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OSTEOPOROSIS, BONE MINERALIZATION, AND STATUS OF SELECTED TRACE ELEMENTS IN TWO POPULATIONS OF MOOSE CALVES IN NORWAY

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ABSTRACT: This study was conducted to clarify the etiology of a high frequency of bone fractures and osteoporosis in the moose (*Alces alces*) population in southern Norway. Liver samples, both metacarpi, and carcass data were collected from 21 and 22 moose calves shot in 1994 in Birkenes (southern Norway), and Nærøy (central Norway), respectively. The liver samples were analyzed for copper, manganese, zinc, cobalt, chromium, molybdenum, and selenium. Bone samples were subject to histologic, radiologic, and chemical examinations. Three of the calves from Birkenes and one calf from Nærøy showed histologic and radiologic evidence of generalized osteoporosis consistent with osteoporosis due to starvation. The calves with osteoporosis had the lowest carcass weights and radio-opacities recorded. There was a positive correlation between carcass weight and bone radio-opacity. Density, ash content, phosphorus, and calcium contents and phosphorous/calcium ratio in bone samples, as well as hepatic trace element status, were within the normal range for all calves in both populations. Two of the osteoporotic calves, were reported to have been orphaned. Our results indicate that the high frequency of bone fractures reported in moose in southern Norway is not associated with regional differences in trace element status or bone mineral balance. We propose that the occurrence of osteoporosis in moose calves in Birkenes may have resulted from inadequate nutrition following general overcrowding and high pressure on feed resources in the southernmost part of Norway.
Key words: *Alces alces,* Cervidae, malnutrition, moose, osteoporosis, radiographic analysis, starvation, trace elements.

INTRODUCTION

From the early 1990s, a high frequency of bone fractures in moose (*Alces alces*) has been reported by hunters in the counties of Aust-Agder and Vest-Agder in southern Norway. The fractures were seen most often in young, growing animals. Punsvik and Jerstad (1994) found that fractures occurred eight times more frequently in moose from the Agder counties compared with reference populations in Hedmark and Nord-Trøndelag counties. Pathologic examinations of 31 moose with limb fractures from Vest-Agder, Aust-Agder, and the neighboring county Telemark, revealed many animals (26%) suffering from a generalized osteoporosis (Stuve et al., 1996).

Osteoporosis is a condition signifying the pathological loss of bone mass but with the remaining bone normally mineralized. Osteoporotic bones are fragile and thus prone to fractures. The condition is usually nutritional in origin and due to complicated nutrient imbalances like starvation and mineral and trace element deficiencies (Palmer, 1993).

Here, we report the results of a comparative study on the bone morphology and mineralization, hepatic trace element status, and carcass weights of moose calves from Birkenes municipality (Aust-Agder County) compared with a reference area at Nærøy municipality (Nord-Trøndelag County) in central Norway. The main objective was to clarify whether the increased frequency of bone fractures and osteoporosis reported in moose in the southernmost part of Norway might be related to regional differences in trace element status or bone mineral balance.

MATERIALS AND METHODS

The material is comprised of 43 moose calves killed during hunting seasons in September and October 1994. The calves originated from the municipalities Birkenes in Aust-Agder (58°25′N, 08°15′E; $n = 21$) and Nærøy in Nord-Trøndelag (64°50′N, 11°45′E; $n = 22$). The carcasses were inspected at slaughter and both metacarpi and a 200 g liver sample were collected from each carcass. The samples were stored at −20 C until laboratory examination. The sex, carcass weight, sibling status, abnormalities, and the presence and location of fractures, were recorded for each calf.

Longitudinal slices, about 3 mm thick, were cut out of the distal metacarpal epiphysis, physis and metaphysis and fixed in 10% neutral buffered formalin. Following decalcification in 14% hydrochloric acid, 5 μm thick paraffin-embedded sections were prepared, stained with hematoxylin and eosin (HE) and van Gieson (vG) (Culling et al., 1985), and examined histologically.

