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NEONATE HEALTH AND CALF MORTALITY IN A DECLINING POPULATION OF NORTH AMERICAN MOOSE (*ALCES ALCES AMERICANUS*)

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ABSTRACT: Moose (*Alces alces americanus*) populations in many areas along the southern extent of the North American moose range, including Minnesota, have experienced decline. Ascertaining neonate health and cause-specific mortality is critical where calf survival is low and understanding underlying causes of population dynamics is important. To investigate moose neonate health and causes of mortality, we studied 43 calves shortly after parturition during 2013–15 and 2018. The observed natural calf mortality rate was 84% by the following January of each calving season. Most natural calf mortalities were caused by black bear (*Ursus americanus*) or wolf (*Canis lupus*) predation or associated injuries (71%) but also included stillbirth (16%), orphaning (7%), generalized bacterial infection (3%), and hunter harvest (3%). Neonate health was evaluated in 27 calves by hematology, serum biochemistry profile, and maternally derived immunoglobulin. General health parameters were mostly within an expected range for normal health and adequate maternal immunoglobulin transfer. Importantly, these data contribute to a growing body of literature on moose neonate health and is the first report, to our knowledge, of maternally derived immunity in moose neonates.

Key words: Hematology, maternal antibodies, moose calf mortality, neonatal ungulate health, passive transfer, serum biochemical profile.

INTRODUCTION

Moose (*Alces alces americanus*) are an important subsistence species used by the Grand Portage Band of Lake Superior Chippewa, historically and presently, but harvests have declined in recent years concomitant with declines in moose populations in surrounding areas of Minnesota. More broadly, declining moose populations have been documented in several regions along the southern cusp of their North American range. These include New Hampshire and Maine, with 50% decline since the 1990s (Jones et al. 2019), Minnesota with >50% decline since the 1990s (Lenarz 2007; DelGiudice 2013), and Ontario, Canada, with 50–60% decline in the past decade (Environmental Commissioner of Ontario 2016; Ranta and Lankester 2017). In Minnesota, moose populations in the northwest plummeted in the 1990s and have not recovered (Lenarz 2007), whereas populations in the northeast have declined since 2004 (DelGiudice 2013) with more recent stabilization (DelGiudice 2020). Drivers of the northwest Minnesota decline were considered high adult mortality and low pregnancy rates, whereas more-recent declines in the northeast have been attributed to high adult mortality and low calf recruitment (Lenarz et al. 2010; DelGiudice 2013; Carstensen et al. 2018).

Causes of low calf recruitment include low pregnancy rates, high pregnancy loss, and high calf mortality. Mean pregnancy rates, estimated by serum progesterone levels of adult females captured in Grand Portage since 2010, were 83% (range: 69–100%), and a pilot study of four pregnant moose implanted with vaginal implant transmitters in 2012 revealed 100% parturition. Further, low body condition and heavy winter tick (*Dermacentor albipictus*) burden of captured cows were consistently observed in the winter of the years before the start of this study. Those observations raised concerns about the influence of

