessing for evidence of ongoing bacterial proliferation at carcass sites (i.e., beyond mere persistence), although we only had multi-year genomic data from 20 carcass sites, which limited our ability to explore this in-depth. However, pairwise SNP distance comparisons failed to show significant differences in at-carcass diversity when comparing instances where multiple samples were collected in the same vs. subsequent years (Figure 4c).

Conclusions

Here, we have presented the results of multi-year sampling efforts (2021 – 2025) during an ongoing outbreak of the Arctic clone of *Erysipelothrix rhusiopathiae* associated with muskox mortalities on Ellesmere and Axel Heiberg Islands in the Canadian Arctic Archipelago and associated genomic analyses. Phylodynamic analyses incorporating AC sequences from 2010 – 2023 allowed us to establish a molecular clock for this clone and showed the likely spread of the

AC from Victoria and Banks Islands in the southwestern CAA over 1000 km to northeastern Ellesmere and Axel Heiberg Islands in approximately a decade. Consistent with work by Vargas-soto et al., our results strongly suggest an important role for alternate host species – particularly Arctic wolves and foxes – in the spread of the bacterium. We also show that viable bacteria can persist at carcass sites for up to seven years in insect larvae/pupae, potentially representing an ongoing infection source well beyond the death of the muskox. The sequencing of recent opportunistically collected *Er* isolates from the northern Canadian mainland and other islands in the CAA show an ongoing absence of the AC, and highlight the importance of continued monitoring to understand the spread and population-level impacts associated with this bacterium. Our study reinforces the powerful role that pathogen genomics can play in elucidating complex epidemiological processes, particularly for diseases of wildlife in remote and hard-to-reach locations such as the Arctic.

Methods

Ethics

Ethical approval for sample collection from animal carcasses was obtained from the University of Calgary Animal Care Committee (AC18-0093, AC22-0060 and AC25-0211). Wildlife Research Permits for the collection and analysis of the samples described herein were approved by the Government of Nunavut (permits WL2021-053, WL2022-055, WL2022-061, WL2023-046, and WL2025-044).

Sample collection and laboratory methods

A full description of the field methods related to sampling from muskox carcasses can be found in[21]. Briefly, field studies occurred on the Fosheim peninsula of central Ellesmere Island during July and August of 2021 through 2025 and Axel Heiberg Island from 2022 through 2023. During these summers, sampling efforts were centred at muskox carcasses that were identified at the time of field deployment, or had been detected in previous years. Samples (e.g., various tissues, bone marrow and soil) from these carcass locations were then taken through a systematic pipeline to (i) selectively isolate *Erysipelothrix* spp. bacteria and, (ii) for those samples not yielding live bacteria, identify if there was DNA evidence that *Erysipelothrix* was present.

In addition to the samples from muskox carcasses, live muskoxen and other potential host organisms were sampled through a variety of means.

From 2021 through 2025, fresh individual faecal deposits were collected from muskox herds as they were observed in the field. Faecal samples were also collected from other species opportunistically as they were encountered. These included samples from Arctic foxes (*Vulpes* lagopus), Arctic wolves (*Canis lupus arctos)*, Arctic hares (*Lepus arcticus*)**,** Lapland longspurs (*Calcarius lapponicus*), northern collared lemmings (*Dicrostonyx groenlandicus*), Peary caribou (*Rangifer tarandus pearyi*), rock ptarmigan (*Lagopus muta*), snow geese (*Anser caerulescens*), and several samples from taxonomically unclassified avian species (presumably scavengers like common ravens *(Corvus corax principalis*)*,* long-tailed jaegers (*Stercorarius longicaudus*), gyrfalcons *(Falco rusticolus),* or snowy owls *(Bubo scandiacus*)*,* as they were collected at muskox carcass sites). Faecal samples from foxes and wolves tended to be collected close to muskox carcass sites but were occasionally found away from these areas. Aside from one sample from an individual animal, lemming faecal samples were composite samples from three or more burrow openings, winter nests, and/or latrines located within zones of about a 5 m radius of a muskox carcass. These were collected both opportunistically and, in 2024, systematically at paired locations within 10 m of muskox carcass sites (excluding the immediate area around the carcass) and at least 100 m away from them.