All the metacarpi collected were radiographed using the same equipment and the same procedure, with an exposure of 46 kV and 4 mAS. Bone samples were laid directly on the film together with a stepladder of aluminum. The stepladder consisted of 10 successively thicker steps, the tenth step being ten times thicker than the first. Radioopacity was evaluated with Neotech Image grabber® (Neotech Limited, Hampshire, England) and Optilab® (Optilab/Pro 2.5, Graftek, Mirmande, France) and a TLC-450 CCD video camera equipped with an EVA zoom lens 6:1 18–108 mm F 2.6 mounted on a RSI Kaiser Copystand with a distance of 79.8 cm from the film. The camera took 32 exposures of each radiograph lying on a light-table. The picture analysis programs mentioned above digitalize a mean of the exposures and divide this picture into pixels. Every pixel is assigned one of 256 possible degrees of blackness, quantified on a scale from 0 to 255. When an area on the picture is defined, the program estimates the average degree of blackness in this area. We defined an area comprising the distal epiphysis of the metacarpi, proximally limited by a line drawn perpendicular to the periosteum and tangential to the proximal end of *Incisura intertrochlearis,* and one standard rectangular area 2,059 pixels on the fifth step of the aluminum stepladder. The average degree of blackness in the defined area on the metacarpi was divided with the average degree of blackness in the defined area on the aluminum stepladder. In this way the radio-opacity of the metacarpus was expressed as the relative blackness of the epiphysis compared to the aluminum stepladder.

A 4 cm long piece of the diaphysis of the metacarpus was cut out with a band saw, cleaned of soft tissue and dried to constant weight. Density was measured by submersion of the bone specimens in glycerol of known density. The bone specimens were ground and ashed at 500 C and the ash dissolved in *aqua regia.* Concentrations of phosphorus and calcium were determined by inductively coupled plasma spectrometry (ICP) (Hellrich, 1990).

Liver samples were analyzed for copper (Cu), manganese (Mn), zinc (Zn), cobalt (Co), chromium (Cr), molybdenum (Mo), and selenium (Se) by atomic absorption spectroscopy after oxidative digestion. The oxidative digestion was carried out in open tubes with a Tecator Digestion System 40/116 Digester (Tecator ab, Sweden) with temperature controller (Godal et al., 1995). The analyses were performed with a Varian SpectrAA 400 AAS with autosampler (Varian, Mulgrave, Australia). Co, Cr, and Mo were analyzed by graphite furnace atomization (Varian GTA 96) after digestion in Scanpure concentrated nitric acid in Teflon tubes (Sivertsen et al., 1995). Samples for the other analyses were digested in a mixture of nitric and perchloric acids in glass tubes. Selenium was determined by a Varian VGA 76 hydride generator system (Norheim and Haugen, 1986), while Cu, Zn and Mn were determined by standard flame atomization techniques. Detection limits on a wet weight basis were 0.5 μg/g for Cu and Zn, 0.1 μg/g for Mn, 0.003 μg/g for Co, 0.01 μg/g for Cr and Se, and 0.2 μg/g for Mo, all on a wet weight basis. A quality control system with regular analyses of certified reference materials (Bovine Liver 1577B and BCR 185) was adopted. All results of the quality control analyses were acceptably close to the certified values.

In statistical calculations, results below detection limits in the chemical analysis were given a numeric value at half of the detection limit. Statistical analyses were performed in Epiinfo® 6, (Center For Disease Control and Prevention, Atlanta, Georgia, USA). Wilcoxon's two sample test was used to compare the two populations (Altman, 1991). Statistical significance in this study, if not further specified, refers to $P < 0.05$. Correlation analysis was carried out using Pearson's product moment correlation coefficient (Altman, 1991).

RESULTS

Three of the 21 calves from Birkenes (calves A, B, C) and one of the 22 calves from Nærøy (calf D) showed distinct os-

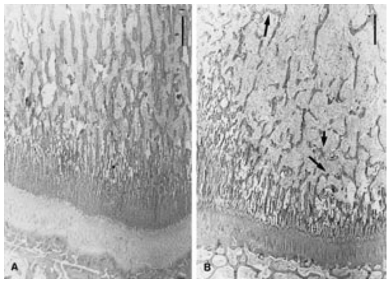

FIGURE 1. The distal diaphyses of metacarpi of (A) a moose calf without any osteoporotic lesions and (B) a moose calf with distinct osteoporosis (calf B in table 1). Note the reduced amount and thickness of longitudinal trabeculae and the presence of transverse trabeculae in calf B (arrows). H&E. Bars = 1 mm.

