”Slutrapport” / end of project report

Belysning av återkommande virusutbrott bland arktiska hunda / Elucidating recurring viral outbreaks among Arctic dogs

N2022-0008

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Summary:

Recurring viral outbreaks among Arctic Greenland sled dogs result in a high number of deaths among puppies and adults. Nevertheless, we still don't know the exact etiology, or which viruses these dogs generally carry (asymptomatically). E.g., canine distemper virus - a likely important pathogen - often utilizes asymptomatic carriers. For the first time, shot gun sequencing (SGS) was used to finally shed light on viruses of Arctic dogs, particularly Greenland Sled dogs. Our efforts are still not finalized, but still underway. Thus far several viral (but also bacterial) elucidations have been achieved through SGS and other molecular tools. Progress is presented herein.

Background

The unique Greenlandic sled dog (GSD) is risking extinction – and with it, it takes a thousand-year-old sled dog culture (> 10.000-year-old if we include the earliest excavations of dogs and dogsleds in Siberia, unpubl. information!) that, in itself, plays a major role in the self-identity and national pride of Greenland (Sonne et al. 2018). The decline of these dogs is due to social- and climatic factors, but also due to frequent outbreaks of infectious diseases (Vernersen and Jensen 2018). These outbreaks are poorly defined and there is a need to understand these events before the population reaches a critical low. Dogs not only die from infectious diseases, however, while virtually no veterinary assistance is attainable for the GSD, many thereby die or are culled, on behalf of minor diseases or trauma, that could be readily treated by a veterinarian. Moreover, animal welfare issues are an ongoing problem in Greenland (pers. comm. S. Wennerberg, 2017-2019, official Greenlandic veterinarian). Knowledge concerning the health and diseases of sled dogs in Greenland is close to non-existing. QimmeqHealth works to protect the Greenlandic sled dog by pro-actively meeting these challenges related to sled dog health and welfare.

Viral disease outbreaks play a great role in relation to modern health risks and fatalities among Greenland sled dogs, but extremely little is known about the origin and exact identity of these viruses, or about the viral carrier status of Greenland sled dogs. On of the consequences of this lack of knowledge, is that the actual efficacy of a national vaccine regimen is unknown. Both viral and bacterial microbiome are even more than ever relevant to elucidate in an Arctic species existing in the intermezzo between humans and nature in the Arctic, while the Arctic is currently changing at a drastic rate, along with it also the dispersal and occurrence of pathogens. Thus an eluciation of viral (but also bacterial) carrier tendencies among Greenland sled dogs is important for several reasons.

This project was a pilot project to identify the parvo and distemper-viruses occurring in Greenland sled dogs and likely causing epizootics among dogs, particularly puppies. Furthermore, we sought to identify unknown or undescribed viruses in Greenland sled dogs in Greenland. In doing so, we were also able to characterize the bacterial gut composition.

Status and results of project

Our project "Elucidating recurring viral outbreaks among Arctic dogs" was fortunate to receive 162,500 SEK from Agria och Svenske Kennelklubbens Forskningsfond ("the Foundation" from here on) in 2022.

This report is regrettably delayed relative to the general guideline for this sort of report to the foundation, and for that I apologize sincerely. The reason is that we have not yet finalized our work. The silver lining of this delay is however that I have managed to merge parallel projects with this so that these projects, together, are achieving more results than initially suggested in the original application. The original aim, in brief, was to search for, isolate and identify distemper and parvovirus from the unique but declining Greenland sled dogs, as well as to perform so called "virus discovery" in fecal matter, meaning the identification of previously un-appreciated viruses in these unique dogs located in a place where climate change is quickly changing pathogen dynamics.

Without actual outbreaks of distemper virus, the virus is difficult to find in apparently healthy dogs, but we have identified and isolated the virus. It has been through an initial sequencing and identification process, but further genetic resolution is needed to differentiate the strain from the vaccine strain. A PhD student, Elsa Brenner, working on another similar project, is highly interested in furthering our genetic identification of the virus. She will approach this work in April 2025.

