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Contagious Ecthyma, Rangiferine Brucellosis, and Lungworm Infection in a Muskox (*Ovibos moschatus*) from the Canadian Arctic, 2014

Matilde Tomaselli,[1,4] Chimoné Dalton,[1] Pádraig J. Duignan,[1,2] Susan Kutz,[1,2] Frank van der Meer,[1] Pratap Kafle,[1] Om Surujballi,[3] Claude Turcotte,[3] and Sylvia Checkley[1] [1]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, 3280 Hospital Drive NW, Calgary, Alberta T2N 4Z6, Canada; [2]Canadian Wildlife Health Cooperative, Alberta Node, Faculty of Veterinary Medicine, University of Calgary, 11877 85th Street NW, Calgary, Alberta T3R 1J3, Canada; [3]Canadian Food Inspection Agency, Ottawa Laboratory Fallowfield, 3851 Fallowfield Road, PO Box 11300, Station H, Nepean, Ontario K2H 8P9, Canada; [4]Corresponding author (email: matilde.tomaselli@ucalgary.ca)

ABSTRACT: An adult male muskox (*Ovibos moschatus*), harvested on 26 August 2014 on Victoria Island, Nunavut, in the Canadian Arctic, had proliferative dermatitis on the muzzle and fetlocks suggestive of contagious ecthyma or orf (*Parapoxvirus*). Histopathologic features of the lesions were consistent with this diagnosis. Orf virus DNA, phylogenetically similar to an isolate from a captive muskox of the Minnesota Zoo, US, was detected in the lesions by PCR using *Parapoxvirus* primers. Additionally, there was a metaphyseal abscess with a cortical fistula in the right metacarpus from which *Brucella suis* biovar 4 was isolated and identification supported by PCR. *Brucella* spp. antibodies were detected in serum. Finally, 212 nodules were dissected from the lungs. Fecal analysis and lung examination demonstrated co-infection with the lungworms *Umingmakstrongylus pallikuukensis* and *Varestrongylus eleguneniensis*. The zoonotic potential of orf and rangiferine brucellosis adds an important public health dimension to this case, particularly given that muskoxen are a valuable source of food for Arctic residents. Careful examination of these pathogens at a population level is needed as they may contribute to muskox population decline and potentially constitute a driver of food insecurity for local communities. This case underscores the importance of wildlife health surveillance as a management tool to conserve wildlife populations and maintain food security in subsistence-oriented communities.

Key words: *Brucella suis* biovar 4, food safety, food security, *Parapoxvirus, Protostrongylidae,* public health, wildlife health and disease surveillance, zoonoses.

Muskoxen (*Ovibos moschatus*) are Arctic ungulates central to Inuit culture and tradition that provide an important source of income through guided sport hunts and sale of meat, qiviut, and handicrafts (Gunn et al. 1991). Recent pathogen emergence and regional population declines are of concern from the perspective of conservation, food safety and security, and the economies of northern communities (Kutz et al. 2015). We describe a case of orf, rangiferine brucellosis, and lungworm infection in a wild muskox and the relevance of these diseases for both wildlife and public health.

On 26 August 2014 a mature adult male muskox of average body condition (2 cm back fat) was shot by a sport hunter in a remote location on Victoria Island, Nunavut, Canada (70°01′48″N, 107°34′10″W). The hunter and his guide reported bleeding scabs on the animal's muzzle (Fig. 1A). On 29 August 2014, when the hunters returned to the community, samples were collected from the affected area on the muskox hide and stored at –20 C. The remaining carcass was revisited by air charter and sampled in the field for histopathology, parasitology, and culture.

Thirteen foci of hyperkeratosis without alopecia (up to 25-mm diameter) and one right hind coronary band ulcer (7×3 cm) were present on the pasterns and coronary bands, while the lesions on nasal planum were proliferative and ulcerated (Fig. 1A). Histologically, the lesions were consistent with orf virus infection in other ruminants (Ginn et al. 2007; Fig. 1B). We extracted DNA from the skin and muzzle lesions using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek Inc, Norcross, Georgia, USA) following manufacturer's protocol. Orf virus (ORFV) was detected by

