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Original Research Article

Estimation of Total Proteins and Minerals Content of Bovine Brucellosis and Anaplasmosis in Some Local Dairy Farms in Kosti, Sudan

Ibrahim Mohammed Adem[1], Nabaa Kamal Alshafei[2], Intisar Hassan Saeed[3], Mona Abdelrahman Mohamed Khaier[4*]

[1]Allzaytona Veterinary Clinic, Khartoum, Sudan
[2]Department of Biochemistry, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
[3]Department of Physiology, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
[4]Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan

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Abstract: Bovine brucellosis, caused by *Brucella abortus*, is a significant bacterial infectious zoonotic disease not only poses a threat to animal health but also presents economic challenges and public health concerns due to its potential transmission to humans. Humans can contract the disease through direct contact with infected animals or consumption of unpasteurized dairy products. Bovine anaplasmosis is a haemolytic disease of cattle caused by the bacterium *Anaplasma marginale* which can cause adult mortality, abortion, weight loss, and a reduction in performance. This paper described the results obtained from the effects of brucellosis and anaplasmosis on serum proteins and the level of the macro elements (Calcium, Phosphorus, Potassium, Sodium, and Magnesium). These minerals play an essential role in animal metabolism. Out of fourteen Bovine serum samples, five samples were found to be positive for Brucella (35.72%) and four samples were found to be positive for Anaplasma (28.57%), the remaining samples were negative. Total protein (Tb) and (Alb) levels were significantly increased (P<0.01) in both brucellosis and anaplasmosis cattle compared with controls. On the other hand Globulin (Glob) was significantly decreased (P<0.01) in brucellosis cattle compared to the controls, also the present study showed that, concentration of Na, Ca, and Mg minerals were significantly increased in case of Brucellosis compared to the controls and significantly low (P<0.05) in potassium level, while animals infected with anaplasmosis showed an increased level of Na and K and decreased level in Mg. In summary, comprehension of laboratory medicine parameters is crucial for monitoring animal health and diagnosing diseases like brucellosis and anaplasmosis. These parameters are essential for implementing effective control measures and ensuring livestock well-being. The objective of this study was to estimate the total proteins and minerals content of Bovine Brucellosis and Anaplasmosis in some local dairy farms in Kosti, Sudan.

Keywords: Anaplasma, Brucella, Bovine, Minerals, Total proteins.

INTRODUCTION

Brucellosis is an important zoonotic disease and has worldwide distribution among humans as well as animals (Pandeya *et al*., 2013). About 500,000 cases of human brucellosis are estimated to occur worldwide every year (Laine *et al*., 2023). It causes heavy economic loss to the animal industry through delayed conception, late-term abortions and retention of placenta and temporary or permanent infertility (Kollannur *et al*., 2007). The study of blood constituents can provide valuable information about the general health of the animal and, therefore, can be used for evaluating the health status of the animal. Brucellosis is a zoonotic disease transmitted by direct or indirect contact with infected animals, soiled pasture and corrals, ingesting infected milk and with semen from male to female animals (Renukaradhya *et al*., 2002).

Anaplasma is a genus of obligate intracellular, gram-negative bacteria that infect blood cells of mammals. These bacteria can cause diseases in vertebrates and the vertebrates may also serve as reservoirs (Rymaszewska and Grenda 2008). Bovine anaplasmosis, caused by Anaplasma marginale, is a rickettsial disease characterized by progressive anemia. Anaplasmosis caused by Anaplasma species which is an obligate intraerythrocytic rickettsial organism belonging

***Corresponding Author:** Mona Abdelrahman Mohamed Khaier
Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan

to the family Anaplasmataceae of the order Rickettsiales (Dumler *et al*., 2001). It is an infectious, non-contagious disease characterized by fever, anemia, jaundice, lethargy and anorexia (Razmi *et al*., 2006). It is the most prevalent tick-borne cattle disease in the world (Whitlock, 2014).

Proteins play a central role in biological processes; some of them are involved in structural support of connective tissues, while others play important roles in biochemical reactions. Proteins also serve as buffers, helping in maintaining the acid-base balance and colloid osmotic structure. Some of them act as carriers of lipids, hormones, vitamins, and minerals in the circulatory system, and are involved in the regulation of cellular activity and immune system. (Anderson and Anderson 2002). Wrotnowski (1998) estimated that up to 10 000 proteins may commonly be found in blood serum. The more heavily represented serum proteins include albumin, immunoglobulins, haptoglobin, transferrin and lipoproteins, and they are found in concentrations that can be measured in g/ml (Burtis and Ashwood 2001).

