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Evaluation of serum enzyme activities and protein fractions in *Brucella*-infected cows

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Abstract: The aim of this study was to evaluate potential changes in serum enzyme activities and protein fractions in *Brucella*-infected cows. A total of 1100 serum samples of dairy cows were screened for *Brucella* infection using the Rose Bengal test (RBT). Serum samples reacting positively to the RBT were subjected to indirect enzyme-linked immunosorbent assay for confirmation. Thirty serum samples from serologically positive cows and 30 from negative (healthy) cows were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) activities and total protein (TP) concentrations and serum protein electrophoresis. Serum ALT, AST, CK, and LDH activities were increased in serologically positive cows and significantly higher than those measured in healthy cows. However, there were no significant differences in ALP and GGT activities. TP and γ-globulin concentrations were increased in serologically positive cows and significantly higher than those of healthy cows, but there were no significant differences in albumin or α1-, α2-, and β-globulin concentrations. Hence, *Brucella* infection has a detrimental effect on dairy cows' health and is accompanied with elevated serum ALT, AST, CK, and LDH activities and increased TP concentrations due to the increase in γ-globulin concentrations only.

Key words: Cows, *Brucella* infection, serum enzymes activities, protein fractions

1. Introduction

Brucellosis is a bacterial zoonotic disease of worldwide importance and of major public health and economic significance, caused by gram-negative facultative intracellular organisms of the genus *Brucella* (1,2). The recognized *Brucella* species are named according to their host preferences, such as *B. abortus* (which infects cattle), *B. melitensis* (which infects sheep and goats), *B. suis* (which infects pigs), and *B. ovis* (which infects sheep) (3,4). Brucellosis is an endemic disease in many developing countries, especially in the Mediterranean, the Middle East, and the Indian subcontinent, and it causes considerable economic loss in livestock production (5–7). Bovine brucellosis is usually caused by *B. abortus* and occasionally by *Brucella melitensis* where cattle are kept together with infected sheep or goats (8). In Africa, brucellosis is more widespread in cattle (9). Brucellosis in cattle is characterized primarily by abortion late in pregnancy, frequently followed by fetal membrane retention, endometritis, metritis, stillbirth, and calf loss in animals and huge economic losses to dairy farmers (10–13).

Abnormal biochemical markers of uteroplacental pathology in dairy cows are scarce in the literature. Elevated levels of serum enzyme activities are reliable indicators of pathological changes in different tissues due to the release of intracellular enzymes from the injured tissue. Similarly, shifts in albumin and/or globulin concentrations, and their evaluation by serum protein electrophoresis, are considered valuable diagnostic tools in domestic animals. Hence, evaluations of serum enzymes activities and protein fractions in combination with other clinical and laboratory data are helpful in understanding the disease process and in making a diagnosis. The purpose of this study was to evaluate potential changes in some serum enzymes activities and protein fractions in cows' sera serologically positive for brucellosis, which may help in understanding the pathophysiology of the adverse effects associated with *Brucella* infection in dairy cows.

2. Materials and methods

2.1. Animals and sample collection

A total of 1100 mature female dairy cows (Holstein-Friesian breed, none vaccinated against brucellosis, 3–5 years age) from private farms (Elbehira Governorate, Egypt) were used. All animal-related procedures were carried out in accordance with the Animal Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University. Blood samples were collected from the jugular vein.

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Approximately 5 mL of blood was taken from each cow into glass tubes without anticoagulant. After clotting the blood samples, serum was harvested by centrifugation at 3500 rpm for 15 min and stored at –80 °C until analysis.

2.2. Serological tests

The Rose Bengal test (RBT) was conducted as described previously (14) and was used to screen sera for anti-*Brucella* antibodies. Only serum samples reacting positively to the RBT were tested using a commercial indirect enzyme-linked immunosorbent assay (ELISA) kit for confirmation. The commercial indirect ELISA kit was BRUCELISA (Veterinary Laboratories Agency, UK). Procedures of indirect ELISA were performed and the results were interpreted according to the instructions of the manufacturer.

2.3. Serum biochemical analyses

Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were determined by colorimetric method using commercially available kits (BioMérieux, France and Spinreact, Spain). Serum concentrations of total protein were determined by the biuret reaction using an automatic analyzer (Cobas 8000, Roche, Germany).

2.4. Serum protein electrophoresis

Serum protein fractions were electrophoresed by using a semiautomated agarose gel electrophoresis system (Helena Laboratories, Helena Biosciences, UK) according to the procedure described by the manufacturer. Serum protein fractions were electrophoresed for 28 min at 450 V. Fixation was done by using an automated system, followed by staining the gel in Coomassie Brilliant Blue for 10 min and then drying at 37 °C. Scanning was performed on a densitometer (Helena Biosciences) after gel destaining and drying. Electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed using computer software (Phoresis, Helena Biosciences). Percentages for each fraction were determined by this software, and absolute values (g/dL) for each fraction were obtained by multiplying the percentage by the total protein concentration. Albumin/globulin (A/G) ratios were calculated by dividing albumin concentrations by the sum of globulin concentrations individually.