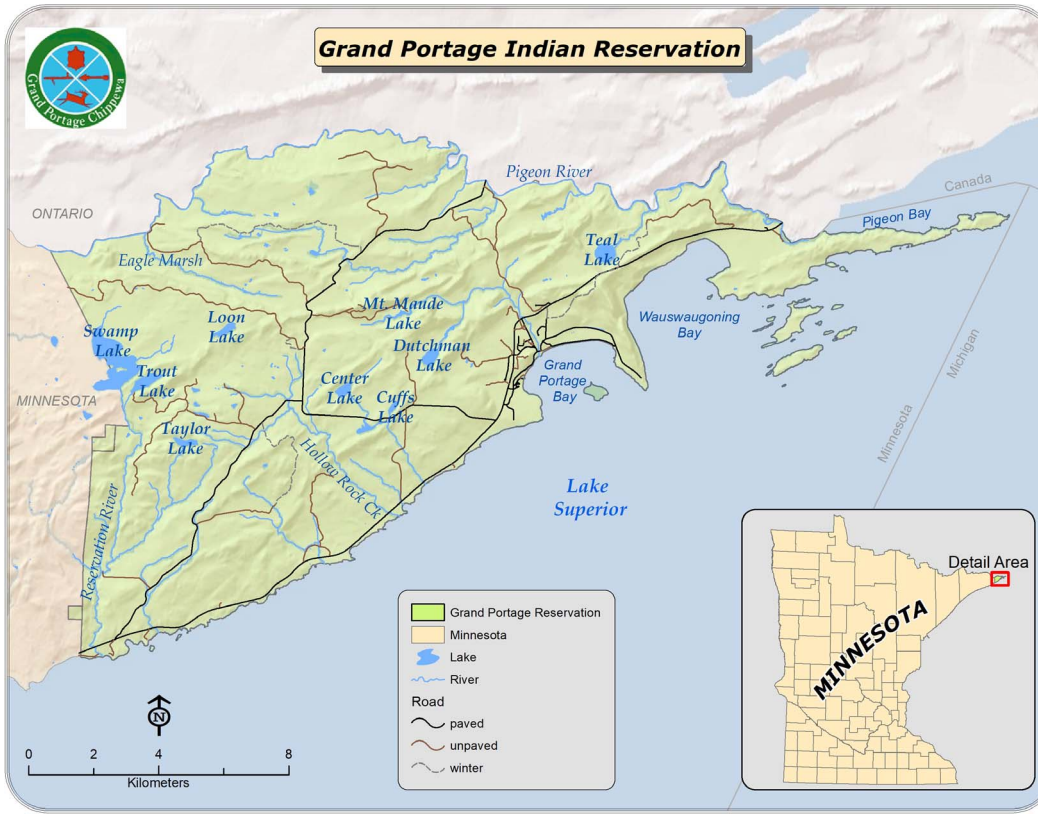

FIGURE 1. Map of study area, Grand Portage Indian Reservation, Minnesota, USA, where 43 moose calves (*Alces alces americanus*) were studied shortly after parturition and to mortality in 2013–15 and 2018.

compromised cow health on neonate health and survival. Thus, either pregnancy loss or high calf mortality or both were considered possible drivers of low annual calf recruitment.

The primary objectives of this study were to evaluate neonatal health and describe cause-specific calf mortality of moose on the Grand Portage Indian Reservation (GPIR) in northeastern Minnesota. Our hypothesis was that high calf mortality was causing low calf recruitment, and compromised neonate health might be a contributing factor. Here, we provide a comprehensive assessment of neonatal health, including serum biochemical profiles, complete blood cell count (CBC) analyses, and maternal antibody transfer, and describe causes of mortality in free-ranging moose calves.

MATERIALS AND METHODS

Study site

The GPIR (227 km^2), located in northeastern Minnesota, in Cook County (Fig. 1), encompasses approximately 19,222 ha, largely comprising undeveloped boreal forest that is bordered on the north by Ontario, Canada, by a mixture of federal, state, and private ownerships to the west, and by Lake Superior to the east and south. Moose were present across the entire reservation (density=0.27/km^2), but the core range was inland from Lake Superior. White-tailed deer (*Odocoileus virginianus*) were also present across the entire reservation but congregated near the lakeshore in winter. Gray wolves (*Canis lupus*; 5–6 wolves/100 km^2) and black bears (*Ursus americanus*; 3.8–4.6 bears harvested/100 km^2) were common and preyed on moose calves and deer fawns (Moore et al. 2013). Annual calf:cow ratios from aerial winter surveys on GPIR were estimated at 0.46 (SD=0.17) from 1994 to 2012, as compared with Minnesota calf:cow ratios of 0.67

(SD=0.14) before the population decline in the mid-2000s (Lenarz 2006).

