Journal of Animal and Veterinary Advances 10 (23): 3169-3171, 2011
ISSN: 1680-5593

Inhibition of Blood Coagulation by Di-Sodium Versenate: A Rapid Method for Estimating Serum Calcium in Dairy Cows

Bhaskar Ganguly
Department of Veterinary Physiology and Biochemistry,
College of Veterinary and Animal Sciences,
G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Abstract: Rapid estimation of serum calcium levels is of pivotal importance in the diagnosis and institution of therapy for milk fever. Several laboratory methods are available for the determination of serum calcium. However, determination of calcium concentrations in serum by these methods is complicating and time consuming. The present study was conducted to develop sequestration of blood calcium by di-sodium versenate as a method for rapid estimation of serum calcium levels. The concentration of di-sodium versenate required for preventing coagulation of known amount of blood was correlated with the serum concentration of calcium using sodium chloranilate method. A simple test for the rapid estimation of serum calcium level by calcium sequestration using di-sodium versenate was developed and used in post-parturient cows for diagnosis of milk fever.

Key words: Di-sodium versenate, hypocalcemia, milk fever, post-parturient, serum calcium, India

INTRODUCTION

Milk fever or parturient paresis is an important production disease of cattle, sheep and goats caused by hypocalcemia, occurring most commonly in high producing adult lactating dairy cattle at or soon after parturition and manifested by changes in mentation, generalized paresis and circulatory collapse. At or near the time of parturition, the onset of lactation results in the sudden loss of calcium through milk. A depression of ionized calcium in tissue fluids is the basic biochemical defect in milk fever. Ionized and total serum calcium levels exist in a dynamic equilibrium with each other. Total serum calcium levels decline from an average normal of 10-12 mg dL^{-1} to below 8 mg dL^{-1}. Commonly, serum magnesium is increased, serum phosphorus is decreased and cows may be hyperglycemic. The disease may occur in cows of any age but is most common in high-producing dairy cows above 5 years of age (Radostits *et al*., 2000).

There are three discernible stages of the disease. In stage one the cows are able to stand but show signs of hypersensitivity and excitability. If calcium therapy is not instituted, cows progress to stage two where they are unable to stand but can maintain sternal recumbency. The third stage terminates in coma and death. Cows in stage three survive only a few hours. Differential diagnoses include toxic mastitis, toxic metritis, other systemic toxic conditions, traumatic injury (e.g., stifle injury, coxofemoral luxation, fractured pelvis, spinal compression etc.), obturator paralysis or compartmental crush syndrome.

Some of these diseases in addition to aspiration pneumonia may also occur concurrently with parturient paresis or as complications. Treatment is directed toward restoring the blood calcium level to normal. Hypocalcemic cows typically respond to therapy immediately. About 75% of cows stand within 2 h of treatment. Cows not responding by 4-8 h should be re-evaluated and treatment repeated if necessary. Of the cases that respond initially, 25-30% relapse within 24-48 h and require additional therapy (Barrington, 2000).

Running a calcium profile of a blood sample is the only definitive way to diagnose milk fever. Any cow with a total serum calcium concentration <2.18 mmol L^{-1}, equivalent to 8.7 mg dL^{-1} should be diagnosed as a case of sub-clinical milk fever. Cows generally do not show clinical signs until the calcium level drops to 5 mg dL^{-1} and often do not become downers until the level reaches 3 mg dL^{-1} (Radostits *et al*., 2000).

Several laboratory methods are available for the determination of calcium (Total and ionized) in blood, serum and plasma including the Kramer and Tisdall method (Kramer and Tisdall, 1921), potentiometric determination (Anker *et al*., 1981), oxalate precipitate (Clark and Collip) method (Clark, 1920), titration methods (Ward *et al*., 1960), flame photometry and Atomic

Absorption Spectroscopy (Burnett *et al*., 2000). For ionized calcium measurement, the International Federation of Clinical Chemistry and Laboratory Medicine (Burnett *et al*., 2000) has described potentiometry as the reference method. However, the use of these methods is greatly limited by the protracted protocol and sophistication of the method.

