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Contagious Ecthyma Dermatitis as a Portal of Entry for *Erysipelothrix rhusiopathiae* in Muskoxen (*Ovibos moschatus*) of the Canadian Arctic

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ABSTRACT: *Erysipelothrix rhusiopathiae* was detected immunohistochemically in contagious ecthyma (orf virus) dermatitis in two muskoxen (*Ovibos moschatus*), harvested and found dead in 2014 and 2015, respectively, on Victoria Island, Canada. This may help target further research on *E. rhusiopathiae* epidemiology and mechanisms of infection in muskoxen, recently associated with widespread mortalities in Canada's Arctic.

Erysipelothrix rhusiopathiae is a facultative, anaerobic, gram-positive bacillus found ubiquitously in nature in both the terrestrial and marine environments (Wang et al. 2010). To date, 28 serotypes within the *Erysipelothrix* genus have been recognized worldwide from a range of vertebrates (including humans), invertebrates, water, and soil (Wang et al. 2010). Depending on intrinsic virulence factors and host immunity, *E. rhusiopathiae* can be a pathogen or a commensal, as well as a saprophyte (Wang et al. 2010). Disease ranges from localized to generalized cutaneous lesions to potentially fatal septicemia associated with endocarditis or polyarthritis (Wang et al. 2010). Immunization against *E. rhusiopathiae* is common in farmed animals—especially in the swine, poultry, and ovine production systems—to avoid losses caused by acute and chronic erysipelosis (Wang et al. 2010). In free-ranging wildlife, *E. rhusiopathiae* has been associated with widespread mortalities of muskoxen (*Ovibos moschatus*) between 2009 and 2013 on Banks and Victoria islands in Canada's Arctic (Kutz et al. 2015; Forde at al. 2016; Tomaselli et al. 2018). Preliminary whole-genome sequencing and phylogenomic analyses of *E. rhusiopathiae* isolates from the muskoxen found dead at those locations suggested limited heterogeneity compared with mainland isolates, supporting the hypothesis of a recent pathogen introduction on these islands (Forde et al. 2016). However, a recent retrospective serology study documented that *E. rhusiopathiae* has probably been circulating longer in muskox populations: seropositive animals were found among the earliest tested sera from different locations (1976 in Alaska, US; 1991 on Banks Island, Northwest Territories, Canada; and 2011 on Victoria Island, Nunavut, Canada); seroprevalence was also greater in association with unusually high mortality rates and population declines (Mavrot et al. 2020). It remains unclear whether a more-infective and virulent *E. rhusiopathiae* genotype has emerged on Banks and Victoria islands in recent years or whether ecologic conditions have changed, triggering a web of disease causation, including novel pathogen interactions.

In the same geographic area of Canada's Arctic, contagious ecthyma (orf virus) has also been recently described in muskoxen (Tomaselli et al. 2016; Dalton 2019; Rothenburger et al. 2021) as an emerging or re-emerging disease (Tomaselli et al. 2018). Contagious ecthyma is caused by the orf virus, a DNA virus belonging to the *Parapoxvirus* genus of the Poxviridae family, which can infect both animals (domestic and wild ungulates) and humans. The virions are extremely resistant in the environment, where they can remain

infective for several years in shed scabs (Hargis and Ginn 2012). Proliferative lesions are generally found on the skin of the head, the lower legs, and on mucocutaneous junctions and appear as a superficial, thick, brown or gray crust, commonly ulcerated (Vikøren et al. 2008). Ulcerated lesions can be extremely painful, limiting the ability of infected animals to feed or walk properly (Vikøren et al. 2008), and affected mothers often abandon their suckling calves, possibly due to painful udder lesions (T. Bretten pers. comm.). Although orf lesions are typically more severe in juveniles (calves and yearlings), natural infection does not confer long-term immunity; animals at any age can be reinfected and develop proliferative dermatitis (Hargis and Ginn 2012). Severe infection can lead to mortality by starvation or predation and may predispose an animal to secondary fatal infections by providing an entry point for opportunistic bacteria, which was recently described in one orf-infected muskox calf from the same study population that died from *Corynebacterium freneyi* septicemia (Rothenburger at al. 2021). In human medicine, orf viral dermatitis has been described, predisposing the patient to cutaneous inoculation of *E. rhusiopathiae*, resulting in a severe form of disseminated erysipeloid in an English sheep farmer (Connor and Green 1995).