Capture and sampling

All capture and handling protocols were conducted in accordance with requirements of the University of Minnesota Institutional Animal Care and Use Committee (protocols 1410-31945A and 1803-35736A) and the guidelines of the American Society of Mammalogists (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). In February–March 2013–18, we captured adult female moose on GPIR by helicopter through a commercial wildlife capture company using a cartridge-fired projector and 2-mL or 3-mL darts with a 14-ga, 38-mm, wire-barbed cannula (Pneu-Dart, Inc., Williamsport, Pennsylvania, USA) as part of ongoing moose habitat and adult mortality studies conducted by the Grand Portage Band of Chippewa. We immobilized moose with either 10-mg thiafentanil oxalate, 4.5-mg carfentanil citrate, or 8.5–10-mg etorphine hydrochloride (Wildlife Pharmaceuticals, Inc., Windsor, Colorado, USA) in combination with 40–50 mg xylazine. We reversed anesthesia with naltrexone (20 mg/mg thiafentanil, 100 mg/mg carfentanil, or 50 mg/mg etorphine) and 800 mg tolazoline (Arnemo et al. 2003). We fitted cows with GPS Plus Iridium collars (Vectronic Aerospace GmbH, Berlin, Germany), equipped with a GPS data-logger that included a movement sensor and mortality signal that was triggered when movement diminished below a programmed threshold after a period of 6 h. An experienced veterinarian examined adult cows by rectal palpation for pregnancy, which was determined by direct palpation of the fetus (Solberg et al. 2003). Using a sterile, disposable vaginal speculum (Jorgensen Laboratories, Inc., Loveland, Colorado, USA), we implanted a vaginal implant transmitter (VIT; Vectronic Aerospace) adjacent to the cervix in 43% of pregnant cows (Johnson et al. 2006; Patterson et al. 2013). The transmitter continuously measured body temperature and motion, transmitting data to the GPS collar at regular intervals.

The GPS location data were transmitted via satellite every 2 h, except from 15 April to 30 June each year, during which time location data transmission frequency was increased to every 30 min to enhance observation of movement behaviors associated with parturition. We monitored cow GPS data daily during the calving season. Parturition was signaled by reduced temperature and cessation of motion of the VIT after expulsion during calving. In cows in which a VIT was not implanted, we recognized parturition by a significant increase in movement, followed by abrupt geographical localization that remained constant over several days to weeks (McGraw et al. 2014).

At 48–72 h after parturition, we captured 44 newborn calves, including 16 males (36%), 26 females (59%), and two of unknown sex (5%) during 2013–15 and 2018. A 2–3-person capture crew approached the cow-calf pair rapidly on foot to induce a flight response from the cow for calf handling (Ballard et al. 1979; Patterson et al. 2013). We blindfolded and manually restrained calves in sternal recumbency to collect approximately 20 mL of blood from a jugular vein using a 21-ga, winged blood collection set attached to a 20-mL syringe, inserted an ear tag, and determined sex. We transferred blood immediately into a Monoject™ tube containing 15% ethylenediaminetetraacetic acid (Tyco Healthcare Group LP, Mansfield, Massachusetts, USA) and serum separator tube (Corvac™, Medtronic, Minneapolis, Minnesota, USA). We placed an expandable, GPS Globalstar collar (Vectronic Aerospace) with an elastic band around the neck, weighing approximately 425 g and designed to break away within 1 yr. Collars were equipped with the same movement sensor and mortality signaling mechanism as the GPS Iridium collars. Calf processing averaged 5.7 min (SD=4.68). At mortality signaling, we investigated the site of mortality and collected the carcass where available.

Blood sample analyses

Within 2–3 h of collection, we created blood smears from whole blood and centrifuged and extracted serum from separator tubes. We stored samples in a refrigerator and shipped samples within 1–3 d to the University of Minnesota Clinical Pathology Laboratory (St. Paul, Minnesota, USA) in 2013 and to Marshfield Labs (Marshfield, Wisconsin, USA) in 2014–15 and 2018 for CBC and serum biochemical analysis. Hematologic analysis in 2013–14 was performed with an Advia 2120 hematology analyzer (Siemens Healthineers, Malvern, Pennsylvania, USA) or a Sysmex XT-2000iV automated hematology analyzer in 2015 and 2018 (Sysmex, Lincolnshire, Illinois, USA). Cell morphology and a six-component leukocyte differential were assessed by peripheral blood smears. Serum biochemical analysis was performed with an AU480 (2013) or AU5800 (2014–15 and 2018) chemistry analyzer (Beckman Coulter, Inc., Brea, California, USA). Aliquots of frozen-stored serum were screened for semiquantitation of total immunoglobulin (Ig) by zinc sulfate turbidity at the Minnesota Veterinary Diagnostic Laboratory and for IgG quantitation through radial immunodiffusion (RID) using a commercial Sheep IgG kit (product 328411, Triple J Farms, Bellingham, Washington, USA; Mancini et al. 1965). For the latter, samples were