2.5. Statistical analysis

Data of each study group were expressed as means ± SD. Data analyses were done using SPSS 17.01 for Windows (SPSS Inc., USA). Repeated measurement of ANOVA was also carried out to determine the difference among the serum parameters. $P < 0.05$ was considered as statistically significant.

3. Results

The data referring to serum ALT, AST, CK, LDH, ALP, and GGT activities are presented in Table 1. Mean values of serum ALT, AST, CK, and LDH activities were increased and were significantly higher in cows serologically positive for brucellosis than those of healthy cows (Table 1; $P < 0.05$). However, there were no significant differences between mean values of ALP and GGT activities in cows serologically positive for brucellosis and those of healthy cows (Table 1; $P > 0.05$).

Table 1. Serum enzyme activities in dairy cows infected with *Brucella*.

Parameters	Groups of cows	
	Brucellosis-positive (n = 30)	Brucellosis-negative (healthy) (n = 30)
ALT (U/L)	25.45 ± 4.31*	19.40 ± 0.30
AST (U/L)	64.70 ± 1.84*	45.05 ± 3.04
CK (U/L)	137.50 ± 23.33*	62.50 ± 2.12
LDH (U/L)	1631.00 ± 2.83*	1595.00 ± 22.63
ALP (U/L)	121.75 ± 43.63	135.60 ± 1.56
GGT (U/L)	22.33 ± 2.10	25.33 ± 1.15

All data are expressed as mean ± standard deviation.
*Values obtained from cows serologically positive for brucellosis are significantly different from those of healthy cows, $P < 0.05$.

Mean values of serum total protein concentrations in cows serologically positive for brucellosis were increased and significantly higher than those of healthy cows (Table 2; $P < 0.05$). Serum proteins were separated by using agarose gel electrophoresis and 5 fractions (albumin, α1, α2, β, and γ) were identified in all serum samples. Absolute values (g/dL) for each fraction were determined by multiplying the percentages of each fraction by the total protein concentrations. Absolute concentrations of γ-globulins were increased in cows serologically positive for brucellosis and significantly higher than those of healthy cows (Table 2; $P < 0.05$). However, there were no significant differences between mean values of absolute concentrations of albumin and α1-, α2-, and β-globulins in cows serologically positive for brucellosis and those of healthy cows (Table 2; $P > 0.05$). Moreover, cows serologically positive for brucellosis had lower A/G ratios when compared to healthy cows (Table 2; $P < 0.05$).

4. Discussion

Brucellosis, a contagious disease of domestic animals, is considered one of the most serious public health problems, especially in developing countries (15). Brucellosis is a chronic bacterial disease of domestic animals and provokes placentitis, abortion, retention of the placenta, and metritis, with devastating economic effects on livestock production (16,17). Serum biochemical references of uteroplacental pathology in dairy cows are scanty. Measurement and evaluation of such parameters may be helpful in elucidating the pathophysiology of the adverse effects associated with brucellosis in dairy cows. The current study was designed to investigate changes that may occur in serum enzyme activities and different protein fractions in dairy cows infected with *Brucella*.

Serum enzyme activities are markers or indicators of pathologic processes and not specific diseases. The results of the present study showed that serum ALT and AST activities in dairy cows positive for brucellosis were increased and significantly higher than those of healthy cows. In the present study, the increased activity of AST and ALT in dairy cows positive for brucellosis was observed, as indicated by Nath et al. (18) in cows and by El-Boshy et al. (19) in camels infected with *Brucella*. The serum activity of the aminotransferases, AST and ALT, are measured to detect hepatocellular injury. ALT is an important liver-specific enzyme in small animals but offers no specificity for detection of liver injury in large animals (20). Liver ALT activity is very low in cattle. Increases in serum ALT activity in these species are likely the result of muscle injury (21). AST is a common marker of hepatocyte damage, but muscle damage also increases AST activities in cattle (22). In cattle, AST activity should be interpreted in conjunction with tissue-specific enzymes such as CK (muscle-specific enzyme) and GGT (liver-specific enzyme) to determine the source of the tissue damage (23).

CK is a leakage enzyme and is considered a muscle-specific enzyme in domestic animals (22). The results of the present study revealed that serum CK activities in dairy cows positive for brucellosis were increased and significantly higher than those of healthy cows. Injury to organs and tissues containing smooth muscle may cause increased serum CK activities, but to a lesser extent than

Table 2. Serum total protein concentration, protein fractions, and albumin/globulin (A/G) ratio in dairy cows infected with *Brucella*.