Minerals are important for milk production of high-yielding cows. Consequently, their role assumes an increasing importance. Homeostasis of calcium, phosphorus and magnesium is primarily affected by the very same homeostatic mechanisms, and as a result, the changes in their concentrations are in most cases mutually linked (Hansard *et al*., 1954). Depending on the amount of minerals found in the organism, they are classified as either major elements (or macro minerals) or trace elements (or micro minerals). In the animal body, major minerals constitute more than 100 mg/kg (Ca, P, Mg, Na, K, Cl, S), while micro minerals or trace elements (Fe, Zn, Cu, Mb, Se, I, Mn, F, Co, Cr, Al, As, Si, V, Ni, Sn) are present in lower amounts (Underwood, 1979)

The objective of this study was to investigate the total proteins and minerals content of animals diagnosed with brucellosis and anaplasmosis to reflect the effect of the diseases on animals' health and performances.

MATERIAL AND METHODS

This study was conducted in a traditional farm in Kosti, Sudan, the farm contains 14 cows, (Kenana breed), with the history of dullness, abortion, ticks' infestation, loss of appetite, low milk production and reduced fertility.

Blood samples

Blood samples were collected from the jugular vein of animals, transported to the College of Veterinary Medicine, University of Bahri Laboratory. For each sample, serum was removed from clotted blood and stored at -20 °C till tested.

Initially sera were examined for bacterial Brucellosis using Rose Bengal Plate test (RBPT). Positive samples were confirmed by serum agglutination test (SAT) as described by (OIE, 2000). The blood smears were fixed with methanol and subjected to Giemsa staining technique, examined under light microscope to check for Anaplasma. Each slide was examined for 5 min and samples with one or more inclusion bodies were classified as positive (Renneker *et al.*, 2013).

To determinate biochemical parameters, Serum calcium was determined by the Photometric Test Condensation Particle Counters (CPC) method according to the procedure described by (Gitelman, 1967), the phosphate concentration (PO4) in serum sample was determined by (Shervedani and Bagherzadeh, 2008) method. Serum sodium and potassium were determined colormetrically according to the method of (Trender, 1951). Serum magnesium was determined by (Howard H Sky-Peck, 1964) method.

Total serum protein was quantified in each serum samples according to the (Lowry *et al*., 1951) method. Serum albumin was determined colorometrically by the Dye binding technique with Bromocresol green (BCG) according to the procedure described by (Young, 1975). Serum globulins concentration was verified by Enzyme linked Immunosorbent Assay (ELISA) according to the method of (Piomelli *et al*., 1998).

Statistical analysis

Statistical analysis was performed using SPSS-version 15 software (2010). An independent sample T-test was used to analyze the effects of brucellosis and anaplasmosis. Parametric data were expressed as means ± S.E.

RESULTS

Bacterial infections precipitate significant alterations in haematological parameters, impacting vital organs such as the liver. Analyzing biochemical markers in cattle holds promise for elucidating the pathophysiology underlying the deleterious effects linked with bacterial infections in bovines. Table (1) and Figure (1), explain the results of blood sample testing for brucellosis and anaplasmosis.

Table 1: Percentage of Brucella and Anaplasma in infected Cattle

Tested samples	Bacteria	Positive	Negative
14	Brucella	5 (35.72%)	9 (64.28 %)
14	Anaplasma	4 (28.57%)	10 (71.42 %)