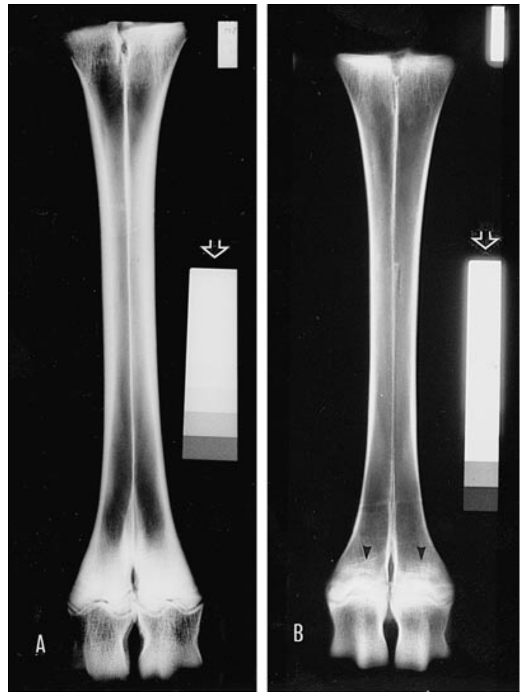

FIGURE 2. Radiography of the metacarpi of (A) a moose calf without any osteoporotic lesions, and (B) a moose calf with distinct osteoporosis (calf B in table 1). Note the reduced radio-opacity and the presence of transverse trabeculae (arrowheads) in calf B. The aluminum stepladder is marked with an open arrow.

teoporotic lesions by histopathologic and radiologic examinations (Figs. 1, 2). The lesions were most severe in calf B. In the metaphyses of this calf, reduced amount and thickness of longitudinal trabeculae and multiple transverse trabeculae were found. The cortical and trabecular bone of the diaphyses and epiphyses was also reduced, and the physeal lines were partially closed. In calves A, C, and D, the lesions were similar but moderate compared to calf B.

The calves with osteoporosis had the lowest bone radio-opacities (0.11–0.38) and carcass weights (23–31 kg) recorded in the two populations (Table 1). All other calves, except for one calf from Birkenes with bone radio-opacity of 0.41, had bone radio-opacities above 0.5. Two calves without osteoporosis, one from Nærøy and one from Birkenes, had carcass weights of 35 and 40 kg. The remaining calves without osteoporosis weighed above 45 kg. A significant positive correlation was found between carcass weight and bone radio-opacity ($r = 0.77$) (Fig. 3). There was no significant difference in bone radio-opacity or carcass weight between the two populations (Table 2).

Calf A and calf B had recent forelimb fractures and healed rib fractures. Two and three of the calves without osteoporosis in Birkenes and Nærøy respectively, had one healed rib fracture. One calf without osteoporosis in Birkenes had a healed limb fracture.

Calf B from Birkenes and calf D from Nærøy, were reported as being orphaned. Half the calves from Nærøy and only one calf from Birkenes were twins. The number of calves per cow was significantly higher in Nærøy compared to Birkenes.

The densities of the bone samples collected in Nærøy were significantly lower ($P < 0.005$) than in Birkenes, but there were no significant differences in the percentage of dry matter and ash (Table 2). The contents of phosphorus and calcium were significantly higher ($P < 0.001$) in the bones from Birkenes, but there was no difference between the two areas in the calcium : phosphorus ratio of the bone samples (Table 2).

No significant differences were found

TABLE 1. Sex; carcass weight; radio–opacity, density, dry matter content, ash content, calcium content, phosphorus content, calcium–phosphorus ratio of bone; and trace element levels in liver (µg/g wet weight) of moose calves with histopathologically identified osteoporosis from Birkenes (A, B, C) and Nærøy (D), Norway in 1994.

	Calf A	Calf B	Calf C	Calf D
Sex	female	female	unknown	female
Carcass weight (kg)	23	25	25	31
Bone analyses				
radioopacity (proportion)	0.28	0.11	0.28	0.38
density (g/cm^3)	1.64	1.76	1.64	1.66
dry matter (g/kg)	996	1,000	987	988
ash content (g/kg)	671	684	681	676
phosphorus (g/kg)	111	117	115	112
calcium (g/kg)	239	240	242	234
Ca : P ratio	2.15	2.05	2.10	2.09
Liver analyses				
copper (µg/g)	54	117	23	51
zinc (µg/g)	73	36	49	22
manganese (µg/g)	3.8	4.3	4.4	4.9
cobalt (µg/g)	0.16	0.091	0.029	0.12
chromium (µg/g)	0.07	<0.01	0.04	0.14
molybdenum (µg/g)	0.5	0.7	0.2	1.0
selenium (µg/g)	1.88	3.58	0.63	0.21

between the hepatic levels of Cu and Cr in the two areas (Table 2). The levels of Mn, Co and Mo were significantly lower in Birkenes than in Nærøy ($P < 0.001$, $P < 0.001$ and $P < 0.025$, respectively), while the levels of Zn and Se were significantly higher in Birkenes ($P < 0.02$ and $P < 0.001$) (Table 2).