Di-sodium salt of Ethylene Diamine Tetra-acetic Acid (Na_2EDTA), commonly called di-sodium versenate or di-sodium edetate, quantitatively binds blood calcium to arrest its coagulation. Thus, determination of the amount of calcium that is bound to a constant amount of di-sodium versenate should enable development of a test where the calcium levels in a blood sample can be estimated by grossly observing the inhibition of blood coagulation in the presence of varying concentrations of di-sodium versenate. Therefore, the present study was taken up with the following objectives:

- To develop a suitable method of blood calcium sequestration for indirect estimation of serum calcium level
- To estimate serum calcium levels in post-parturient cows (within 6-12 h of calving) by calcium sequestration method

MATERIALS AND METHODS

Development of blood calcium sequestration method for indirect estimation of serum calcium level: The proposed test method was standardized against sodium chloranilate method (Webster, 1962) as per the following steps.

The 1% (10 mg mL^{-1}) stock solution of di-sodium versenate was prepared by dissolving 1 g of di-sodium versenate (S.D. Fine Chem, India) to 100 mL with triple glass distilled water. Di-sodium versenate solutions of concentration 0.6, 0.7, 0.8, 0.9, 1.0 and 1.1 mg mL^{-1} were prepared. The solutions hence obtained were poured in glass vials and the vials were placed in a hot air-oven at 60°C until dry. Successively, the glass vials were grouped into sets of six vials, one each of concentrations 0.6, 0.7, 0.8, 0.9, 1.0 and 1.1 mg mL^{-1}.

About 10 mL blood was collected by jugular venipuncture from an apparently healthy dairy cow. About 1 mL blood was transferred in each of the six vials of a set. The vials were stoppered and observed for clotting of blood.

For standardization of the test procedure, serum obtained from the remaining 4 mL of blood was used in triplicate for estimation of calcium level by sodium chloranilate method (Webster, 1962). The Optical Density (OD) of the test and standard was read against the blank at 520 nm. Serum calcium concentration was calculated using the following equation:

$$\text{Serum } Ca^{+} \text{ Conc.} = \left[\frac{OD_{Test} - OD_{Blank}}{OD_{Std} - OD_{Blank}}\right] \times Conc._{Std}$$

Estimation of serum calcium in post-parturient cows: The test was used for determination of serum calcium in 10 post-parturient cows at Instructional Dairy Farm, G.B. Pant University of Agriculture and Technology; blood samples were obtained by jugular venipuncture. The sets of vials were taken and blood was collected in them; 1 mL blood in each of the six vials of a set and observed for clotting inhibition.

RESULTS AND DISCUSSION

During the clotting inhibition studies following observations were made (Table 1). The clotting of blood was found to be inhibited at a di-sodium versenate concentration of 0.9 mg mL^{-1}.

Using sodium chloranilate method, following OD readings were obtained for the same blood sample (Table 2). Thus, the serum calcium concentration of the sample worked out to:

$$\text{Serum } Ca^{+} \text{ Conc.} = \left[\frac{0.103 - 0.038}{(0.070 - 0.038}\right] \times 5 \text{ mg } dL^{-1}$$

$$\text{Serum } Ca^{+} \text{ Conc.} \approx 10.15 \text{ mg } dL^{-1}$$

Now, the results were correlated the serum calcium level determined by sodium chloranilate method was 10.15 mg dL^{-1} and since 0.9 mg of di-sodium versenate inhibited the coagulation of 1 mL blood that contained 10.15 mg dL^{-1} of calcium, it was concluded that 1 mg of di-sodium versenate would inhibit the coagulation of 1 mL blood with about 11.3 mg dL^{-1} of serum calcium concentration.

