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Seasonal and individual variation in hepatic copper concentrations in a flock of Norwegian Dala sheep

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ABSTRACT

This study focused on a flock of Dala sheep with recurrent cases of chronic copper (Cu) poisoning. The seasonal variation in hepatic Cu concentration was followed in individual sheep with repeated liver biopsies, four times per year, in two consecutive years. Thirty-six ewes were included, yielding a total of 279 biopsies. Cu concentrations were measured by atomic absorption spectroscopy. Hepatic Cu concentrations remained almost stable from December to March, fell substantially from March to June, and rose sharply during the summer pasture period from June to October. There were large individual differences in hepatic Cu levels. These differences remained stable through the two years. Treatment with ammonium tetrathiomolybdate (3 × 3.4 mg per kg bodyweight (bw) s.c.) in June had only weak and inconsistent effect on hepatic Cu levels in October. The results may partly explain why chronic Cu poisoning in sheep in Norway predominantly occur in the autumn and winter months.

1. Introduction

Chronic copper poisoning in sheep is well known in Norway (Nordstoga, 1962). In much of the world, outbreaks of this disease are most commonly seen in housed lambs or milk sheep, when they are fed concentrates with an improper mineral balance (Greene and Huston, 1999; Underwood and Suttle, 1999). In Norway, chronic Cu poisoning is an endemic, low-frequency, spontaneous disease, encountered under normal grazing conditions. It is primarily seen in inland districts, during the autumn and early winter months, in sheep that have been on mountain or woodland pasture in the summer. The condition is seen mostly in ewes above one year of age (Nordstoga, 1962; Sivertsen et al., 1995; Søli, 1980). Previous research has indicated that a main background for these disease cases is a severe hepatic copper accumulation in a high percentage of sheep in some Norwegian inland districts (Frøslie, 1980), which seems to be related to low molybdenum concentrations and high copper/molybdenum ratios in local grass (Frøslie and Norheim, 1983) and mountain pasture plants (Garmo et al., 1986).

From the 60s to the 90s, cases of chronic copper poisoning was fairly common, and led to much concern in the sheep industry (Frøslie, 1980; Sivertsen et al., 1995). Since then, the prevalence seems to have subsided, though cases are still diagnosed by the Norwegian Veterinary Institute almost every year (Bernhoft, 2013). The reasons for this apparent change in prevalence is not fully understood.

A recent nation-wide survey of hepatic trace element concentrations in slaughtered Norwegian sheep and lambs did however confirm that serious hepatic Cu accumulation in the autumn is still widespread in sheep in Norway (Sivertsen et al., 2009). Hepatic Cu concentrations up to 690 μg/g wet weight (ww) were observed, and 13% of sampled livers from adult sheep had Cu concentrations

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above 150 μg/g ww. This led us to reconsider the previously unpublished results from a study of the seasonal variation of hepatic Cu concentrations performed in the 1990's, and prepare it for publication.

Though most clinical cases of chronic Cu poisoning in Norway have always occurred sporadically, the prevalence might in these years be considerable in some flocks (Sivertsen and Wie, 1996), and one of these flocks was used in the investigation.

Several important questions concerning Cu accumulation and poisoning in Norwegian sheep have remained unanswered by previous research. Among these are the reasons for the seasonal occurrence of the disease, and the individual factors that determine which animals within the flock that will be clinically affected. One of the limitations of previous studies is that they have all been based on samples collected at slaughter, and in Norway the great majority of sheep are slaughtered in a few autumn months, from September till November. In an attempt to improve the knowledge on the seasonal variation in hepatic Cu accumulation, we used repeated liver biopsies to track hepatic Cu concentrations in a number of sheep from the selected flock; through two consecutive years. We also tested the effect of three s.c. injections with ammonium tetrathiomolybdate (Humphries et al., 1988) in June on the hepatic Cu concentration in October, and of similar injections in November upon the hepatic concentrations two weeks later. Tetrathiomolybdate (TTM) is an effective chelator of Cu ions, and parenteral TTM injections have been used successfully to reduce hepatic Cu levels in flock outbreaks of chronic Cu poisoning (Gooneratne et al., 1981; Humphries et al., 1988). In order to evaluate the possible effect of the mineral content of the winter feed, we analyzed Cu, zinc (Zn), molybdenum (Mo) and sulfur (S) concentrations in the hay and silage fed to the flock in the indoor season.

Permission to conduct the liver biopsy study was granted by the National Animal Research Authority in Norway. Some preliminary results from the first year of the study were presented at two symposia in Norway in the nineties (Sivertsen et al., 1995; Sivertsen and Wie, 1996).

2. Materials and methods

2.1. Animals, feeding and pasture

The flock used in this study consisted of about 60 winter-fed ewes, of the Norwegian Dala breed. In four out of six years before the study was started, the owner had lost ewes from copper poisoning. A total of 7 ewes were lost during the worst year. All the lost ewes were adult, all died in the autumn, and all had shown the typical signs of copper-induced hemolytic crisis and hemoglobinuric nephrosis (Moeller, 2004; Søli, 1980). Mean hepatic copper concentrations in clinically normal ewes from the flock slaughtered in autumn had in these years varied from 257 to 465 μg/g ww (Sivertsen et al., 1995).