We present findings from two muskoxen, one adult bull hunter-harvested on Victoria Island (70°01′48″N, 107°34′10″W) in August 2014, and one adult cow found dead in the same region (69°06′29″N, 105°18′6″W) in June 2015. The bull had proliferative dermatitis of the right hind limb (Tomaselli et al. 2016), whereas the cow had perioral lesions (Fig. 1A, a, B, b). In both cases, orf virus infection was confirmed by histopathology and conventional PCR targeting the major envelope protein gene (*B2L*) performed at the Veterinary Virology Laboratory of the University of Calgary following methods described in Tomaselli et al. (2016). Formalin-fixed, paraffin-embedded skin lesions were then processed by immunohistochemistry (IHC) using *E. rhusiopathiae* polyclonal antiserum at the Veterinary Diagnostic Laboratory of Iowa State University, as described by Opriessnig et al. (2010), and tested positive for *E. rhusiopathiae* (Fig. 1C, D).

For completeness, we report that orf skin lesions of four other muskoxen found dead in 2015 in the same geographical area were negative for *E. rhusiopathiae* by IHC. However, the IHC technique currently available specifically targets the *E. rhusiopathiae* serotypes 1a, 1b, and 2—those that are most frequently associated with clinical disease in pigs. Although limited information is currently available on the serotypes of the *E. rhusiopathiae* isolates associated with the muskox die-offs on Banks and Victoria islands, one belonged to serotype 1b (Forde et al. 2020) and another to serotype 5 (Forde at al. 2016). Genomic analyses revealed that the latter was likely the most-prevalent serotype among the die-off isolates (Forde at al. 2016), but it is not targeted by the IHC test. Given the limitations of the current IHC technique available, we cannot exclude the possibility of false-negative results for at least some of the other animals.

To understand drivers of recent muskox mortalities, we suggest further exploration of whether the orf virus may have a significant role in predisposing animals to fatal infections from *E. rhusiopathiae*, given that it produces wounds that can facilitate secondary bacterial infection with subsequent septicemia. This could be important considering that orf virus appears to be increasingly found in muskoxen at different locations across their range (Afema et al. 2017; Tomaselli et al. 2018; Dalton 2019), including in areas of both Canada (Banks and Victoria Islands) and Alaska (eastern North Slope), in which declines and mortalities have been observed and were temporally associated with high *E. rhusiopathiae* seroconversion rates (Mavrot et al. 2020).

