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Serum iron as an indicator of acute inflammation in cattle[1]

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ABSTRACT

The aim of this study was to assess the value of serum iron concentration in the diagnosis of acute inflammation in cattle. The diagnostic value of this approach was compared with that of various other hematological tests, including commonly used techniques that measure the levels of various other acute-phase proteins. The study population comprised 10 cows with acute traumatic reticuloperitonitis (RPT group) and 10 cows with acute mastitis (mastitis group) admitted to the Veterinary Teaching Hospital at Firat University (Elaziğ, Turkey). Ten cows from local barns, kept and fed under same conditions as the diseased animals, were used as controls. After the clinical examination, blood samples were collected for biochemical, hematological, and acute-phase protein (haptoglobin, serum amyloid A, α-1 acid glycoprotein, and fibrinogen) analyses. The mean levels of serum iron in the RPT, mastitis, and control groups were 6.00, 7.82, and 26.78 μmol/L, respectively. Serum iron level was significantly reduced in the RPT and mastitis groups. The results of this study indicate that serum iron analysis, preferably in combination with other markers of inflammation, may be a useful diagnostic tool for acute inflammation in cattle. Because serum iron measurement is individually available and easily applicable, it may be used for clinical cases as well as the determination of herd health.
Key words: acute inflammation, acute-phase protein, cattle, iron

INTRODUCTION

Inflammation is a complex response to cell or tissue injury. This response dilutes, eliminates, or incapacitates agents that are injurious to the organism and then initiates the reconstruction and recovery of damaged cells and tissues (Cheville, 1999; Baron and Lee, 2006). Inflammation is usually classified as acute or chronic (Ward, 2010).

Acute inflammation develops over minutes or hours, depending on the type and severity of the tissue damage and generally lasts hours to days (Kumar and Wakefield, 2010). Acute inflammation involves the leakage of water, salt, and proteins from the vascular compartment; endothelial cell activation; adhesive interactions between leukocytes and the vascular endothelium; leukocyte recruitment; macrophage activation; platelet activation and aggregation; activation of the complement, clotting, and fibrinolytic systems; and the release of proteases and oxidants from phagocytic cells to cope with the injury (Ward, 2010). Clinically, the integration of these phases manifests as redness, heat, swelling, pain, and a loss of function (Baron and Lee, 2006; Ward, 2010).

Chronic inflammation lasts longer than acute inflammation (weeks or months) and often results from the organism's failure to eliminate an acute inflammatory irritant or an autoimmune response to a self-antigen. The persistence of an innately chronic irritant of low intensity can also trigger chronic inflammation. Chronic inflammation is characterized by simultaneous inflammation and repair, including the recruitment and activation of macrophages, lymphocytes, and other cells. These processes are regulated by the coordinated action of cytokines and growth factors (Ward et al., 1996; Baron and Lee, 2006).

Numerous bovine diseases are associated with acute inflammation, but the arsenal of the practicing veterinarian includes only a limited number of laboratory tests for the diagnosis of inflammation. Total and differential white blood cell (**WBC**) counts and acute-phase proteins (**APP**) analyses are the tools most commonly used for the diagnosis of bovine inflammatory diseases (Hirvonen, 2000; Murata et al., 2004; Petersen et al., 2004; Ceciliani et al., 2012). Studies indicate that serum Fe concentration could also be used as a marker of acute inflammation in humans and certain other animal species (Ward et al., 1996; Sunder-Plamann et al., 1999; Neumann, 2003; Borges et al., 2007). Serum Fe concentration decreases rapidly in response to inflammation. This host defense mechanism aims to withhold the Fe required for bacterial virulence and replication (Borges et al., 2007). Serum Fe concentration has been evaluated as a marker of inflammation in dogs

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(Neumann, 2003), cats (Neumann, 2003), and horses (Jacobsen et al., 2005; Borges et al., 2007), but limited data exist about whether serum Fe concentration can be used to diagnose acute inflammation in cattle.

The present investigation was undertaken to assess the value of serum Fe concentration in the diagnosis of acute inflammation in cattle. The diagnostic value of this approach was compared with that of the hematological tests and APP used currently.