As for canine parvovirus (CPV), we have thus far had interesting results. One of the biggest problems and frustrations for sleddogs owners in Greenland is the great loss of puppies due to infectious disease resembling parvovirus – in spite of vaccination efforts! In a pilot screening for parvovirus in fecal samples from 13 apparently non-diseased puppies, parvovirus was found in 9 of them! Moreover, it has been theorized (Faraji et al., 2022; Thaiwong et al., 2016) that disease related to parvovirus is exacerbated by co-occurrence of canine circovirus. We therefore also screened for canine circovirus and found that 10/13 were positive, and circovirus co-occurred in all puppies positive for parvovirus! See also ***Figure 1***.

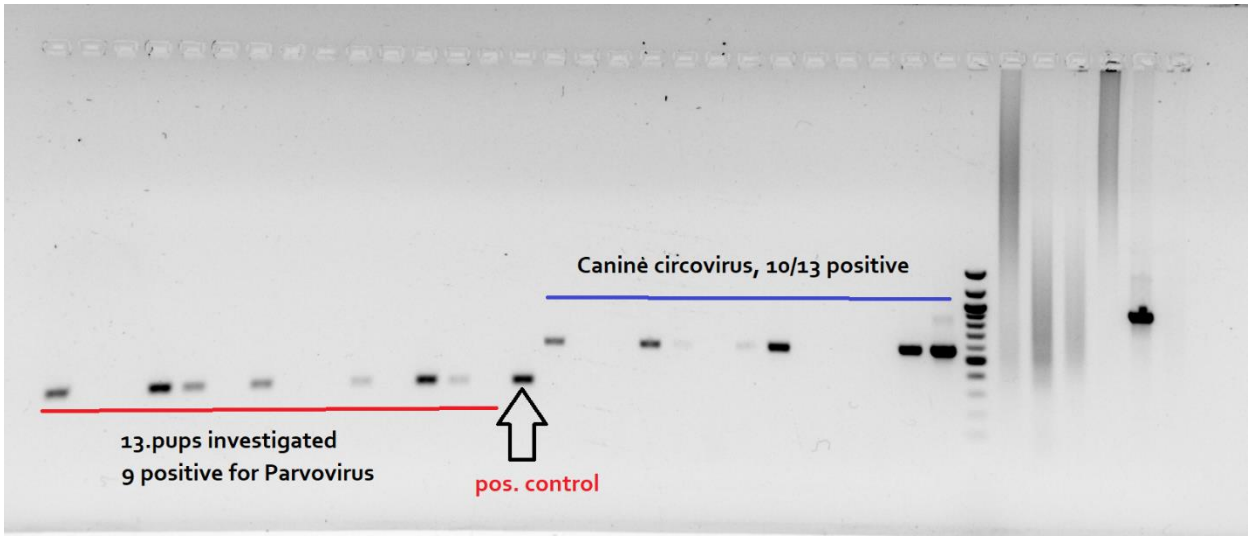

***FIGURE 1** SCREENING GREENLAND SLEDDOG PUPPIES FOR CANINE PARVO- AND CIRCOVIRUS WHICH ARE LIKELY CAUSES OF HIGH FATALITY RATES AMONG THESE PUPPIES*