Avian castings (regurgitant of indigestible or impassable materials like fur and bones) were collected opportunistically from 2023 through 2025 as well. Prominent rock outcrops and high points where predatory and scavenging avian species will often rest were targeted for collection. As with the avian faecal samples collected at muskox carcass sites, taxonomic identification for the species of origin of these castings was unknown.

From 2022 through 2025, whole carcasses, tissues, bones, and/or swabs were also collected opportunistically, as available or appropriate depending on animal size and degree of decay, from non-muskox species including Arctic hares, an Arctic fox, Arctic wolves, northern collared lemmings, Lapland longspurs, a red knot (*Calidris canutus*), and snow buntings (*Plectrophenax nivalis*). Northern collared lemmings were also sampled via lethal trapping with snap traps baited with a mixture of oats and peanut butter in 2022. A total of 176 traps were set across 5 different carcass sites for between 2-4 nights, resulting in a total of 512 trap nights. For those animals that

were collected as whole carcasses (lemmings and passerine bird bycatch) and were not in a state of advanced decay, subsampling of specific organs or organ systems using sterile instruments was performed prior to *Er* testing. For lemmings, the gastrointestinal (GI) tract from stomach to large intestine, spleen, liver, lungs, and urinary bladder were all individually excised, as available. For birds, the GI tract from proventriculus to large intestine and liver were individually excised, as available.

Finally, both developing and adult insects were collected in 2022 through 2025. Direct collection of larval, pupal, and adult flies from decaying muskox carcasses occurred as they were encountered. Flying insects were also actively trapped and preserved in either 95% ethanol or 99.5% propylene glycol, as described in **Supplementary Methods**.

In the lab, all non-muskox faecal, carcass/tissue, casting, and insect samples were taken through the same culture/PCR protocol utilized for the muskox carcass and soil samples, excluding those stored in bactericidal solutions (e.g., Malaise trap insects, mosquitoes stored in ethanol). Due to large sample size, live muskox faecal samples were pooled by herd prior to testing. In the event pooled samples returned positive by culture or PCR, the herd samples were tested individually. For the adult flies collected from carcasses and mosquitoes, samples totalling more than five or six individuals were pooled by carcass or sampling location and time into groups of no more than 15 individuals prior to DNA extraction as described in Wooten et al.[21] Larval/pupal samples were processed in pools by carcass.

Sequencing, bioinformatics and phylogenomic analyses

Er isolates (n = 156) obtained from muskoxen and other host and environmental samples during the outbreak investigations on Ellesmere and Axel Heiberg Islands from 2022-2023 (Table 2, Supplementary Table S1) were whole genome sequenced, adding to the WGS data initially published relating to this outbreak[20] (Table 3, Supplementary Table S1). Additional sequencing was conducted of *Er* isolates obtained from throughout the CAA (2015-2022; n = 20) and from the Canadian mainland (2022-2023; n = 5) from muskoxen and other mammalian host species. Moreover, a subset of *Er* isolates from 2010–2021 (n = 8) were re-sequenced to obtain higher depth of coverage (Table 2). Isolates were sequenced at MicrobesNG (Birmingham, UK) on an Illumina NovaSeq 6000 platform, generating 250 base-pair, paired-end reads with a minimum average coverage depth of 30X. Sequences were adapter trimmed with Trimmomatic v0.30 [45]

with a quality cutoff of Q15, and assembled using SPAdes version 3.7[46]. Kraken[47] was used to confirm bacterial species identity.

Table 3 Previously sequenced *Erysipelothrix rhusiopathiae* Arctic clone genomes included in phylogenetic and molecular clock analyses. Full details for these sequences can be found in Supplementary Table S1.