diluted 1:4 in phosphate buffered saline and tested in duplicate. The standard used was IgG from an adult moose, isolated by Triple J Farms by oceanic acid with a concentration determined by biuret. Rings grew to equilibrium by 48 h and were measured and compared with the standard. Samples at the low range of the plate measurement were retested undiluted.

Postmortem examination and disease screening

We submitted intact carcasses to the Minnesota Veterinary Diagnostic Laboratory (St. Paul, Minnesota, USA) for postmortem examination, histopathology, and pathogen screening. Necropsies included gross examination of the entire carcass and histologic examination of intestine, abomasum, liver, spleen, kidney, lung, heart, lymph node, brain, tongue, and skeletal muscle. Samples for aerobic culture included lung and liver of two stillborn calves and one neonatal calf with evidence of generalized infection, as well as joint swabs from two approximately 6-mo-old calves with evidence of infected lesions on gross exam. In selected cases, eyes, adrenal glands, thyroid glands, thymus, and skin were examined histologically. Real-time PCR was used to screen a tissue pool (kidney, lungs, intestine, and spleen) for bovine herpes virus-1 and bovine viral diarrhea with primers and a probe target, as previously described (Mahlum et al. 2002), and kidney samples for *Leptospira interrogans* with previously published primers (Merien et al. 1992). The latter PCR can detect and discriminate the following *L. interrogans* serovars: Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohemorrhagica, and Pomona. We submitted liver tissue for measurement of heavy metals (arsenic, cadmium, lead, and mercury) and mineral elements (i.e., copper, iron, and zinc) through the Michigan State University Veterinary Diagnostic Laboratory (Lansing, Michigan, USA). All tissue mineral concentrations were measured by either inductively coupled plasma atomic emission spectrometry or mass spectrometry. We based cause of death on a comprehensive site investigation, necropsy or histopathologic examination, and disease screening or a combination thereof.

Data analysis

We used SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) software to analyze hematologic, serum biochemistry, and maternal antibody parameters. We examined the statistical distributions of all parameters with histograms and the Shapiro-Wilk test for normality (normal distribution $P>0.05$). We checked for and removed outliers using Dixon's range statistic at a confidence level of $\alpha=0.10$ or Tukey's method (Friedrichs et al. 2012). For cause-specific mortality analysis, seven additional calves (three males, three females, and one unknown), whose mortalities were known (e.g., found dead at capture) were also included, whereas calves that were abandoned subsequent to capture and sampling were removed, for a total of 43 calves. We report Wilson score confidence intervals for mortality estimates, which is appropriate for small sample size and sample proportions close to 0 or 1 (Brown et al. 2001). Unfortunately, our sample size was not sufficient for robust statistical analysis of neonatal health parameters in association with calf mortality.

RESULTS

All calves appeared healthy at time of capture. Of 44 captured calves, blood was collected from 27 (61%) for hematologic, serum biochemical, and/or maternal antibody measurement. The serum biochemical and hematologic values we observed (Tables 1, 2) were comparable to the few reports of free-ranging neonate moose and white-tailed deer (Sams et al. 1996; Powell and Delgiudice 2005; DelGiudice and Severud 2016). Although total Ig correlated well with IgG ($r=0.93$, $n=24$), the semiquantitative measurement of total Ig trended lower than the quantitative IgG measurement (Table 1). Most moose calves (83%; 20/24) had IgG levels (1,771±406.5 mg/dL) consistent with adequate passive transfer in domestic species and white-tailed deer fawns (Parish 1996; Edmondson et al. 2012; Evers et al. 2017), whereas four (16%; 4/24) had levels consistent with partial transfer (Table 3).