The flock was from a small mountain farm in Namdalen, an inland area in the county of Nord-Trøndelag, at 64° 40′ latitude and 450 m above sea level. As in most mountain areas in Norway, the surface varies between bare rock and thin deposits of glacial origin. Soils are predominantly podzols, with patches of swamp soils in between (Låg, 1983). Climatically, the area lies mainly within a low alpine vegetation zone (Moen, 1999). From the middle of October to the middle of June the sheep were kept indoors in an unheated barn and mainly fed silage and hay grown on the farm. Concentrates were used in small amounts, mainly in the months before and after lambing. The compound concentrates and the mineral supplements used in the flock were without added copper, but contained standard recommended amounts of other trace elements (Zinc, cobalt, selenium, iodine, manganese). From the start of July till the middle of October the flock was grazing in the surrounding mountain area. No changes due to this study were made in the normal management of the farm, nor with the owner's recruitment and slaughter policy.

The study was performed in the years 1994–96. It was begun in late November. Ten adult ewes aged 1½–7 years and 10 ewe lambs born in the spring were selected for inclusion. Liver biopsies were taken at the start in late November, and in March, June, October and December the following year. As three of the original animals selected were slaughtered in the spring, three new ones were recruited.

In December of the second year, 10 adult ewes (age 1½–7 years) and 10 new ewe lambs were again selected. Seven of the adult ewes had also been sampled the first year, while 3 were new. The second year samples were taken in December, and in March, June and October in the following season. Altogether 36 animals were sampled. Two of the animals we managed to sample only once. As they would give no information on temporal change, the results from these animals are not included in the tables, figures and main statistical calculations. Due to logistical problems, the liver samples from four of the ewes in October the last year had to be collected at the abattoir, after slaughter. An overview of the sampling, treatment and fate of each of the 34 animals included in the results are shown in Table 1.

Twenty-nine samples of the silage and hay fed to the sheep in the winter season were collected at intervals both winters (Tables 1 and 3).

2.2. Ammonium tetrathiomolybdate treatment

In each of the seasons studied, half of the animals included in the study were treated with ammonium tetrathiomolybdate (TTM) in June, to see if this affected the levels in October (Table 1). In assigning the animals to the treatment and non-treatment groups, efforts were made to obtain an even distribution between groups. The distribution of adult and one year old ewes as well as the hepatic Cu levels recorded in March were taken into account.

In the first autumn, half of the animals that had been sampled in October were given a new TTM treatment in November, ending two weeks before the sampling of new liver biopsies in December. None of these animals were included in the second year of the study (Table 1). This November TTM treatment did therefore not influence the results reported in Table 2.

The treatments comprised three s.c. injections of 3.4 mg TTM per kg bw, with 48 hours' intervals (Humphries et al., 1988). The TTM had been bought as crystalline ammonium tetrathiomolybdate from Rowett Research Services, Aberdeen, UK, and kept dry and cool till it was used. Immediately before usage it was dissolved in sterile 0.9% saline solution to a concentration of 40 mg/ml. The dissolved batch was stored in a refrigerator between treatments and discarded after the third treatment.

2.3. Liver biopsy method

The biopsies were taken in the barn, using a modified version of the percutaneous approach described by Harvey et al. (1984) and Humann et al. (1999). No sedation was necessary. An area above the 10th and 11th costae on the right side of the animal was clipped and shaved. To select the most suitable place to insert the biopsy needle, and the appropriate depth from the skin surface, a portable ultrasound scanner (Aloka Echo Camera SSD-500 with 7.5 MHz scanner head, Aloka Co ltd, Tokyo, Japan) was used. Usually a point in the 11th intercostal space was chosen, about a third of the length from the top of the palpable costa. In some sheep, especially when pregnant, a point slightly further down in the 10th intercostal space was found to be more suitable. After disinfection with 2% iodine tincture, and local anesthesia with 5 ml of 20 mg/ml Lidocaine, a small incision was made in the skin. The biopsies were taken with a sterile disposable biopsy needle with depth markings (Bard® Biopty-cut 14 G × 160 mm) mounted on an automatic biopsy pistol (Bard® Biopty, Bard biopsy systems, Tempe, Arizona). In late November the first year, only one biopsy was taken from each animal. At the subsequent sampling dates, we attempted to take two biopsies each time. After the last biopsy, the incision wound was closed with a wound clip. Immediately after excision, the biopsy was dropped into an isolated box filled with liquid nitrogen. Thereafter, each of the frozen biopsies was put into a closed and pre-marked plastic vial (Nalgene Cryoware 1.2 ml, Nalge (UK) Ltd., Rotherwas, UK). The vials were