Location	Years of isolation	Host species (number of unique individuals)	Sample types*	# isolates (# included in molecular clock analyses)	Reference(s)
Banks Island	2012	Muskox (6)	BM, Spleen, Kidney, Liver	15 (12)	Forde et al., 2016[17]
Ellesmere Island	2021	Muskox (8)	BM, Faeces, Heart, Gut, Kidney, Liver, Lung, Spleen	24 (19)	Seru et al., 2024[20]
Victoria Island	2010-2011	Muskox (7)	Faeces, Gut, Lung, LN, Heart, Tonsil, Liver	14 (8)	Forde et al., 2016[17]

Note. *BM- Bone marrow, LN – Lymph node

To situate newly sequenced *Er* isolates from across northern Canada within a global phylogeny, a core-genome alignment using Parsnp v1.2[48] was performed. In addition to the newly sequenced isolates in this study, genomic data (raw reads) from a total of 115 previously-sequenced isolates available on NCBI GenBank were accessed. This comprised the 53 AC sequences in Table 3, as well as 62 non-AC sequences representing the global *Er* diversity (Supplementary Table S1).

To evaluate the evolution of the *Er* AC over space and time, this study included all genome sequences available from current and past outbreaks that passed the quality thresholds described below (n = 225). This included isolates from Victoria, Banks, Prince Patrick, Ellesmere and Axel Heiberg Islands (2010-2023) (Figure 1, Supplementary Table S1). Read mapping and variant

calling were conducted on the CLIMB platform for microbial genomics[49], using a pipeline available at https://github.com/tlforde/OHRBID. A newly assembled complete genome of the AC isolate AMX7 (from a muskox from Banks Island) was used as a reference (Accession GCF_977118275.1). This genome was assembled using the hybrid assembly service of MicrobesNG (Birmingham, UK), using short reads with a minimum average coverage depth of 30X generated by the same Illumina platform as above and long reads with a minimum 50X depth of coverage generated by Oxford Nanopore Technologies-based services on a GridION with R10.4.1 flowcells. For all other sequences, trimmed reads were aligned to this AMX7 reference genome using bwa-mem v0.7.17. Picard v2.27.4 (http://broadinstitute.github.io/picard) was used to mark/remove duplicates, add read group info and index BAM files. VarScan v2.4.4[50] was used to detect SNPs with parameters of minimum read depth 8, base quality ≥20, and variant allele frequency ≥0.8. A maximum-likelihood phylogeny was estimated from concatenated SNPs with IQ-TREE v2.2.2.3[51], using the K3P+ASC nucleotide substitution model as the best-fit model. Pairwise SNP differences between isolates were calculated with snp-dists v0.6 (https://github.com/tseemann/snp-dists), and subsequent comparative analyses were carried out in R v4.4.2 (R Core Team, 2024). Mean pairwise SNP differences were compared from isolates obtained within vs. between islands using Welch's two-sample t-test with unequal variances.

Analysis of Genetic Diversity on Ellesmere and Axel Heiberg Islands

To explore the genetic diversity associated with different types of samples collected during the in-depth outbreak investigations on Ellesmere and Axel Heiberg Islands (2021 – 2023), pairwise SNP differences were calculated between all genomes sequenced from these two islands. Pairwise comparisons were classified into five categories: "Within carcasses" comprised comparisons between isolates recovered from the same muskox carcass; "Carcass vs Faeces" comprised comparisons between isolates from a muskox carcass and faecal samples from other host species collected at the same carcass site; "Carcass vs Larvae/Pupae" comprised comparisons between isolates from a muskox carcass and insect larvae or pupae collected at the same carcass site; "Carcass vs Soil" comprised comparisons between isolates from a muskox carcass and soil samples collected at the same carcass site; and "Different carcasses" comprised comparisons between isolates recovered from different muskox carcasses.

Differences in pairwise SNP differences among comparison categories were assessed using a negative binomial Generalized Linear Model (GLM) fitted with the *glmmTMB* package[52]. Pairwise SNP difference was included as the response variable and comparison category as a fixed effect. Because carcasses with larger numbers of sampled isolates generated disproportionately more pairwise comparisons, each comparison was assigned an inverse-frequency weight (weight = $1/n$), calculated within each carcass-site combination and comparison category, to prevent heavily-sampled carcasses from disproportionately influencing model estimates. Model assumptions were assessed using the *DHARMa* package[53], and post hoc pairwise comparisons among categories were performed using Tukey-adjusted contrasts.