The natural mortality rate of study calves ($n=43$) was 84% (Wilson score 95% confidence interval [CI]=70–92%, $n=36$, annual range: 75–100%). We were able to determine a cause of mortality for 31 of 36 dead calves (86%). Most of these deaths were due to predation or predation-associated injuries (71%, $n=22$, Wilson score 95% CI=53–84%) by wolves and black bears (Fig. 2). Carcasses were recovered and necropsied from three predator-related mortalities (Supplementary Material Table S1). One 3-d-old calf died as a result of injuries sustained during a predation attempt by wolves, as evidenced by substantial

TABLE 1. Serum biochemistry profiles and maternally derived immunoglobulin of neonatal moose calves (*Alces alces americanus*) captured 48-72 h post-parturition in 2013–15 and 2018 in Grand Portage, Minnesota, USA.

Analyte[a]	*n*	Mean	SD	Range
BUN (mg/dL)	23	15	3	7–20
Creatinine (mg/dL)	24	0.6	0.2	0.4–1.4
Calcium (mg/dL)	24	10.8	0.6	9.3–11.8
Phosphorus (mg/dL)	24	9.4	1.1	6.8–11.7
Total protein (g/dL)	24	4.8	0.4	4.1–5.8
Albumin (g/dL)	24	2.0	0.2	1.7–2.2
Globulin (g/dL)	24	2.8	0.5	1.9–3.7
Sodium (mmol/L)	23	142	2	139–145
Chloride (mmol/L)	24	96	2	93–102
Potassium (mmol/L)	23	4.5	0.3	4.0–5.0
Iron (μg/dL)	18	147	70	29–292
GGT (U/L)	25	63	23	34–108
SDH (U/L)	18	43	24	8–80
AST (U/L)	24	56	12	36–80
CK (U/L)	20	81	24	47–138
Glucose (mg/dL)	24	139	36	59–198
Total immunoglobulin[b] (mg/dL)	27	995	419	291–2072
Total IgG[c] (mg/dL)	24	1620	517	754–2650

[a] BUN = blood urea nitrogen; GGT = γ-glutamyltransferase; SDH = sorbitol dehydrogenase; AST = aspartate aminotransferase; CK = creatine kinase; IgG = immunoglobulin G.

[b] Total immunoglobulin determined by zinc turbidity test.

[c] Total IgG determined by radial immunodiffusion assay using commercial Sheep IgG kit (no. 328411, Triple J Farms, Bellingham, Washington, USA; Mancini et al. 1965).

disturbance to the scene, persistence of the cow with the calf even after death of the calf, and identification of wolf hair at the scene. Postmortem examination revealed anemia; an open, transverse fracture of the left radius; transverse fracture of the left metatarsus; multifocal, moderate subcutaneous hemorrhage; and a skull puncture with mild, acute cerebrocortical hemorrhage. These injuries and the site investigation were consistent with blunt force trauma (e.g., trampled by cow) and perforating trauma (e.g., wolf bite). A 6-mo-old calf died from cachexia and dehydration associated with infected wounds that included chronic, suppurative arthritis and periarthritis of the right elbow and suppurative and necrotizing cellulitis of the left front limb in the metacarpal region. *Pasteurella multocida* was cultured from the right elbow, left metacarpus, lung, kidney and liver; *Trueperella pyogenes* from the right elbow and left metacarpus; and *Staphylococcus aureus* from the left metacarpus. These wounds were considered consistent with injuries from a predator attack, although other puncture-like injuries leading to infection cannot be excluded. A second 6.5-mo-old calf died of cachexia, also accompanied by multiple chronic, suppurative lesions with draining tracts, suggestive of predator-inflicted trauma with subsequent infection. *Trueperella pyogenes* and *Streptococcus* sp. were cultured from an affected left carpal joint. The remaining predator-related mortalities were characterized by scant remnants of calf hair, tissue, bone fragments, or blood, as in cases of wolf predations, or pelt or cached portion of a carcass, as in bear predations.

