DIAGNOSIS OF MILK FEVER BY A WATER HARDNESS TEST KIT IN EWES

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ABSTRACT

The aim of the study was to evaluate the measurement of total calcium levels in sera of sheep with and without milk fever by using a commercial water hardness test kit. Thirty sheep with findings of milk fever from 9 different farms were used in the present study. Total serum calcium concentrations were determined by using a commercial water hardness test kit and a laboratory automated biochemical analyzer. Results of the test kit and laboratory methods were significantly ($P < 0.001$) correlated (Spearman's $p = 0.896$). In conclusion, it has been determined that total calcium levels in sheep sera may be determined with a water hardness test kit as used in this study, and that data are in concordance with the clinical findings and the other laboratory results.

Key words: diagnostic, milk fever, sheep, water hardness test kit.

INTRODUCTION

Milk fever - parturient paresis or hypocalcaemia - is a metabolic disorder that occurs around the time of parturition or during early lactation in sheep (1). The disease commonly occurs in outbreaks, in groups of ewes exposed to forced exercise, long-distance transport, and sudden deprivation of food and grazing on oxalate-containing plants or green cereal crops (2). Although it is may be seen occasionally as epidemic in the herd where up to 25% of the herd may be affected, its course is mostly sporadic and generally affects less than 5% of the herd (1, 2, 3, 4).

The disease is characterized by a low serum calcium (Ca) level (2, 5, 6). As a result of inadequate calcium intake during the last periods of pregnancy or first periods of lactation, the body may meet the required need of calcium through calcium mobilization from bones. If hormonal mechanisms are inadequate, for example in the case of an inactive parathyroid gland, mobilization is delayed and blood calcium concentration is reduced with the resultant development of milk fever. In small ruminants, hypocalcaemia may develop secondarily during the course of other periparturient diseases, particularly in pregnancy toxemia (7). The diagnosis of the disease is based on clinical or laboratory methods or based on the presence of risk factors which have been reported (8). Therefore, monitoring the calcium level in sheep during periparturient period may be useful for the identification of the development risk of both milk fever and other hypocalcaemia related periparturient diseases. The definitive diagnosis is made through measurement of total and /or ionized serum calcium concentration (7). Serum total or ionized calcium levels may be measured by laboratory and/or portable biochemical analyzers. In veterinary practice chemistry analyzers are often unavailable in the field. Therefore, the aim of the present study was to measure the total calcium levels in sheep sera with and without milk fever using a commercial water hardness test kit (WHTK) compared to a routine laboratory method.

MATERIAL AND METHODS

Animals

Thirty ewes were used in this study had at least one clinical findings of milk fever such as depression, increased respiration, muscular tremor, weakness, sternal recumbence and extended or twisted head. Clinical examinations were made by the same veterinarian. The sheep were 3.3±1 years of age (range 2 and 5 years) were taken from 9 different farms. Fifteen sheep were pregnant and 15 sheep were lactating.

Approximately 20 ml blood was collected from the jugular vein. Blood samples were allowed to clot for minimum 30 minutes at ambient temperature. The samples were then centrifuged and sera were collected. The sera were kept at -20°C until the analysis was performed. All samples were analyzed within 4 weeks.

Serum calcium analyses

Total serum calcium concentrations were determined by spectrophotometric method using an automatic biochemical analyzer (Cobas 6000 analyzer, Roche, Switzerland). All samples were analyzed at the same time. Based on the results obtained with the automatic chemical analyzer the values were found to be in the range of 6.1 to 10.1 mg/dl.