We subsequently sequenced positive samples and found that the parvovirus was a so called CPV-2a strain and that it was genetically closer to a wildlife racoon strain than to CPV-2a in Danish dogs. CPV2 is the most common parvovirus to cause severe gastroenteritis in dogs world wide, particularly prevalent in- and dangerous to puppies. It is highly contagious, and the 2a-strain is one of the dominant disease-inducing strains among dogs. The circovirus seemed to be a recombinant strain (hybrid strain) between a dog and wildlife circovirus strain originating from North America. We have now gone on to a "virus discovery" step, where we will be able to identify unknown viruses in the samples. We are yet to receive these results for the puppy fecal samples, but in 2023-24, with so-called "shot gun sequencing", we conducted very similar investigations on ca. 40 fecal samples from East Greenland sled dogs, which revealed very interesting results! Here we also found evidence of both CPV1 and 2, but also an array of other DNA viruses. RNA viruses were, however, not targeted in this shot gun sequencing due to the higher cost, and distemper virus discovery was therefore not possible in this particular analysis. However, interestingly, civilian owned dogs in Ittoqqortoormiit had a significantly higher amount of virus in their feces compared to military owned dog in Daneborg. Moreover, the civilian owned dogs carried more Adenovirus, Papillomavirus and parvovirus than military owned dogs. See also Figures 2-6. The difference between these groups is most likely due to differences in management. The difference in management of these two groups include veterinary assistance for military owned dogs (MOD), which in unattainable for civilian owned dogs; sufficient feeding/good body condition score of MOD; clean water offered to MOD for hydration in clean bowls; frequent sanitizing of dog yards, thus frequent removal of feces (for MOD); regular deworming (MOD); less feeding of wildlife to MOD dogs compared to civilian owned dogs; dog houses (MOD); and a higher amount of exercise for MOD. Apart from these management related differences, the two groups of dogs are *not* genetically diverged populations.

These DNA sequencing results also included intestinal parasite DNA, and interestingly, the findings corroborated parallel findings of classical parasitological fecal screenings for parasites. A further ancillary result of this study was not only the characterization of the dogs' intestinal viral microbiome, but also its' bacterial microbiome, and even a characterization of these bacteria's functions. For example, it was shown that dogs in Ittoqqortoormiit, to our surprise, carried bacteria that presented with antibiotic resistance! The most obvious explanation for this, was the fact the dogs were kept close to a creek, for which, upstream, garbage was dumped including human waste… In contrast to this, there were no antibiotic-resistant bacteria found in the intestinal microbiome of military working dogs in the Sirius sleddog patrol located further north on the Greenlandic east coast. This is a significant find for Greenlandic communities because it underlines the importance of proper waste disposal! See also *Figure 3*.