The sex distribution was similar in the two municipalities: 11 female, 9 male, and one calf with unknown sex from Birkenes, and 9 female, 10 male, and 3 calves with unknown sex from Nærøy. The female calves had significantly higher percentage of dry matter of bone than the males ($P < 0.01$). No other significant sex differences were found.

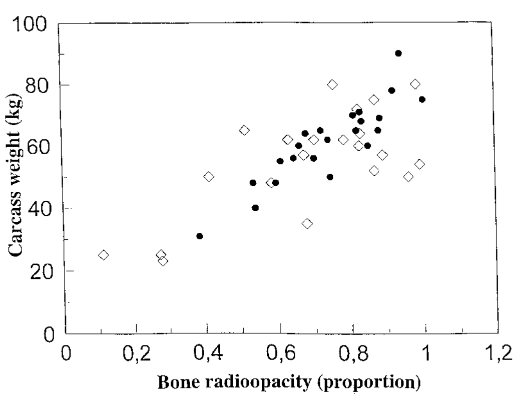

FIGURE 3. Correlation between bone radio-opacity and carcass weight of 21 moose calves in Birkenes (◇) in southern Norway, and 22 moose calves in Nærøy (●) in central Norway.

DISCUSSION

Four of the 43 moose calves, three from Birkenes and one from Nærøy, were suffering from a generalized osteoporosis. We are aware of only a few reports of osteoporosis in free-ranging cervids. Leader-Williams (1980) suggested that dietary deficiency and undernutrition was the cause of severe osteoporosis of the mandible seen in reindeer (*Rangifer tarandus*) living on the subantartic island of South Georgia. Hindelang and Peterson (1996) evaluated osteoporotic skull lesions in skeletal remains of moose collected in Isle Royale National Park, Michigan, USA. The number of lesions increased with age and were more prevalent among males. These le-

TABLE 2. Carcass weight; radioopacity, density, dry matter content, ash content, calcium content, phosphorus content, calcium–phosphorus ratio of bone; and trace element levels in liver (μg/g wet weight) of moose calves from Birkenes and Nærøy, Norway in 1994.

	Birkenes $n = 21$		Nærøy $n = 22$	
	Median	Range	Median	Range
Carcass weight (kg)	57	23–80	63	31–90
Bone analyses				
radioopacity (proportion)	0.76	0.11–0.99	0.74	0.38–1.00
density[a] (g/cm^3)	1.78	1.64–1.85	1.74	1.66–1.78
dry matter (g/kg)	989	981–1,000	993.5	983–1,000
ash content (g/kg)	686	671–704	691	653–700
phosphorus[a] (g/kg)	118	111–121	114	110–119
calcium[a] (g/kg)	242	239–250	238.5	227–242
Ca : P ratio	2.06	2.01–2.15	2.08	2.03–2.14
Liver analyses				
copper (μg/g)	42	13–117	33.5	6–75
zinc[a] (μg/g)	26.0	19–73	22.0	19–31
manganese[a] (μg/g)	3.9	2.7–4.9	5.0	4.0–6.6
cobalt[a] (μg/g)	0.09	0.03–0.16	0.14	0.04–0.46
chromium (μg/g)	0.07	<0.01–0.13	0.04	<0.01–0.14
molybdenum[a] (μg/g)	0.6	<0.2–1.2	0.8	0.3–1.4
selenium[a] (μg/g)	1.20	0.31–3.58	0.13	0.08–0.21

[a] The differences between the groups were statistically significant (*P*-values specified in the text).

sions were interpreted as cumulative manifestations of osteoporosis associated with physiological changes in mineral demands. Frank and co-workers (Frank et al., 1994; Frank, 1998) have suggested secondary Cu deficiency as the cause of a complex wasting disease syndrome occurring in Swedish moose, where osteoporosis is one of the lesions observed.