Total calcium concentrations in the sera samples of all sheep were determined using a commercial WHTK which was designed for measurement of calcium carbonate concentrations in water samples (CHEMetrics. Inc Cat. No: K-1705. Calverton, VA, USA). The test was carried out as follows: Calcium in the test sample was allowed to react with a zinc salt in the presence of a color indicator. The determined blue and blue-green color of the resulting zinc-zincon complex was assigned as the endpoint of the reaction. According to the manufacturer the test range for the kit was between 2 to 20 mg/dl. Briefly, all samples were thawed at room temperature (approx. 20°C) during the test period. In order to achieve proper concentration, 1 drop of indicator solution were dropped in 6.7 ml of sera sample and mixed. The content turned an orange color. This sample was drawn into an ampoule by using a titrator apparatus and the content of the ampoule was shaken gently back and forth. This application was performed until the orange color of the ampoule content was transformed into blue or blue-green color. The calcium carbonate concentration (ppm) was inversely correlated to volume of sera reaching the endpoint titration on the scale of the ampoule. The result was further multiplied by 0.04 to calculate total calcium concentration in mg/dl. The samples were assayed with the WHTK in a blind manner without prior knowledge of the laboratory results.

Statistical analyses

Linear regression test was performed in order to determine the relationship between the sera total calcium levels obtained from the laboratory versus the WHTK analyses. Sperman's rank correlation test was applied to calculate the correlation between the results obtained by these two methods. Two samples were excluded from statistical analysis due to hemolysis.

In order to evaluate the WHTK capacity to diagnose milk fever, calcium concentrations of less than 8 mg/dl was determined as a laboratory-derived value for defining milk fever. The cutoff reference value was used according to results of Bickhardt et al. (9).

Sensitivity and specificity were calculated on the basis of actual test kit measurements using the defined total Ca concentration for milk fever, the cutoff value and predicted total Ca concentrations, derived from the regression equation. All statistical analyses were performed with computer based software for statistics (Sigma Stat).

RESULTS

Based on the results obtained in the laboratory, sera total calcium values were between 6.1 mg/dl and 10.1 mg/dl (median, 8.5 mg/dl) and the concentration in 6 of 30 samples was below 8.0 mg/dl.

There was a significant relationship between the laboratory results and WHTK results in the 28 samples tested (R^2= 0.7743; Figure). The regression formula was: laboratory value = 0.4324 + (0.953 x test kit value). The 95% confidence intervals for the regression equation were 0.4 to 1.6, the intercept and slope and 0.8 to 1, respectively. Results of the WHTK and laboratory methods were found to be significantly ($P < 0.001$) correlated (Spearman's p = 0.896).

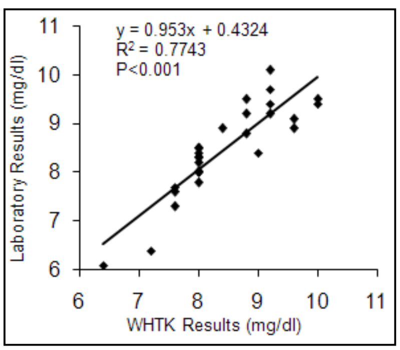

Figure - Linear regression of total serum Ca concentrations derived by use of a laboratory based-method and by use of WHTK for 28 sheep.

When comparing the two methods, 6 samples of the laboratory results were evaluated to fall into the range designated for the diagnosis of milk fever (Ca< 8 mg/dl), while only 5 samples analyzed by the WHTK method were found to fall into this range (sensitivity = 83.3%). Laboratory results showed that when 22 samples which were found to be greater than 8 mg/dl sera total calcium concentration were compared to the WHTK method results, all samples were found to be above the cutoff value of 8 mg/dl, therefore giving a specificity of 100% for this range. Thus, predictive value of a negative test result was 100% (22/22) and predictive value of a positive test result was 83% (5/6). The accuracy of WHTK to indicate the diagnosis of milk fever was verified by the predicted total calcium concentration of < 8 mg/dl and the regression equation results.

Analysis of two the hemolyzed serum samples did not react with the WHTK giving no color change where laboratory method was able to calculate a calcium value.