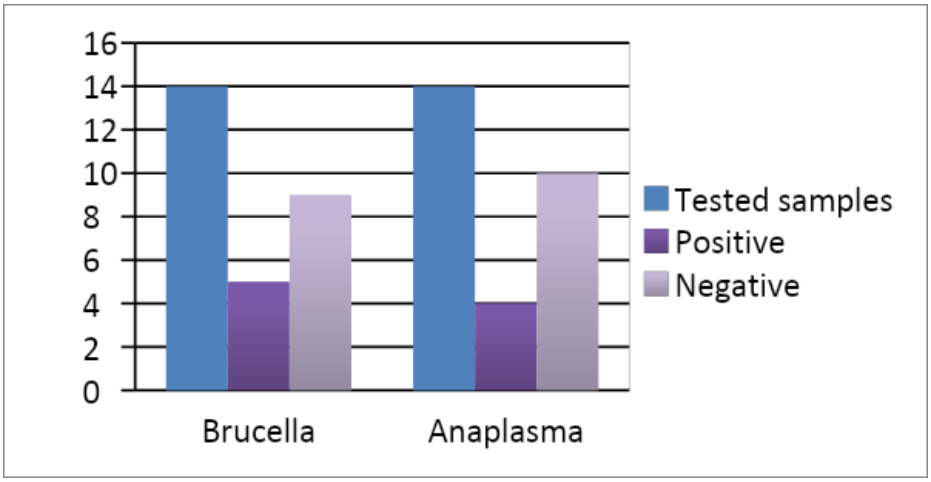

Figure 1: Tested samples of Infected Cattle Brucellosis and Anaplasmosis

For the total protein concentrations of negative, (used as control) and positive samples infected with Brucella and Anaplasma, the results were shown in Table (2) and Figure (2, a and b).

Table 2: Mean values ± S.E. of serum protein concentration in g/L in cattle with anaplasmosis and brucellosis infection

Parameters	Brucellosis		Anaplasmosis	
	Negative (Control)	Positive	Negative (Control)	Positive
TP **g**/l	73.01±2.98b	81.10±11.38a**	74.76±6.09	78.75±2.21
Alb **g**/l	29.97±3.33	39.10±4.03	32.22±3.65	35.77±3.59
Glob **g**/l	42.48±3.04a	41.99±14.57b**	42.53±7.36	42.55±1.60

TP= Total proteins, Alb= Albumin, Glob = Globulin
Data are presented as mean ± S.E, ** = significant (P<0.01)

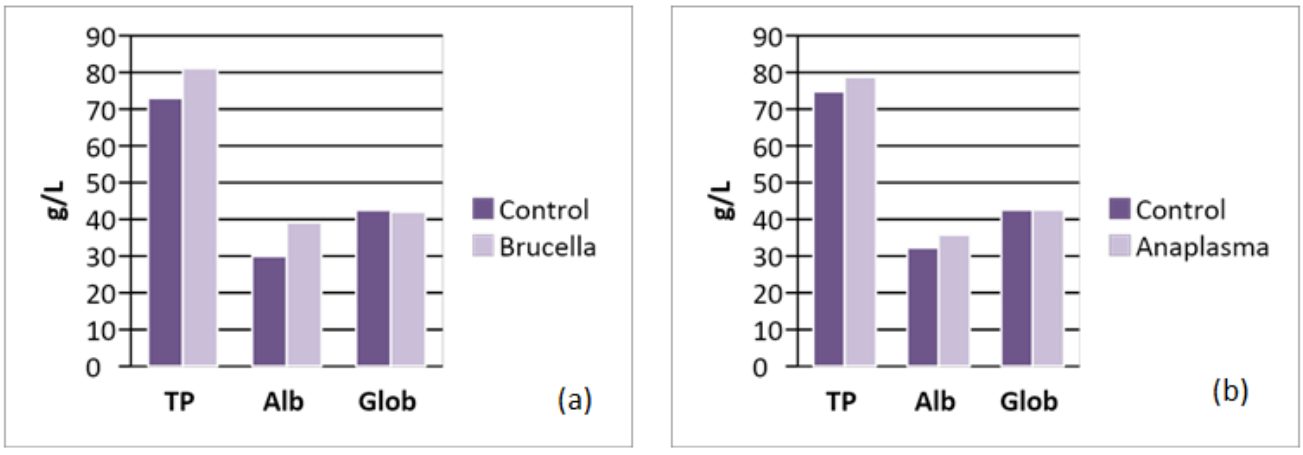

Fig 2: Serum protein concentration in g g/L in cattle with brucellosis and anaplasmosis infections

Concentration of minerals Na, Ca, Mg and PO4 for bovine Brucellosis and Anaplasmosis were shown in Table (3), Figure (3, a and b) and Figure (4, a and b).