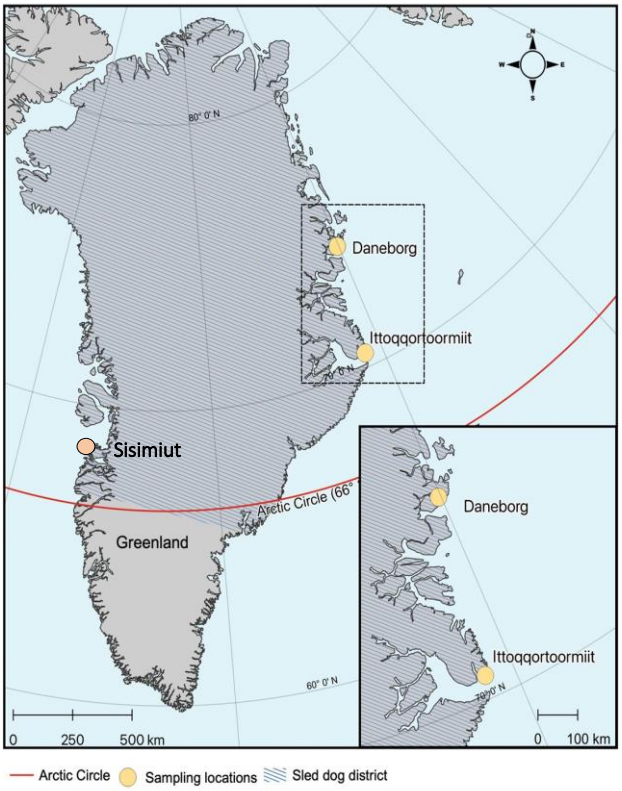

FIGURE 2 MAP OF STUDY SITES. GREENLAND SLED DOG (GSD) FECAL SAMPLES WERE COLLECTED IN SISIMIUT IN DECEMBER 2024 AND IN DANEBORG AND ITTOQQORTOORMIIT AUGUST-SEPTEMBER 2022. DANEBORG MAINTAINS A POPULATION OF 78 MILITARY-MANAGED GSDS, WHILE ITTOQQORTOORMIIT HAS A POPULATION OF CIVILIAN-OWNED GSDS. BOTH SITES ARE WITHIN THE SLED DOG DISTINCT (HASHED LINES) OF GREENLAND, ABOVE THE ARCTIC CIRCLE (RED). FIGURE CREATED USING QGREENLAND (V. 3.0) IN QGIS.

FIGURE 3 DOGS LOCATED IN ITTOQQORTOORMIIT ADJACENT TO A CREEK WHICH WAS CONTAMINATED WITH WASTE WATER AND GARBAGE RESULTING IN THE OCCURRENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN THE FECES OF THESE DOGS.

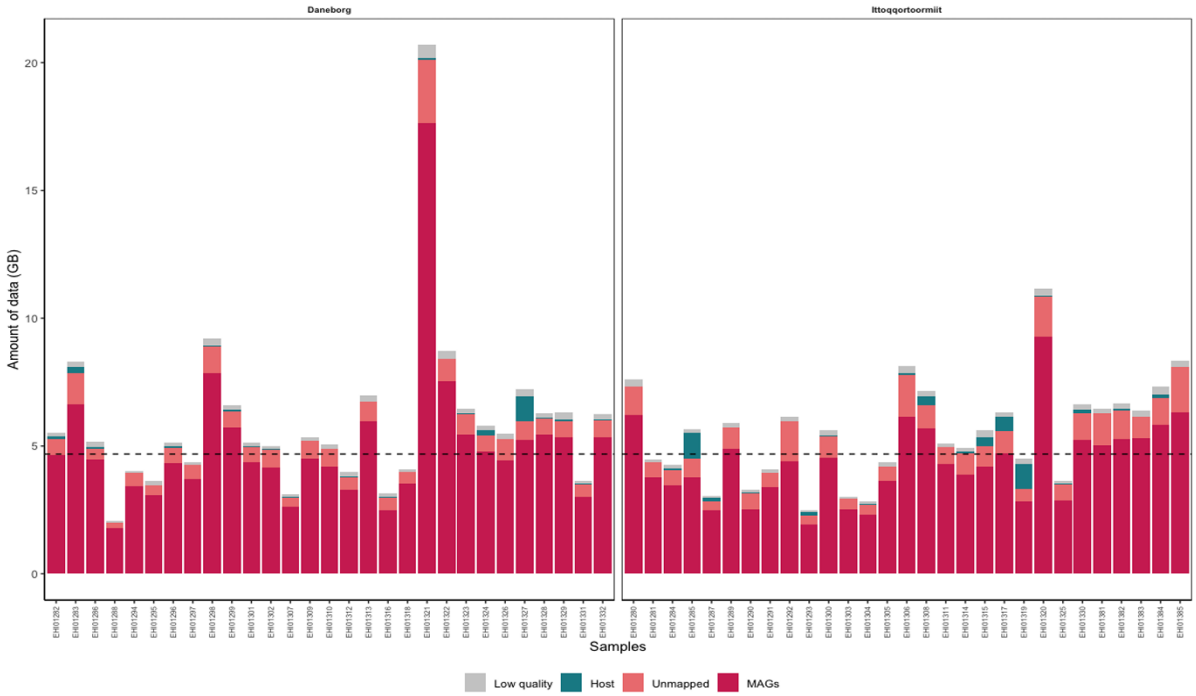

FIGURE 4. 58 FECAL SAMPLES ANALYSED TO CHARACTERIZE THEIR MICROBIOME. THE AMOUNT OF GENETIC DATA PER SAMPLE IS SHOWN AS BARS (DARK PINK). GREEN: HOST DNA (HIGHER AMOUNTS CAN SIGNIFY ENTERITIS), LIGHT PINK: UNMAPPED GENETIC SEQUENCES (UNKNOWN ORIGIN), GRAY: LOW QUALITY SEQUENCES (USELESS). DOTTED LINE: AVERAGE SEQUENCING DEPTH. (BRENNER, MASTER'S THESIS 2024)