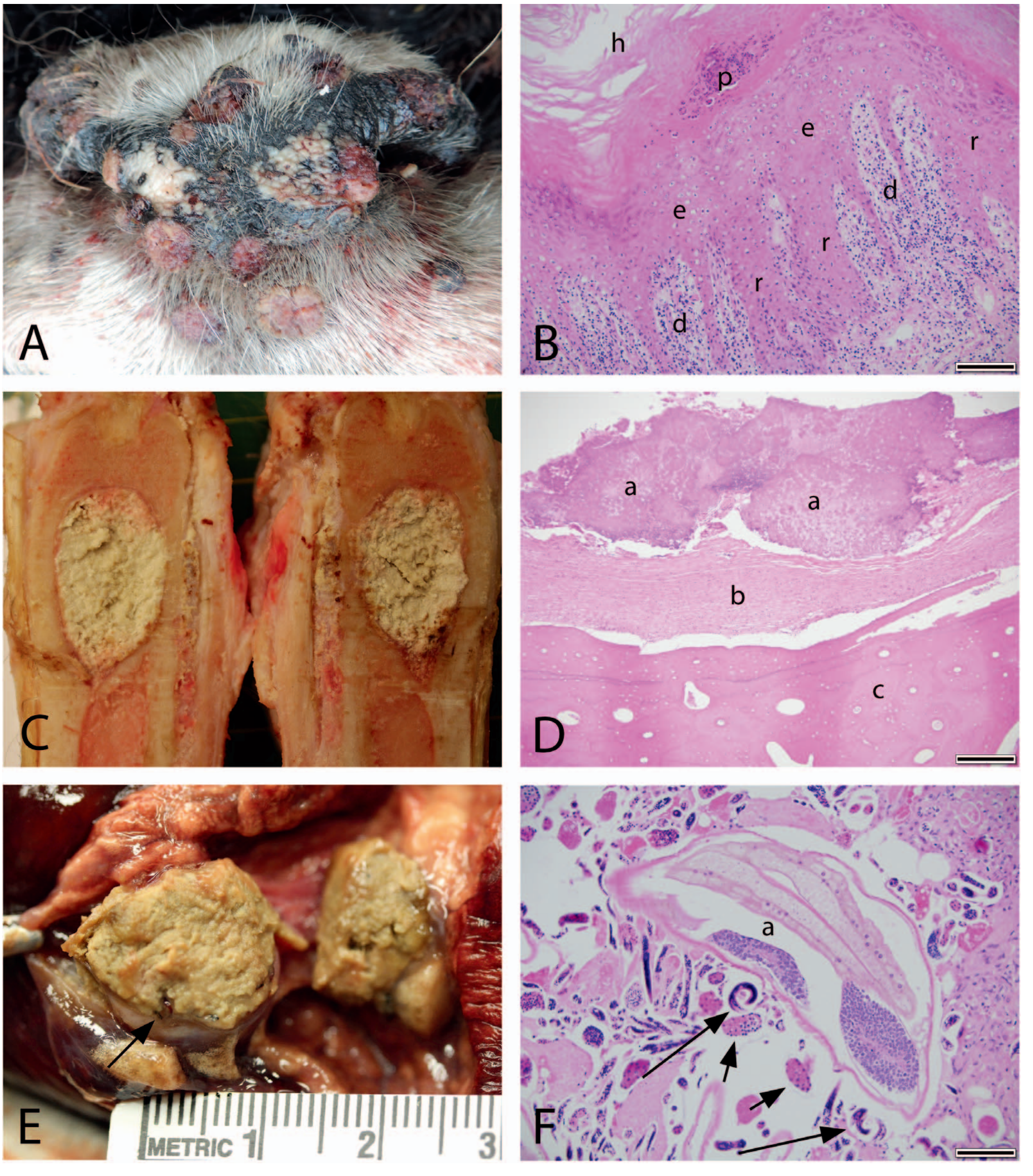

FIGURE 1. Gross pathology and histopathology of an adult male muskox (*Ovibos moschatus*) harvested on Victoria Island, Nunavut, Canada, August 2014. (A) Nasal planum: Multifocal hyperkeratosis interspersed with raised papillary to verrucous nodules often with an eroded or ulcerated and hemorrhagic surface. (B) Skin: The dermis has superficial edema, capillary dilatation, proliferation of dendritic cells, and influx of variable numbers of neutrophils, lymphocytes, and plasma cells (d). The epidermis is hyperplastic with long rete ridges projecting deep into the dermis (r), hydropic degeneration characterized by vacuolation and swelling of the keratinocytes (e), intra-epidermal pustule formation (p), and hyperkeratosis (h). H&E. Bar=100 µm. (C) Right metacarpus: Sagittal section showing a medullary abscess in the metaphysis surrounded by a thin capsule of granulation tissue. (D) Right metacarpal abscess: Caseous debris containing bacterial colonies (a), abscess capsule (b), cortical bone (c). H&E. Bar=200 µm. (E) Lung nodule section: Adult nematode (*Umingmakstrongylus pallikuukensis*, black arrow) embedded in caseous debris and surrounded by a thin fibrous capsule. (F) Lung: Adult protostrongylid nematode *U. pallikuukensis* (a), larvae (long arrow), and developing eggs (short arrow). H&E. Bar=100 µm.