MATERIALS AND METHODS

The study population comprised 10 cows with acute traumatic reticuloperitonitis (**RPT**) and 10 cows with acute mastitis admitted to the Veterinary Teaching Hospital at Firat University (Elazığ, Turkey). Ten cows from local barns, kept and fed under the same conditions as the diseased animals, were included as controls. All the cows underwent a detailed clinical examination (Radostits, 2000). The tentative diagnosis of acute RPT was based on the results of physical, ferroscopic (Hauptner Ferroscope, Art-Nr 39500; H. Hauptner & Richard Herberholz GmbH & Co. KG, Solingen, Germany), and ultrasonographic (LOGIQ Book XP Vet; GE Medical Systems Co. Ltd., Wuxi, China) examinations. A rumenotomy was conducted in the hospital to confirm this diagnosis. Acute mastitis was diagnosed clinically based on the results of the clinical examination. Microbiological examinations were performed to confirm a California mastitis test (**CMT**) diagnosis. The animals that were not treated with any drugs and in which the course of the clinical disease was not longer than 72 h, were included in RPT or mastitis groups. All animals were fed a ration of concentrate diet, straw, and dried alfalfa. Estimated levels of NE_L and CP of the diet were 5.9 MJ/kg of DM and 146 g/kg of DM, respectively.

Blood and Milk Sample Collection

Blood samples were taken from the jugular vein of each cow into the Vacutainers (Becton Dickinson, Franklin Lakes, NJ) with and without EDTA for hematological and biochemical (including Fe) analysis, respectively. The serum was separated by centrifugation at 750 × *g* for 15 min at 4°C within a period of 2 h maximum after sampling and stored at −20°C until use. Blood samples were used for hematological and fibrinogen analyses within a period of 2 h maximum after sampling. Milk samples were stored in plastic CMT wells for the CMT and in sterilized tubes for the microbiologic culture antibiogram.

CMT

Samples from each of the cows in the mastitis and control groups were submitted to the CMT described by Schalm and Noorlander (1957).

Biochemical Examinations

Serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, ammonia (NH_3), total protein (**TP**), total bilirubin, BUN, creatinine, creatine kinase, glucose (**GLU**), albumin (**ALB**), and Fe levels were determined through biochemical analysis by using specific test slides for a dry system autoanalyzer (Vitros DT 60; Johnson & Johnson Clinical Diagnostics Ltd., Buckinghamshire, UK).

The method for measuring serum Fe is described briefly below. The Vitros Fe DT slide is a multilayered, analytical element coated on a polyester support. A drop of serum sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Iron (as ferric ion) is removed from transferrin at acidic pH and migrates to the reducing layer, where ascorbic acid reduces Fe to the ferrous form. The ferrous ion then is bound to the dye and forms a colored complex. Within-laboratory coefficients of variation for Fe were 3.0 and 2.8%.

Hematologic Examinations

Red blood cell and white blood cell (**WBC**) count, hemoglobin (**Hb**) level, packed cell volume, mean corpuscular volume, mean corpuscular Hb (**MCH**), and MCH concentration measurements were obtained by using manual techniques (Morris, 2009). Blood smears were prepared on slides and stained using the May-Grünwald-Giemsa solution in phosphate buffer (pH 6.8). The blood smears were examined to identify WBC subtypes [neutrophils (band), neutrophils (segmented), lymphocytes, monocytes, eosinophils, and basophils] using a light microscope (Harvey, 2001; Thrall, 2006).

Analysis of APP

Serum haptoglobin (**Hp**) was measured using an Hb-binding biochemical reaction, which can be used on a microtiter (ELISA) plate (Tridelta Development Plc, Greystones, Co. Wicklow, Ireland). Serum amyloid A (**SAA**; Tridelta Development Plc) was measured using a solid-phase sandwich ELISA (ESR 200 ELISA Plate Reader; Medispec Ltd., Germantown, MD). Serum α-1 acid glycoprotein (**AGP**) levels were measured using