Table 3: Mean values ± S.E. of serum minerals concentrations in m.mol/L in cattle with anaplasmosis and brucellosis infection

Parameters	Brucellosis		Anaplasmosis	
	Negative (Control)	Positive	Negative (Control)	Positive
Na m.mol/L	128.14±3.43b	137.33±2.03a*	129.22±3.21	136.92±2.98
K m.mol/L	4.51±0.65a	4.20±0.22b*	3.86±0.18b	5.76±1.26a**
Ca m.mol/L	2.34±0.57	2.60±0.03	2.42±0.06b	2.47±0.05a*
Mg m.mol/L	0.76±0.06b	0.83±0.01a***	0.75±0.04a	0.70±0.20b*
PO_4 m.mol/L	1.22±0.02	1.27±0.01	1.22±0.02	1.29±0.01

* = significant (P<0.05), ** = significant (P<0.01), *** = significant (P<0.001)

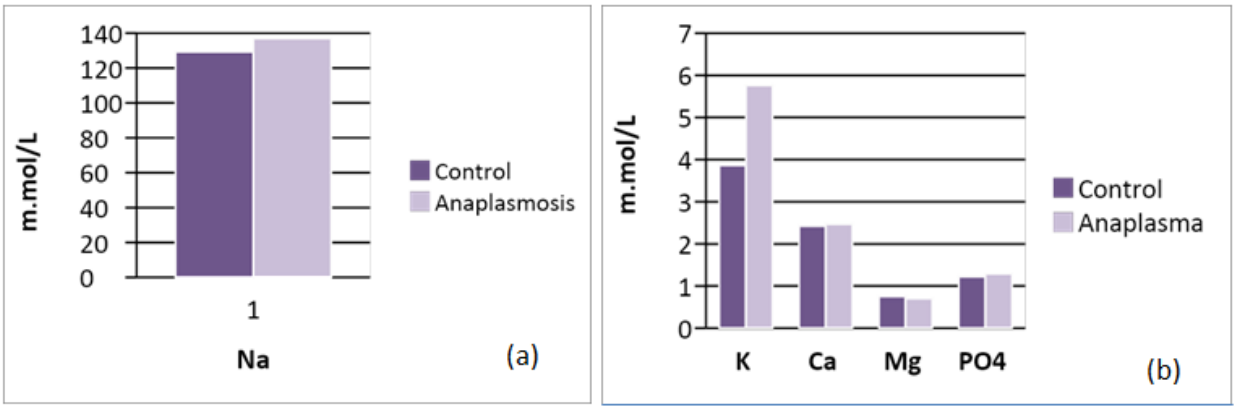

Figure 3: Serum minerals concentrations in m.mol/L in cattle with Anaplasmosis infection

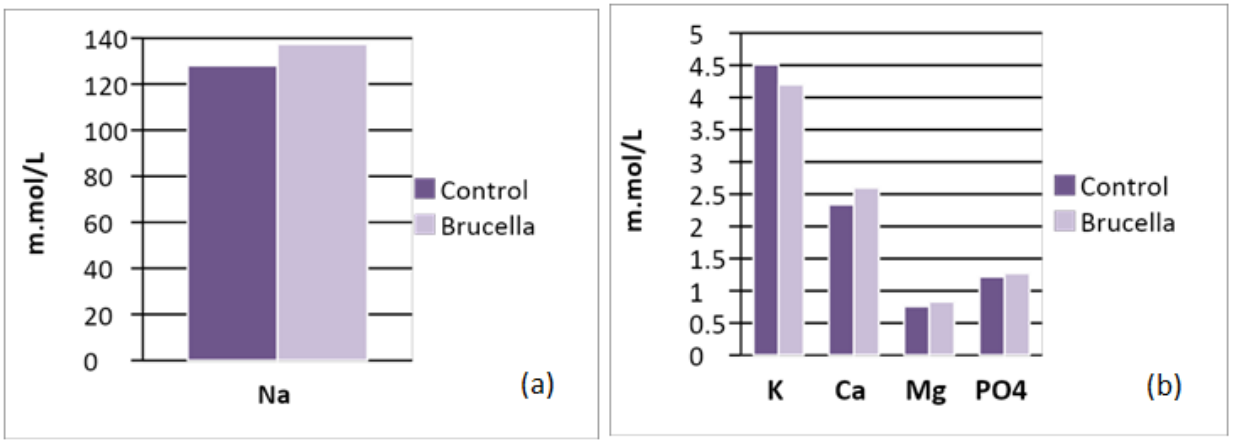

Figure 4: Serum minerals concentrations in m.mol/L in cattle with Brucellosis infection

DISCUSSION

Laboratory medicine parameters are an important tool that helps dairy practitioners to monitor cow health at the individual and herd level. To identify anomalous situations in a given dairy herd, values from blood analysis are generally compared with the population average or ranges of standard values (Herdt, 2000). Serology can help diagnose clinical cases or screen herds in most species; however, it is not considered to be reliable for diagnosis in individuals. There are no established serological tests yet for some of the more recently recognized Brucella (Jacques *et al*., 2010).