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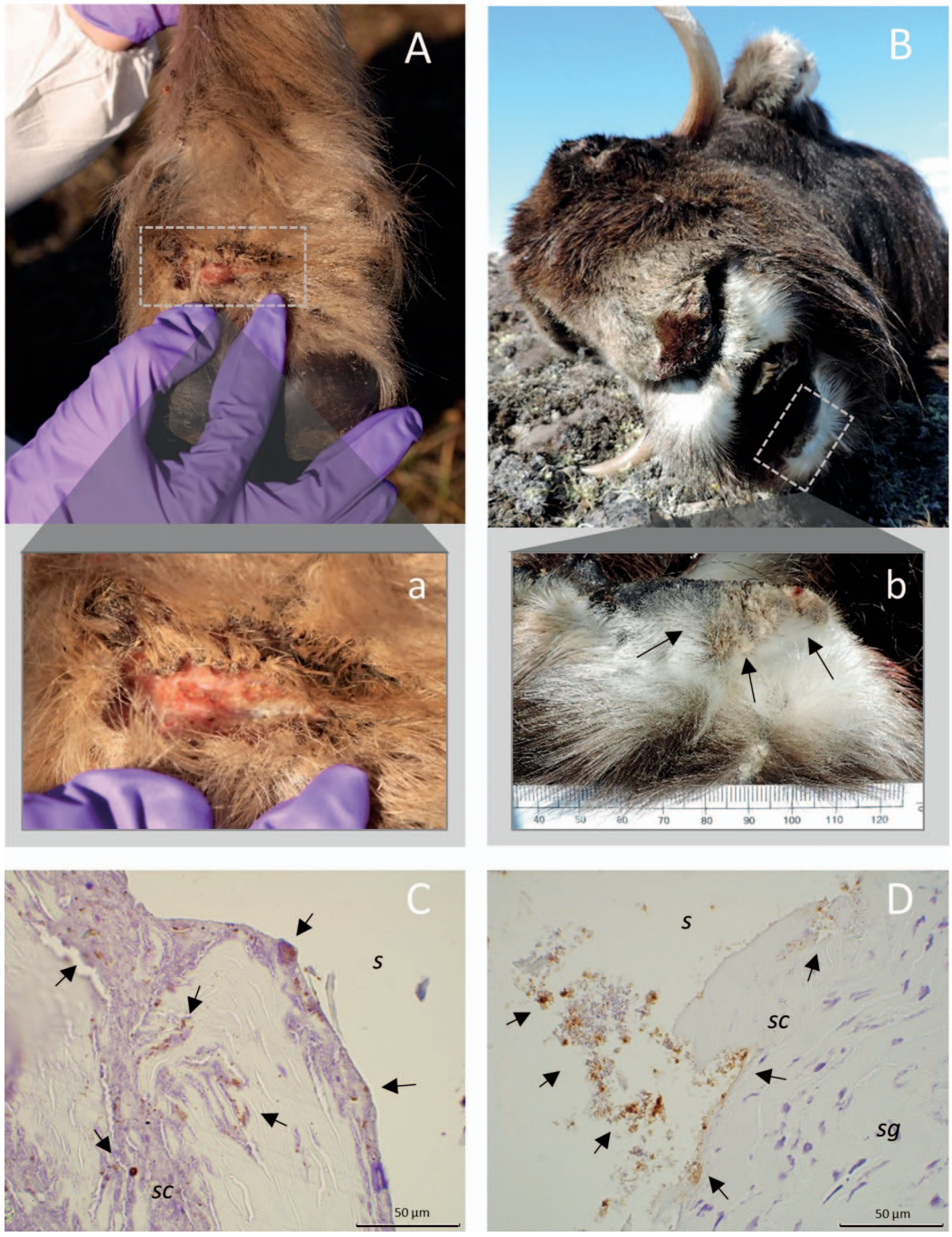

FIGURE 1. Gross pathology and *Erysipelothrix rhusiopathiae* immunohistochemistry staining of skin lesions on the foot (A, a, C) and mouth (B, b, D) of two adult muskoxen (*Ovibos moschatus*) harvested (bull) and a found dead (cow) in 2014 and 2015, respectively, on Victoria Island (Nunavut, Canada). (A, a) Adult bull: tarsus, plantar aspect, crust, and hyperkeratosis surrounding an ulcerative lesion (7×3 cm) on the coronary band. (B, b) Adult cow: thick crusts with fissures (3.5×2 cm) on the lower lip (black arrows). (C) Superficial part of crusted skin proliferations from the coronary band with dark-brown staining of *E. rhusiopathiae* (black arrows). Immunohistochemistry using a polyclonal *E. rhusiopathiae* antiserum and hematoxylin counterstain. (D) Skin from the lower lip showing positive *E. rhusiopathiae* staining in the superficial crust (black arrows). Immunohistochemistry staining as above. *s*=surface; *sc*=stratum corneum; *sg*=stratum granulosum of the epidermis. Scale bars as shown.

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