Nonpredation calf mortalities included stillbirths (16%, n=5, Wilson score 95% CI=7–33%), orphaning (6%, n=2, Wilson score 95% CI=2–21%), generalized bacterial infection (3%, n=1, Wilson Score 95% CI=0.6U–16%), and hunter-harvest (3%, n = 1, Wilson Score 95% CI=0.6–16%). Six of these carcasses were recovered and underwent partial or complete postmortem examination (Supplementary Material Table S1). We observed five neonates in this study confirmed as stillbirths based on complete fetal pulmonary atelectasis. Two stillborn carcasses underwent a complete necropsy and pathogen screening. No additional lesions were identified on necropsy; however, the presence of keratin squames in numerous alveoli of one of the stillborn calves suggested that the animal died during dystocia with inhalation of amniotic fluid. β-Hemolytic *Escherichia coli* was isolated from the liver of the other stillborn calf examined by necropsy. Pooled tissues were negative for bovine viral diarrhea virus and bovine herpes virus-1, and the kidney was negative for *L. interrogans* on both stillborn calves. One 4-d-old calf had evidence of a generalized bacterial infection, and the umbilicus was suspected as the portal of entry. Bacterial

TABLE 2. Hematology of neonatal moose calves (*Alces alces americanus*) captured 48–72 h after parturition in 2013–15 and 2018 in Grand Portage, Minnesota, USA.

Analyte[a]	*n*	Mean	SD	Proportion zero[b]	Range
WBC ($\times 10^3/\mu L$)	22	5.0	1.2		2.8–6.9
Neutrophils ($\times 10^3/\mu L$)	22	4.07	1.04		2.32–6.21
Bands ($\times 10^3/\mu L$)	22	0.02		0.91	0.00–0.42
Lymphocytes ($\times 10^3/\mu L$)	22	0.78	0.35		0.04–1.43
Monocytes ($\times 10^3/\mu L$)	22	0.08		0.48	0.00–0.39
Eosinophils ($\times 10^3/\mu Lv$	22	0.02		0.82	0.00–0.26
Basophils ($\times 10^3/\mu L$)	22	0.01		0.91	0.00–0.06
RBC ($\times 10^6/\mu L$)	22	5.37	0.52		4.41–6.09
Hemoglobin (g/dL)	22	8.9	1.1		6.8–10.7
Hematocrit (%)	22	30.0	4.0		21.4–37.1
MCV (fL)	22	56.0	5.9		47.2–67.8
MCH (pg)	22	16.5	1.1		14.6–18.4
MCHC (g/dL)	22	29.6	2.3		25.5–33.1
RDW (%)	21	23.3	1.6		20.1–26.2
Platelets ($\times 10^3/\mu L$)	20	461	122		190–714
nRBC (per 100 WBC)	21	6		0.19	0–26

[a] WBC = total leukocytes; bands = immature neutrophils with unsegmented nuclei; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; nRBC = nucleated red blood cells.

[b] For parameters with a substantial number of measurements of zero, the proportion that was zero is reported instead of the standard deviation.

overgrowth because of advanced postmortem autolysis precluded identification of the causative bacterial agent by culture. Hepatic mineral element and heavy metal concentrations from the six calves that underwent complete postmortem examination are summarized in Supplementary Material Table S2. Copper concentration ranged higher in neonates (*n*=4, range: 629–1,009 ppm) than in the 6-mo-old calves (*n*=2, range: 42–57 ppm), as would be expected in newborn mammals (Owen 1982). Two calves that were 111-d-old were presumed to have died after the death of the cow, and a single 5-mo-old calf was killed along with the cow by a hunter in Ontario, Canada.

TABLE 3. Comparison of 24 moose calf (*Alces alces americanus*) serum immunoglobulin G levels collected in 2013–15 and 2018 in Grand Portage, Minnesota, USA, with reference levels of the domestic species goats (*Capra aegagrus hircus*), sheep (*Ovis aries*), and captive white-tailed deer (*Odocoileus virginianus*) for maternal antibody transfer. Four moose calves had antibody levels classified as partial, and 20 moose calves had antibody levels classified as adequate.

Maternal antibody classes[a]	Immunoglobulin G (mg/dL)		Immunoglobulin G (mg/dL±SD)	
	Domestic sheep and goats[b]	Domestic cattle[c]	White-tailed deer[d]	Moose
Deficient	<500	<1,000		
Partial	500–1,000		680±140	851±79.5
Adequate	>1,500	>1,000	951±66	1,771±406.5

[a] Maternal antibody classes based on frameworks established for domestic species.