Study results in Table 1 and Figure 1 illustrate the microscopic analysis of Giemsa-stained blood smears, where out of 14 smears examined, inclusion bodies indicative of anaplasmosis were observed in four samples, constituting 28.57% of the total, while the remaining ten samples tested negative, representing 71.42%. Velusamy *et al.*, (2014) asserted that seasonal variations do not exert influence on the prevalence of anaplasmosis. It is noteworthy that despite the presence of inclusion bodies in erythrocytes, certain samples tested negative for anaplasmosis through polymerase chain reaction (PCR) analysis. This discrepancy may arise from the potential misinterpretation of inclusion

bodies under light microscopy. Inclusion bodies can bear resemblance to Howell-Jolly bodies, other intra-erythrocytic parasites, and staining artifacts (Torina *et al*., 2012).

The outcomes of this investigation, delineated in Table 2 and Figure 2 (a and b), elucidate the haematological profiles concerning total protein (TP), albumin (Alb), and globulin (Glob) concentrations in cattle afflicted with brucellosis and anaplasmosis relative to the controls. Significantly heightened TP and Alb levels ($P < 0.01$) were evident in brucellosis-infected cattle, whereas globulin levels exhibited a significant reduction ($P < 0.01$) compared to the control group. Notably, the serum albumin concentration for brucellosis was determined to be (39.10±4.03) g/L in this study, agreeing with (Anju and Hari 2016) who mentioned that chronic or sub-acute bacterial infections can cause increases in globulin levels, particularly the gamma globulins resulting from production of antibodies in response to chronic antigenic stimulation. (Hamada *et al*., 2013) and (Abenga and Anosa 2005), they discussed that, increased serum globulin level in Brucella affected cows indicating that the infection increased serum gamma globulins in infected animals that may be due to the host parasite interaction.

In this study, serum albumin values were determined to be 39.10±4.03 g/L and 35.77±3.59 g/L for cattle afflicted with brucellosis and anaplasmosis, respectively. These values are consistent with the findings reported by Alberghina *et al*., 2011, who indicated that albumin (Alb) is typically present in bovine plasma at concentrations ranging approximately from 22 to 39 g/L. Additionally, the observed albumin concentration of 31.60±4.39 g/L aligns closely with the findings reported by Alberghina *et al*., 2011.

A decrease in albumin concentration is a common occurrence in dysproteinemias, with hypoalbuminemia often attributed to reduced hepatic production due to liver diseases such as chronic hepatitis, cirrhosis, or liver failure (Lee, 2012). Furthermore, hypoalbuminemia may manifest in renal diseases and nephrotic syndrome, where glomerular damage leads to increased protein loss in urine (Kodner, 2009). Additionally, low albumin concentrations may indicate chronic malnutrition, inadequate protein intake, or may be associated with gastrointestinal diseases, internal parasitism, and protein-losing enteropathy. Conversely, hyperalbuminemia, characterized by increased serum albumin concentration, may be observed in cases of severe dehydration (Diogenes *et al*., 2010).Our results were also diverging from the findings of Al-Kaysi *et al*., 2010, who reported a reduction in albumin levels among Brucella-infected animals.

Liver damage caused by Brucella results in the heightened release of liver enzymes into the plasma, accompanied by reduced albumin synthesis by reticuloendothelial cells in the liver (Giambartolomei, and Delpino, 2019). Additionally, Brucella infection can extend to the kidneys, exacerbating protein excretion in urine and inducing hypoalbuminemia (Al-Hussary and Al-Zuhairy, 2010). This phenomenon is attributed to hepatic damage induced by brucellosis, leading to decreased synthesis of albumin and urea in the liver, consequently resulting in a significant decrease in albumin levels (Hamada *et al*., 2013).