We followed the same procedure to investigate differences in SNPs for pairwise comparison focussing on isolates from muskox carcasses and faecal samples, separating those by species (Arctic fox, Arctic wolf, birds and lemmings), using a negative binomial GLM. The response variable was pairwise SNP differences and the fixed effect was the different species for faecal sampling. The same inverse-frequency weighting procedure described above was applied, model diagnostics were assessed using the *DHARMa* package, and Tukey-adjusted post hoc comparisons were used to compare model-estimated means.

To investigate temporal changes in genetic similarity, pairwise comparisons were further classified according to sampling year. Comparisons were assigned to either "Year one only", in which each pairwise isolates were collected during the initial sampling of a fresh muskox carcass, or "Year one vs subsequent year", in which, for each pairwise comparison, one isolate was collected during the initial sampling and the other was collected from the same carcass site during a subsequent year. This analysis was restricted to the Within carcass, Carcass vs Larvae/Pupae, and Carcass vs Soil comparison categories. A negative binomial GLM was fitted with pairwise SNP difference as the response variable and the interaction between comparison category and sampling-year category as fixed effects. The same inverse-frequency weighting procedure described above was applied and we conducted the same model diagnostics and post-hoc Tukey tests.

Reconstructing the spatiotemporal spread of the AC

The alignment of concatenated SNPs was used for molecular clock and phylogeographic analyses after excluding a subset of identical or highly similar sequences; these were excluded

using a cut-off value based on the mean pairwise SNP differences between isolates obtained from a single carcass in a single year. Based on the observed within-carcass diversity and the mean pairwise SNP difference of 2.93 SNPs between tissue isolates from the same carcass (see Results), the number of unique sequences used for these analyses was refined to 131 sequences (including the AMX7 reference genome) as follows:

1. For each animal with multiple tissue isolates / sequences, all sequences with 3 or more SNP differences compared to other sequences from the same animal were included. Where multiple sequences from the same carcass had < 3 SNPs difference, one sequence was arbitrarily selected. Where possible, bone marrow isolate sequences were prioritised, as bone marrow was the most common tissue source of isolates in the study dataset.

2. Where carcasses were sampled across multiple years (2 or more years between 2021 and 2023, which may not be consecutive), only isolates from the first sampling year were included.

3. For sequences from soil, scavenger faeces or carrion fly larvae samples, all sequences with < 3 SNP differences compared to sequences chosen above were excluded (i.e. those closely related to a carcass-derived isolate).

The remaining alignment was reduced to contain only variable sites, separately recording the number of non-varying sites involving each nucleotide base based on the AMX7 reference genome to allow correction of mutation rates during the phylogeographic reconstruction below. Next, a test for the most appropriate substitution model was performed using IQ-TREE version 2.3.6[51,54], restricting the set of models considered to those compatible with any version of BEAST 1. The best model according to both BIC and AIC was a General Time Reversible (GTR) model with empirical base frequencies. Finally, a discrete phylogeographic reconstruction [28] was performed using BEAST version 1.10.4[55], allowing for asymmetric rates of movement between islands. This analysis used a Bayesian SkyRide demographic prior[56,57] along with a log-normal uncorrelated relaxed molecular clock[58], and the GTR substitution model selected above. Since the mutation rate of *Er* remains unknown, the mean clock rate was given a broad uniform prior between 10^{-8} and 10^{-5}, broadly in line with values seen for *Escherichia coli*[59]. The reconstruction was performed across two independent MCMC chains of 50 million steps each,

saving values every 5000 steps. After discarding the first 10% of samples and visually confirming convergence and appropriate effective sample sizes in each chain with Tracer version 1.7.2[60], the combined posterior set of phylogenies across both chains was summarized as a maximum clade credibility tree, with clade ages summarized using the common ancestor algorithm[61] in treeannotator version 1.10.4[55]. Phylogenies were visualized using ggtree version 3.14.0[62] in R version 4.1 (R Core Team, 2024).

Data Availability

All data included in this manuscript are available in the supplementary materials and in the following open datasets: https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02164; https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02179; https://data.bas.ac.uk/full-record.php?id=GB/NERC/BAS/PDC/02275

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