[b] Values from Edmondson et al. (2012).

[c] Values from Parish (1996).

[d] Values from Evers et al. (2017).

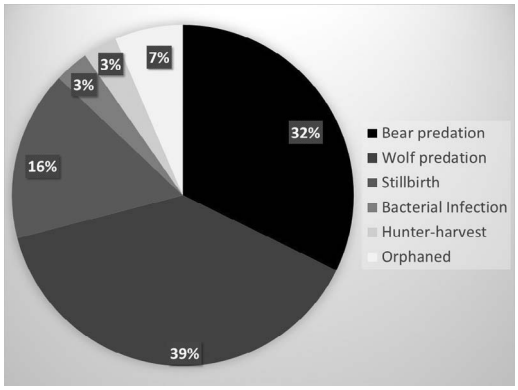

FIGURE 2. Cause-specific mortality of moose calves (*Alces alces americanus*; $n = 31$) in Grand Portage, Minnesota, USA, 2013–15 and 2018. The total mortality rate of study animals (n=43) was 84% (95% confidence interval: 68–92%), where the causes of five mortalities were undetermined and excluded from the figure.

DISCUSSION

In this study, we observed a high rate of natural mortality of study calves (84%; 36/43), and predation by wolves and black bears was determined to be the primary cause of mortality. Calf mortality was high compared with other studies of moose calf mortality, and the proportion of predation-associated mortality (71%; 22/31) was similar (60–94%; Osborne et al. 1991; Bertram and Vivion 2002; Keech et al. 2011). Although we were limited by a small sample size in examining associations between health and survival, we did not identify any clear trends in neonate health or maternal Ig levels that would suggest comprised health as a predisposition to mortality, as has been observed elsewhere (Sams et al. 1996).

We report maternal Ig transfer in wild moose, an important factor in the early survival of young ungulates. Although we were unable to associate Ig levels with survival, the mean levels of IgG observed were above those associated with survival (951±66 mg/dL) in captive white-tailed deer fawns (Evers et al. 2017) and were consistent with what is considered adequate passive transfer in domestic species (Table 3; Parish 1996; Edmondson et al. 2012). Most of the moose neonates tested (83%; 20/24) had IgG levels >1,000 mg/dL by RID, considered the gold standard for assessing maternal Ig transfer in calves (Hogan et al. 2015). In contrast, we found semiquantitative total Ig levels trended lower than the more accurate IgG concentrations. Although total Ig correlates with IgG and demonstrates high sensitivity (99%) in detecting failure of passive transfer, test specificity is much lower (30%), resulting in false-positives (Hogan et al. 2015). Despite that limitation, semiquantification of total Ig is readily available through commercial laboratories and is not limited by the use of species-specific reagents. Commercial RID kits are available for purchase and use outside of the commercial laboratory setting; however, because these were developed and validated for use in domestic species, they should not be used in nondomestic ungulates without a species-specific standard for test validation and interpretation. In this case, a standard was developed with the serum from an adult moose.

γ-Glutamyl transferase (GGT) is also an indicator of colostral uptake in calves (Parish 1996; Edmondson et al. 2012), but we observed mean GGT levels (63 U/L) that were lower than what has been considered sufficient for the health and survival of deer and captive nondomestic ungulates (Sams et al. 1996; Howard et al. 2005). For example, white-tailed deer fawns that survived to 45 d of age in Oklahoma had significantly higher mean GGTs (150 U/L) than those that died (77 U/L). Howard et al. (2005) documented mean GGT at 24–48 h after birth among calves and fawns in five captive, nondomestic ruminant species and found that the mean GGT levels of individuals assessed as clinically normal within 5 d of age ranged from 75 to 1,519 U/L, whereas mean GGT of clinically abnormal individuals ranged from 43 to 452 U/L. Clinically normal white-lipped deer (*Cervus albirostris*), the closest taxonomically to moose, had mean GGT of 224 U/L. Despite the low GGT levels observed in this study, maternally derived immunity appeared to be sufficient. Thus, GGT may not be a reliable indicator of colostral intake and maternal

antibody uptake in moose calves. Ultimately, the relationship between maternally derived immunity in moose calves and calf survival warrants further study, particularly in populations in which population health is in question.