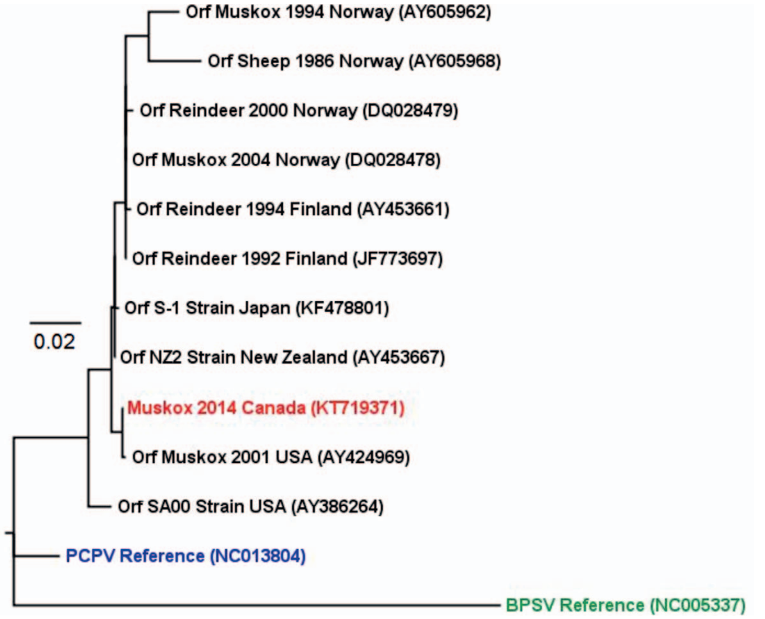

FIGURE 2. Maximum likelihood nucleotide phylogenetic tree of the major envelope protein gene (B2L) sequences from an orf virus (ORFV) identified in an adult male muskox (*Ovibos moschatus*) harvested on Victoria Island, Nunavut, Canada in August 2014, and additional *Parapoxvirus* sequences from GenBank. Viral DNA sequences are indicated by strain name/animal, year, and location of isolation, and GenBank accession numbers in parentheses. The Victoria Island ORFV sequence is shown in red, *Pseudocowpox virus* (PCPV) reference strain is shown in blue, and *Bovine papular stomatitis virus* (BPSV) reference strain is shown in green. The Victoria Island ORFV sequence shares a recent ancestor with the viral sequence identified in a muskox from the Minnesota Zoo, Apple Valley, Minnesota, US, in 2001. The scale bar indicates nucleotide substitutions per site.

PCR, which targeted the major envelope protein gene (B2L), and sequences were aligned with 12 other parapoxvirus B2L sequences published in GenBank using the MUSCLE algorithm (Inoshima et al. 2000; Edgar 2004). We constructed a maximum-likelihood phylogenetic tree with a relative divergence scale using the RAxML software in the program Geneious 8.1 (Kearse et al. 2012; Stamatakis 2014). The Victoria Island ORFV sequence shared >99% nucleotide identity with an ORFV from a muskox at the Minnesota Zoo, Apple Valley, Minnesota, US (Guo et al. 2004; Fig. 2).

A medullary abscess (3.5 cm) was found in the right distal metacarpal metaphysis connected by a fistulous tract through the cortical bone to a smaller abscess (0.5 cm) beneath the deep digital flexor tendon (Fig. 1C). Histologically, the abscess had colonies of Gram-negative bacteria (Fig. 1D). Samples from the bone abscesses and serum were submitted to the Canadian Food Inspection Agency, Brucellosis Centre of Expertise, Ottawa (Ontario, Canada). *Brucella* spp. antibodies were detected by indirect ELISA (Nielsen et al. 2004). Typical *Brucella* spp. colonies were detected at 5 d postinoculation and identified as *Brucella suis* biovar 4 based on biotyping (Alton et al. 1988). Identification was supported by a multiplex AMOS PCR (US Department of Agriculture protocol) followed by a multiplex Bruce-ladder PCR performed in a reaction mixture containing primers (Lopez-Goni et al. 2011) and the master mix and Q-Solution supplied in a Qiagen Multiplex PCR kit (Qiagen, Toronto, Ontario, Canada) following the manufacturer's protocol.

A total of 212 nodules (0.5–5 cm) representing 25.9% of the total lung weight were distributed throughout the parenchyma and contained the protostrongylid *Umingmakstrongylus pallikuukensis* (Hoberg et al. 1995; Fig. 1E, F). A total of 574 protostrongylid larvae per gram of feces were isolated and identified as *U. pallikuukensis* and *Varestrongylus eleguneniensis* based on morphology and sequencing (Kafle et al. 2015).