Taxa	DB mean	DB sd	IT mean	IT sd	padj (BH)
Eukaryota	8.9	1.7	8.2	2.2	
Cryptosporidium spp.	0.0058	0.0012	0.0076	0.0032	0.037
Echinococcus spp.	0.013	0.017	0.010	0.0059	
Giardia spp.	0.00036	8.0E-04	0.00051	5.3E-04	0.037
Sarcocystis spp.	2.0E-05	NA	0.00025	1.9E-04	
Taenia spp.	0.0012	0.0021	0.0017	0.0029	
Toxascaris leonina	2.3E-05	5.8E-06	8.8E-04	9.4E-04	
Toxoplasma gondii	0.0044	0.0013	0.0053	0.0050	
Trichinella spp.	0.010	0.0028	0.0083	0.0017	0.0033
Uncinaria spp.	0.0012	0.0047	7.2E-05	7.3E-05	
Viruses	0.073	0.033	0.38	0.80	0.012
Adenoviridae	0.012	0.0095	0.082	0.11	0.017
Papillomaviridae	0.027	0.02	0.21	0.26	0.0025
Parvoviridae	0.26	0.15	0.41	1.9	9.0E-07

FIGURE 5 SHOT GUN SEQUENCING RESULTS OF PARASITES AND VIRUSES IN GREENLAND SLED DOGS. DB: DANEBORG, MILITARY OWNED DOGS IN NE GREENLAND, IT: ITTOQQORTOORMIIT, CIVILIAN OWNED DOGS EAST GREENLAND. NUMBERS RELY ON PERCENTAGES OF VIRAL OR PARASITIC DNA IN THE SAMPLES. (BRENNER, MASTER'S THESIS 2024)

Family		DB	IT	p-value (Fisher's exact)
Adenoviridae		19	27	0.021
	Canine mastadenovirus *	0	3	
	Human adenovirus ^	3	24	3.38E-08
Papillomaviridae		28	29	
	Human papillomavirus ^	28	29	
	Tick associated papillomavirus lsx	0	25	2.72E-12
Parvoviridae		28	27	
	Bocaparvovirus	23	27	
	Bufavirus 1 ^	9	10	
	Canine bufavirus *	0	1	
	Canine minute virus (CPV1) *	0	1	
	Canine parvovirus (CPV2) *	1	0	
	Canine protoparvovirus *	0	1	
	Human bocaparvovirus ^	19	1	6.40E-07

FIGURE 6 DETECTED ABUNDANCE OF 3 VIRAL FAMILIES IDENTIFIED IN FECAL SAMPLES FROM GREENLAND SLED DOGS. NUMBERS REPRESENT THE TOTAL NUMBER OF SAMPLES FROM DANEBORG (DB) AND ITTOQQORTOORMIIT (IT) WHERE VIRAL FAMILIES WERE PRESENT. * CANINE INFECTION POTENTIAL, ^ HUMAN INFECTION POTENTIAL, I.E., POTENTIALLY ZOONOTIC (BRENNER, MASTER'S THESIS 2024)

Continued analyses

Our efforts are currently focused on further characterization of parvovirus strains, as well as distemper strains. We are also conducting more virus discovery analyses on a new batch of samples from West Greenland. This work is primarily being carried out by senior veterinarian Emilie A.-Ranberg (author), Ass. Prof. Marta Canuti and PhD-student Elsa Brenner (all Uni. of Cph). We expect to have further parvoviral results and virus discovery results during late spring or summer this year, and further distemper results by the fall 2025.