El Azab (2015) conducted an assessment of protein fractions in Brucella-infected cows, revealing that total protein and gamma globulin concentrations were elevated in serologically positive cows, surpassing levels observed in healthy counterparts. Notably, the escalation in total protein concentrations was primarily attributable to increased gamma globulin concentrations.

Minerals play a vital role in maintaining homeostasis. Minerals are involved in all living processes, either in the capacity of structural elements or as regulators of almost all metabolic processes (Bauman and Currie, 1980). Table (3) illustrates the mean concentrations of various minerals in the blood serum of cows afflicted by both brucellosis and anaplasmosis. Specifically, sodium concentrations were found to be 137.33±2.03 mmol/L for brucellosis and 136.92±2.98 mmol/L for anaplasmosis, while potassium concentrations were 4.20±0.22 mmol/L for brucellosis and 5.76±1.26 mmol/L for anaplasmosis. These values fall within the proximate range of 136 – 134 mmol/L for sodium and 3.7 - 4 mmol/L for potassium, as reported by Cozzi *et al*., (2011). Notably, alongside water, sodium, potassium, and chlorine are vital components of sweat, with sweating serving as a primary thermoregulatory mechanism to dissipate excessive body heat in lactating cows exposed to hot environments (Kadzere *et al*., 2002). This acclimation strategy likely accounts for the lower mean sodium values observed in samples collected during the summer.

In this study, the concentrations of minerals Na, Ca, and Mg were observed to increase significantly in cases of Brucellosis compared to the controls. Conversely, there was a significant decrease ($P<0.05$) in potassium levels. Conversely, animals infected with anaplasmosis exhibited increased levels of Na and K but a decreased level of Mg.

Literature indicates that the reference values for phosphorus in cows typically range from 1.81 to 2.1 mmol/L (Kaneko, 2008; Radostits *et al*., 2000; Whitaker, 2000) and 1.4 to 2.5 mmol/L (Merck, 2003), aligning closely with our findings of 1.29±0.01 mmol/L for anaplasmosis and 1.27±0.01 mmol/L for brucellosis. The observed increase in PO4 levels may be attributed to dietary adjustments aimed at enhancing energy intake and the provision of sodium through Na-phosphates. This increase, correlated with heightened growth hormone activity, promotes intestinal phosphate

absorption and renal phosphate re-absorption (Meyer and Harvey, 2004). However, an opposing trend was observed by Calamari *et al*., 2007, suggesting that phosphorus levels are not directly influenced by mineral losses induced by hot environments (NRC, 2001).

In terms of calcium, the reference range for cows typically spans from 2.2 to 3.0 mmol/L (Jovanovic *et al*., 1997), 2.1 to 2.8 mmol/L (Merck, 2003), and 2.43 to 3.1 mmol/L (Kaneko, 2008; Radostits *et al*., 2000), consistent with our study's findings of 2.60±0.03 mmol/L for brucellosis and 2.47±0.05 mmol/L for anaplasmosis. Certain fluctuations in calcium concentration have been noted depending on the reproductive cycle of cows, with the highest values recorded during the dry period in summer samples, while lactating and postpartum cows exhibit significantly lower concentrations during the same periods (Kaneko, 2008).

Magnesium concentrations vary widely in the literature, ranging from 0.8 to 1.3 mmol/L (Whitaker, 2000), 0.7 to 1.2 mmol/L (Merck, 2003), and 0.74 to 0.95 mmol/L (Kaneko, 2008; Radostits *et al*., 2000). However, our recent study aligns with these references, showing magnesium concentrations within the normal range. Serum magnesium levels are consistent across cows (Blosser *et al*., 1951), although maternal plasma magnesium concentrations increase at parturition (Wilson *et al*., 1977).

CONCLUSION

Anaplasmosis and brucellosis pose significant threats to cattle herds, resulting in substantial financial losses amounting to millions of Sudanese pounds annually. Despite extensive research efforts, there remains much ambiguity surrounding these diseases, particularly regarding the role of wildlife reservoirs. However, advancements in modern research methodologies offer promising avenues for addressing these uncertainties. In the foreseeable future, conducting controlled research focusing on factors such as nutrition and mechanical transmission can provide valuable insights into the epidemiology of these diseases. This, in turn, will facilitate the development of more effective strategies for diagnosing, treating, preventing, and controlling them.

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