Nonpredation mortality was high (29%; 9/31) as compared with other studies of moose calf mortality (2–12%; Ballard et al. 1981; Keech et al. 2011; Patterson et al. 2013; Severud et al. 2015), which was largely driven by the number of stillbirths we observed. Nonpredation mortalities reported in these other studies include malnutrition and exposure, drowning, congenital defect, injury by the cow, pneumonia, and natural abandonment. Only one study reported stillbirth as a cause of neonatal mortality in free-ranging moose (Bertram and Vivion 2002). Our approach to capture (i.e., visiting known calving sites vs. visually searching for cow-calf pairs by air) likely contributed to the high level of detection of stillbirths in this study, though two other studies that used the same capture methodology had no detections of stillbirths (Patterson et al. 2013; Severud et al. 2015).

Full necropsies on two stillbirths with histopathologic, virologic, and bacteriologic screening did not reveal an underlying cause of perinatal death, except for a possible *E. coli* infection of one calf. The presence of meconium in the placenta and keratin squames in numerous alveoli of one stillborn calf suggested the animal likely died during dystocia with inhalation of amniotic fluid. Other reports of stillbirth in free-ranging ungulate species are rare, and although frequently undetermined, underlying causes have included severe congenital defects and disseminated lymphocytic inflammation with focal encephalomalacia in caribou (*Rangifer tarandus*; Roffe 1993) and infectious disease, such as brucellosis in bison (*Bison bison*), elk (*Cervus elaphus*), and caribou (Neiland et al. 1968; Thorne et al. 1997; Proffitt et al. 2010) and equine piroplasmosis in the Przewalski horse (*Equus przewalskii*; Robert et al. 2005).

The hepatic copper concentrations (median=700 ppm) of the neonatal moose calves of our study were markedly higher than published concentrations in cattle (*Bos taurus*) fetuses (30–200 ppm) and goat fetuses (30–114 ppm) but resembled concentrations in deer fetuses (100–1,200 ppm; Puls 1994). The comparatively low hepatic concentrations of both 6-mo-old calves of this study were within the range of published concentrations of moose in Minnesota (Wünschmann et al. 2015). Our data align with previous research that demonstrated hepatic copper concentrations of moose fetuses that increased markedly up to birth and rapidly declined within 6 mo of life (Hyvärinen and Nygrén 1993). This observation is in accordance with data from cattle fetuses, albeit the degree of copper concentration in the fetal liver in the third trimester of pregnancy is not quite as dramatic in cattle as it is in the moose. The high copper concentration in the fetal liver helps to support rapid fetal growth in late pregnancy and early postnatal life in the face of a relatively low milk copper concentration (Suttle 1987). We speculate that the same physiologic purpose operates in moose that have an approximately 1,000% growth rate in the first 6 mo of life (Jensen et al. 2013).

Hematologic and serum biochemical parameters were comparable to the few reports of free-ranging neonate moose and white-tailed deer (Sams et al. 1996; Powell and Delgiudice 2005; DelGiudice and Severud 2016). Although several reports describe CBC and biochemical parameters of adult moose (Franzmann and LeResche 1978; Keech et al. 1998; Kreeger et al. 2005), there is limited information for calves (Franzmann and LeResche 1978; Rostal et al. 2012; DelGiudice and Severud 2016). Comparisons of similarly aged animals are critical to ensure comparison is made among animals at the same physiological stage of development. Only one study, also from Minnesota, generated reference ranges for similarly aged, neonate calves (DelGiudice and Severud 2016), and those aligned with the parameter estimates we observed.

It is critical to understand the role of health in mortality as the dynamics of moose

populations change along the southern edge of the moose range. This is particularly true in populations in which calf recruitment is low and population health is a concern. The challenge is the paucity of information on hematologic, serum biochemical, maternally derived immunologic, and other health parameters in free-ranging, neonatal ungulates (Parkinson et al. 1982; Sams et al. 1996). Although our study helps to fill that gap, more work is needed to determine how these values relate to calf survival and recruitment.

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SUPPLEMENTARY MATERIAL

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