Significance

We are looking forward to disseminating our findings with a broader scientific audience when the article in writing is finally published (Brenner et al. in writing). While our findings are novel both in the sense of our concrete findings but also in the sense of the methodology of using metabarcoding/SGS to screen Greenland sled dogs for viruses and other microbiota. From our pilot, we have among others found an extremely high carrier rate of parvovirus among Greenland sled dog puppies that are otherwise asymptomatic, which shows that the general infection pressure is very high in Greenland for this virus which has high epizootic potential. It also shows that puppy health and survival are highly dependent on maternal health and immunity as well as an environment with few other biological stressors, i.e., shelter, high quality and sufficient quantity of maternal lactation and other types of nutrition.

We have found several viruses in the sled dogs which have not been acknowledged previously, see Figure 5-6. The significance of these in terms of sled dogs' health is however still largely unknown.

In societal terms, our greatest discovery has perhaps been the finding of antibiotic-resistant bacteria in sled dogs, because it clearly prompts further societal action towards improved sanitation and garbage disposal. The current way waste is handled creates a high risk of transmission of antibiotic-resistant bacteria from e.g. dogs, or from marine wildlife to humans handling these animals. Antibiotic resistant bacteria were otherwise intuitively not believed to be a problem in remote areas of Greenland because of the low no. of humans living there, and the assumed relatively low use of antibiotics, but our study has proved this intuition and assumptions wrong.

Our work has also spurred further interest and studies on the sled dogs, and multiple parallel projects are currently underway. For example, the author's (Emilie A.-Ranberg) initiative to offer veterinary assistance in various shifting locations in Greenland (as well as one semi-permanent location in Sisimiut), has tremendously increased our access to samples from sled dogs while at the same time giving back to the community by offering free veterinary assistance.

In more general terms, our work helps to sustain a political, scientific and legislative focus on the unique dog which has been pinned down by diseases, policies, legislation, demographic-, social and climatic changes.

Publications

Thus far, our results have been published in:

Brenner E, Master's Thesis 2024. Investigating associations between pack management strategies and the intestinal microbiota of Greenland sled dogs (Canis lupus familiaris borealis). University of Akureyri, Faculty of Business and Science University Centre of the Westfjords, Master of Resource Management: Coastal and Marine Management, Ísafjörður.

And partially in:

Andersen-Ranberg E. PhD submitted Nov. 2024 defended and granted in Feb. 2025: Contemporary health hazards of the Greenland sled dog in Greenland. University of Copenhagen, Department of Veterinary Clinical Sciences.

A scientific paper is however currently in writing aimed at one of the more renowned microbiological journals, and the working title is thus far: Intestinal microbiota of two different populations of Greenland sled dogs in Greenland using metagenomics by Brenner E, Andersen-Ranberg E and Aizpura O.

Parvoviral and distemper focused results will be most thoroughly presented in a final paper which will be written as a cooperation between E Andersen-Ranberg (the author) and Ass. Prof. Marta Canuti (Uni. of Cph).

Text References

Brenner E, Master's Thesis 2024. Investigating associations between pack management strategies and the intestinal microbiota of Greenland sled dogs (Canis lupus familiaris borealis). University of Akureyri, Faculty of Business and Science University Centre of the Westfjords, Master of Resource Management: Coastal and Marine Management, Ísafjörður.

Sonne C, Langebæk R, Dietz R, et al (2018) Greenland sled dogs at risk of extinction. Science 360:1080–1080

Vernersen ES, Jensen JJ (2018) Bachelor's Thesis: Hundesyge i Historisk Perspektiv

Wennerberg SE (2017) pers.comm., official veterinarian for the Greenlandic Government, only veterinarian physically present in the sled dog district, responsible for reporting cases of neglect among other responsibilities. Contact: sawe@nanoq.gl