We did not find any indications of bone mineral imbalance being involved in the disease etiology. In our study, the bone density, ash content, phosphorous and calcium contents, and the calcium : phosphorus ratio were within normal ranges (Staaland et al., 1991; Schultz et al., 1994) for all animals in both locations. Arnemo (1996) reported hypophosphataemia in 15 adult moose from Aust-Agder. However, these findings were not confirmed in later studies (J. M. Arnemo, pers. comm.).

Seven trace elements were selected for analysis in the present study. Of these; Cu, Mo, and Cr have been set in relation to other chronic moose disease problems in Scandinavia (Frank et al., 1994). The other elements were included because of their known importance in the nutrition of domestic ruminants. For all the trace elements included, hepatic concentrations were either found to be within ranges previously observed in normal moose populations in Norway (Frøslie et al., 1984; 1987; Sivertsen et al., 1995), or within an accepted normal range for ruminants (Radostits et al., 1994). No differences in hepatic levels of Cu and Cr were found between the two areas.

A considerable difference in the hepatic level of selenium was observed; the Se concentrations in Birkenes were ten times higher than in Nærøy. The highest hepatic Se concentrations in moose calves from Birkenes might have been indicative of selenium poisoning in domestic ruminants (Osweiler, 1996). However, moose and some other wild cervides are apparently able to accumulate selenium at this level without signs of toxicity. Hepatic Se concentrations in Norwegian moose vary over a wide range, and levels well above the highest concentration observed in this study have regularly been recorded in samples from normal moose (Frøslie et al., 1987; Sivertsen et al., 1995). Experimental feeding of captive pronghorn antilope with high-selenium hay has led to an average hepatic Se concentration above 8 μg/g ww without symptoms of disease (Raisbech et al., 1996). In the present study, no macroscopic signs of hoof abnormalities, liver cirrhosis or other findings typical of chronic selenium toxicity were noted in the carcasses of any of the moose calves.

Thus, in our opinion, the most plausible explanation of the osteoporosis observed in moose calves in our study is protein-energy deficiency. The trabecular lesions found by histopathologic examination of the metaphyses were consistent with osteoporosis due to starvation in farm animals (Platt and Stewart, 1962; Palmer, 1993). The positive correlation found between carcass weight and bone radio-opacity in all our calves supports this interpretation.

The osteoporotic calf from Nærøy and one of the osteoporotic calves from Birkenes were orphaned. These calves most likely did not meet their nutritional requirements when denied mother's milk and the necessary guidance for selection of forage of optimal dietary quality. However, this explanation is not valid for the other two osteoporotic calves from Birkenes.

There was a significantly lower rate of twinning in Birkenes compared to Nærøy, and the median carcass weight was 6 kg lower in Birkenes. Recent studies of the moose population in Agder have revealed a general reduction in reproduction rates (including twin-rate) and carcass weights during the last decade (Stuve et al., 1996). These results have been confirmed in a national moose population surveillance program running from 1991 to 1995 (Solberg et al., 1997). Simultaneously, the moose density has increased dramatically. The local wildlife management authorities have estimated an average winter population density of 2 to 3 animals per km^2, and in some areas the density may even reach 5 to 6 animals per km^2 (Stuve et al., 1996).

Hofmann (1989) classifies the moose as a concentrate selector that strongly selects forage after nutritional quality to meet a high demand for protein and energy (Belovsky and Jordan, 1978). The fitness of individual animals improves with increased intake of energy on the summer range (Hjeljord et al., 1990). The presence of enough high quality forage in summer is therefore important for the maintenance of a healthy and vital moose population. Recent studies of the moose pasture areas and feeding habits in Aust-Agder have shown that highly preferred bushes, for instance rowan (*Sorbus aucuparia*), were utilized almost to extinction (Damli and Roer, 1995). Oak (*Quercus* spp.) and birch (*Betula* spp.), which are not regarded to be among the preferred moose feeds, seemed to be the most important dietary component and were heavily browsed. In addition to low nutritional value, oak, and to some degree birch, contain high concentrations of tannins and other anti-browsing substances (Maxie, 1993; Sunnerheim-Sjöberg and Knutsson, 1995). These anti-browsing substances may have a negative influence on digestive processes (Cooper and Owen-Smith, 1985; Robbins et al., 1987). In conclusion, we did not find any indication that trace element or bone mineral imbalances may be involved in the increased occurrence of bone fractures reported in moose in the Agder counties. We propose that the osteoporosis found in two dam-raised calves from Agder is a result of inadequate nutrition, following a general overcrowding and high pressure on feed resources in the southernmost part of Norway.

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