Tabel 1: Observations of clothing inhibition

Na_2EDTA concentration (mg mL^{-1})	Clotting of blood*
0.6	+
0.7	+
0.8	+
0.9	-
1.0	-
1.1	-

*+: Clotting present, -: Clotting absent

Table 2: OD readings

Tube	Test	Standard (5 mg dL^{-1})	Blank
OD	0.103	0.070	0.038

Table 3: Determination of serum calcium in post-parturient cows

Cow number	Minimum concentration of Na_2EDTA showing clotting inhibition (mg mL^{-1})
2582 C	0.6
2588	0.7
2689	0.6
2699	0.6
2775	0.6
X 119	0.6
X 127	0.6
X 167	0.6
X 19	0.6
X 47	0.7

The test was used for determination of serum calcium in 10 post-parturient cows and the observations of the test are shown in Table 3.

Inhibition of coagulation occurred with di-sodium versenate at a mean concentration of 0.62±0.012 mg mL^{-1}. Since, 1 mg of di-sodium versenate chelated 11.3 mg dL^{-1} of calcium, 0.62±0.012 mg of di-sodium versenate would correspond to 7.006±0.143 mg dL^{-1} (11.3×0.62±0.012) of calcium.

Serum calcium level was estimated in post-parturient cows (within 6-12 h of calving) by calcium sequestration method and was found to have an average value of 7.006±0.143 mg dL^{-1} or below. These levels are significantly <8.7 mg dL^{-1} therefore, the cows were diagnosed to be suffering with sub-clinical milk fever. For most samples, inhibition of coagulation occurred with 0.6 mg mL^{-1} of di-sodium versenate corresponding to serum calcium levels of 6.78 mg dL^{-1} or below.

The inherent simplicity and compact set-up of the test, requiring only six 1 mL volume wells and one pipetting aid, implies a ready adaptation to a kit based assay for the rapid estimation of serum calcium in cattle as well as in other animal species. Though limited by small frame of standardization and a small sample size, the test shows good internal validity.

CONCLUSION

A simple test for the rapid estimation of serum calcium level by calcium sequestration using di-sodium versenate was developed and used in post-parturient cows for diagnosis of milk fever. Due to ease and quickness of performing, the test appears to be useful for the rapid estimation of serum calcium towards diagnosis of milk fever.

ACKNOWLEDGEMENTS

The researchers acknowledge the Departments of Veterinary Clinical Medicine and Veterinary Biochemistry, College of Veterinary and Animal Sciences and Joint Director, Instructional Dairy Farm, G.B. Pant University of Agriculture and Technology, Pantnagar for extending the necessary facilities and resources required for the study.

REFERENCES

Anker, P., E. Wieland, D. Ammann, R.E. Dohner, R. Asper and W. Simon, 1981. Neutral carrier based ion-selective electrode for the determination of total calcium. Blood Serum Anal. Chem., 53: 1970-1974.

Barrington, G.M., 2000. Parturient Paresis in Cows. In: The Merck Veterinary Manual, Aiello, S.E. (Ed.). 8th Edn., Merck and Co. Inc., Whitehouse Station, New Jersey, ISBN: 9780911910803.

Burnett, R.W., T.F. Christiansen, A.K. Covington, N. Fogh-Andersen and W.R. Kulpmann *et al.*, 2000. IFCC recommended reference method for the determination of the substance concentration of ionized calcium in undiluted serum, plasma or whole blood. Clin. Chem. Lab. Med., 38: 1301-1314.

Clark, G.W., 1920. Effect of hypodermic and oral administration of calcium salts on the calcium content of rabbit blood. J. Biol. Chem., 43: 89-95.

Kramer, B. and F.F. Tisdall, 1921. A simple technique for the determination of calcium and magnesium in small amounts of serum. J. Biol. Chem., 47: 475-481.

Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Production Diseases, Veterinary Medicine. 9th Edn., W.B. Saunders, Philadelphia, pp: 1420-1434.

Ward, G.M., R.C. Lindsay and C.A. Vain, 1960. Calcium and magnesium determination in bovine blood by EDTA titration. J. Dairy Sci., 43: 1014-1015.

Webster, W.W. Jr., 1962. A simple microspectrophotometric method for the determination of serum/plasma calcium. Tech. Bull. Registry Med. Technol., 32: 32-35.