Orf is caused by a zoonotic parapoxvirus with a worldwide distribution in domestic and wild ruminants and directly transmissible between hosts (Frölich 2000). Affected individuals generally recover; however, death can occur in juveniles as a result of secondary infections or starvation (Frölich 2000). Orf has been identified in wild muskox populations in Alaska (Zarnke et al. 1983; Afema 2008; M. Tryland pers. comm.) and Norway (Vikøren et al. 2008). Morbidity and mortality in calves and adults have been documented in captive muskoxen in North America and Europe (Frölich 2000; Guo et al. 2004; Vikøren et al. 2008). Orf-like lesions on muskoxen have previously been reported by hunters on Banks Island in the Northwest Territories, Canada (M. Branigan pers. comm.) and by residents of Cambridge Bay on Victoria Island on both muskoxen and caribou since the mid-2000s (M.T. unpubl. data). This case is the first laboratory confirmed report of ORFV infection in a wild muskox in Canada. While contact with domestic sheep was hypothesized to have been a possible source of infection in free-ranging Alaskan and Norwegian muskoxen (Zarnke et al. 1983; Vikøren et al. 2008) and in the captive animal (Guo et al. 2004), in our case this route of transmission is unlikely because domesticated ruminants are not present on Victoria Island. Further investigation, including obtaining more isolates from Arctic and subArctic ungulates, is needed to elucidate ORFV epidemiology in Arctic wildlife.

Brucella suis biovar 4, the etiologic agent of rangiferine brucellosis, is enzootic in Holarctic reindeer and caribou (Gates et al. 1984). Typical clinical signs include stillbirth, abortion, and orchitis, leading to reproductive failure, and articular hygromas causing lameness (Forbes 1991). In the Arctic, where caribou and reindeer represent a major source of food for communities, brucellosis is a serious public health concern (Forbes 1991). Brucellosis has rarely been reported in muskoxen. The disease was found in two adult male muskoxen on the Canadian Arctic mainland near Garry Lake and Kugluktuk in the 1980s (Forbes 1991), two animals harvested on Victoria Island (Minto Inlet, NWT and Ekalluk River, NU) between 1996 and 1998 (B. Elkin pers. comm.), and in four animals from the eastern North Slope in Alaska between 2004 and 2007 (Ingebjørg et al. 2016). To our knowledge, the presentation of an intramedullary bone abscess, in the absence of bursitis or orchitis, has not been previously reported. This may suggest that some infections in wild ungulates may go undetected without detailed examination including sectioning of bones. Thus, brucellosis in muskoxen is likely under-reported and may increase the risk of this zoonotic disease for hunters and consumers of traditional food.

The lungworms *U. pallikuukensis* and *V. eleguneniensis* are emerging pathogens on Victoria Island (Kutz et al. 2013). *Umingmakstrongylus pallikuukensis* is a large nematode, which forms granulomas in the pulmonary parenchyma of the host, with infection accumulating with host age (Hoberg et al. 1995). *Varestrongylus eleguneniensis* is a small nematode found deep in the airways (Verocai et al. 2014). These parasites were only recently confirmed in Victoria Island muskoxen (Kutz et al. 2013), and their distribution is expanding with increasing prevalence and intensity of infection (P.K. unpubl. data). This case has the highest *U. pallikuukensis* intensity reported for Victoria Island muskoxen (P.K. unpubl. data).

Our findings are relevant in the context of both muskox and public health, and they increase available knowledge on pathogen diversity for Victoria Island muskoxen, where population decline and unusual mortalities have been reported (Kutz et al. 2015). Orf and brucellosis may contribute to population decline by affecting recruitment and reproductive success (Afema 2008). High lungworm intensities may have energetic costs, thus enhancing susceptibility to predators and diseases (Kutz et al. 2013). In the Arctic, rapid climate warming is having an impact on pathogen and disease transmissions by altering host-parasite interactions, and cold-adapted muskoxen are considered extremely vulnerable to these changes (Post et al. 2013; Ytrehus et al. 2015). Recent and widespread die-offs with concurrent population declines of muskoxen in the Canadian Arctic (Kutz et al. 2015), together with reports of increasing morbidity (M.T. unpubl. data) and the detection of multiple significant pathogens in this muskox, including clinical orf in an adult animal, may suggest declining host resilience in this region that warrant further investigation.

From a public health perspective, the zoonotic potential of brucellosis and orf is important given that muskoxen are a source of food for local people, who are unfamiliar with the presence of these pathogens in local muskox populations (M.T. pers. obs.). Atypical or subclinical presentation of disease, as described here with brucellosis, may also decrease the detection of infected animals by hunters and increase the potential for exposure. Public health education is an important measure to mitigate these risks, ensuring safe harvesting practices. This case highlights the importance of thorough wildlife disease investigations undertaken by qualified health professionals in collaboration with local residents and harvesters. Such partnerships can enhance the capacity of wildlife surveillance efforts and promote both wildlife and public health.

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