DISCUSSION

Milk fever in sheep develops mostly in the last 4-6 weeks of pregnancy and the first 6 weeks of lactation (2, 4). The most frequently observed clinical findings of the disease involve ataxia, tremor, tetania, constipation and reduced ruminal motility, increased respiration and pulsation, regurgitation, tympani, depression, sternal recumbency and extended or twisted head (2, 5). In the study presented, 30 sheep, with at least one clinical finding of milk fever, were tested during the periods between the last 4 weeks of pregnancy and the first 4 weeks of lactation.

The clinical signs of milk fever are nonspecific and therefore cannot be relied upon. The differential diagnosis of milk fever includes hypomagnesemia, osteomalacia, pregnancy toxemia and enterotoxemia (2, 10).

In cows, milk fever may coexist with hypermagnesemia or hypomagnesemia. Relative hypermagnesemia may occur by shifting the ratio of Ca:Mg from 6:1 to 2:1. Hypophosphatemia in milk fever may be secondary to the hypocalcemia rather than being a concurrent event. Woldemeskel et al. (5) found that serum while total calcium may be decreased phosphorus and magnesium were normal in sheep with milk fever. In contrast, El-Khodery et al. (1) reported both total calcium and magnesium levels were low in milk fever.

The clinical pathological diagnosis of milk fever is based on the measurement of the total serum calcium concentration which is comprised of the sum of ionized calcium and the calcium bound protein. Ionized calcium is important for immediate metabolic functions however the analysis of ionized calcium in the field is not practical due to the unavailability of equipment and the high expense involved (2). Practically this is not required due to the correlation between the concentrations of ionized and total sera (2, 4, 11). In the last decade, there have been many reports of diagnosis of milk fever using total serum total calcium level in cows (12, 13, 14), buffalo (15), and sheep (1, 5, 6, 16). Bickhardt et al. (9) showed that serum total calcium concentrations are less 8 mg/dl in sheep with milk fever.

In the field the drawing blood from the animal with suspected milk fever, delivering the sample to the laboratory and waiting for the result lead to a loss of valuable time which is disadvantageous to the health of the patient. In the

1990's, portable biochemical analyzers developed for humans were used in animals, particularly in cats, dogs and horses providing more accessible results in a shorter time frame (18). Later, portable biochemical analyzers were produced specific for animals including sheep, which are still being used successfully. The disadvantage of these analyzers are their high costs.

In this study, sera total calcium concentrations were determined by using a commercial water hardness test kit which is highly affordable and the results correlate well with the standard laboratory method ($P < 0.001$) (Spearman's p = 0.896). Previous studies have been undertaken to determine the calcium levels in sera using commercial water hardness test kits. A commercial kit designed for water quality analysis was used by Ley et al. (17) in order to obtain the quantitative value of calcium concentration in mare milk. Matsas et al. (16) further determined sera total calcium levels in dairy cattle by using such a kit in order to obtain a diagnosis of milk fever. They found sensitivity of 100% and specificity of 73% when comparing the laboratory method with the data obtained by using WHTK indicating that there was a significant correlation between two methods ($P<0.001$). Comparing the laboratory results and the results obtained by using WHTK in the present study we found a sensitivity of 83.3% and a specificity of 100%. There was a significant correlation ($P<0.001$) between two methods as was shown Matsas et al. (16) working with dairy cattle.

The WHTK lacks the ability to determine magnesium and phosphorus concentration which is a disadvantage. The use of non-hemolysed blood is a prerequisite for using the WHTK analysis. The advantages of the kit lies in the fact that the test is a rapid (less than 5 minutes) and inexpensive method for measuring serum total Ca concentrations in sheep. This offers a benefit ovine practitioners and veterinarians who do not own portable clinical analyzers or blood chemistry machines, allowing diagnosis of milk fever in individual sheep and monitoring of postpartum Ca concentrations of a herd. The assay range of WHTK (2 to 20 mg/dl) is wide enough to measure total calcium level in serum samples. It can be assumed that this test may be used in other situation other than milk fever which has been correlated in this study with a standard laboratory method.

In conclusion, it has been determined that total serum calcium levels in sheep may be measured with the water hardness test kit used in this study, and that data are in concordance with standard laboratory results.

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