Parameters	Groups of cows	
	Brucellosis-positive (n = 30)	Brucellosis-negative (n = 30)
Total protein (g/dL)	8.61 ± 0.50*	7.71 ± 0.30
Albumin (g/dL)	3.23 ± 0.18	3.26 ± 0.10
α1-globulin (g/dL)	0.28 ± 0.01	0.28 ± 0.01
α2-globulin (g/dL)	0.94 ± 0.06	0.92 ± 0.04
β-globulin (g/dL)	0.95 ± 0.10	1.06 ± 0.01
γ-globulin (g/dL)	3.22 ± 0.34*	2.18 ± 0.27
A/G ratio	0.60 ± 0.03*	0.73 ± 0.01

All data are expressed as mean ± standard deviation.
*Values obtained from cows serologically positive for brucellosis are significantly different from those of healthy cows, $P < 0.05$.

with striated muscle damage. It was reported that uterine tissue contains high CK and AST activities, with CK being 20-fold higher than AST, and cows with endometritis have elevated CK and AST activities in serum (24). CK and AST activities in serum of buffalo cows with endometritis could be a valuable aid for the determination of uterine tissue atrophy and degeneration (25).

LDH, similar to AST, is found in many types of cells and should not be considered liver-specific. Serum LDH activities were also increased in dairy cows positive for brucellosis and were significantly higher than those of healthy cows. Increased LDH activity may result from hemolysis, muscle damage, or hepatocellular injury (21). Lactate dehydrogenase activity provides additional information that may help explain activities of more tissue-specific enzymes (i.e. CK). Dutta and Dugwekar (26) reported that an increase in LDH activities could be a useful indicator of the presence of uterine and placental pathology.

The current investigation also showed no statistically significant differences between mean values of ALP and GGT activities in cows positive for brucellosis and healthy cows. ALP is a membrane-bound glycoprotein mainly found in various animal tissues and used as a biochemical marker to diagnose osteoporosis and hepatobiliary disorders as well as fatty liver disease (27–29). Similarly, GGT, a membrane-bound enzyme, is found primarily in cells with high rates of secretion or absorption, and significant GGT activity is present in the liver, kidneys, pancreas, and intestine. GGT activity is relatively high in the livers of cows. Remarkable elevations in serum GGT activity are observed primarily in diseases of the hepatobiliary system associated with cholestasis in cows (23). Increased GGT activity is generally considered to have better diagnostic sensitivity than ALP for detecting cholestasis or other biliary disorders in cattle (30).

Regarding the result of protein analyses, mean values of total protein and **γ**-globulin concentrations in cows serologically positive for brucellosis were increased and significantly higher than those of healthy cows. However, there were no significant differences between mean values of albumin and α1-, α2-, and β-globulin concentrations in cows positive for brucellosis and healthy cows. Furthermore, cows serologically positive for brucellosis had lower A/G ratios when compared with healthy cows. The result of the present study is partly in agreement with previous reports that documented an increase of serum globulin level in *Brucella*-affected cows (18), ewes (31), and camels (19), whereas Nath et al. (18) reported a significant decrease in albumin concentrations and nonsignificant changes in total protein and A/G ratio between the brucellosis-affected and healthy cows. However, Hamada et al. (31) found a nonsignificant difference in albumin and total protein between brucellosis-affected and healthy ewes. El-Boshy et al. (19) reported a significant decrease in serum albumin between brucellosis-affected and healthy camels.

Serum protein measurement constitutes a vital component of laboratory diagnostic evaluations in animals. Increased total protein concentration can result from increased concentrations of albumin, globulin, or both. In the present study, the higher globulin concentrations led to higher total serum proteins and lower A/G ratio. Elevation of γ-globulin concentration is common during chronic inflammatory diseases. Chronic or subacute bacterial infections can cause increases in globulin fractions, particularly the γ-globulins resulting from production of different immunoglobulins by plasma cells in response to chronic antigenic stimulation (32).

In conclusion, the present study provides helpful clinicopathological findings in dairy cows serologically positive for brucellosis. *Brucella* infection in dairy cows is associated with harmful effects on animal health leading to a disturbance in some vital organs functions. Consequently, serum ALT, AST, CK, and LDH activities tend to increase in the sera. The increased serum activities of these enzymes could be related to a retained placenta or pathological changes of the uterine wall (chronic endometritis) commonly associated with *Brucella* infection in dairy cows. Furthermore, serologically positive cows had higher total protein and globulin concentrations as well as lower A/G ratios. The increased globulin concentrations (particularly γ-globulins) are most likely due to chronic antigenic stimulation caused by the organism.

References

1. Kakoma I, Oluoch AO, Baek BK, Rahman MS, Matsuda K. More attention warranted on *Brucella abortus* in animals. J Am Vet Med Assoc 2003; 222: 284.
2. Heller MC, Watson JL, Blanchard MT, Jackson KA, Stott JL, Tsolis RM. Characterization of *Brucella abortus* infection of bovine monocyte-derived dendritic cells. Vet Immunol Immunopathol 2012; 149: 255–261.
3. Meyer ME. Evolutionary development and taxonomy of the genus *Brucella*. In: Adams LG, editor. Advances in Brucellosis Research. College Station, TX, USA: Texas A&M University Press; 1990. pp. 12–35.
4. Corbel MJ. Brucellosis: an overview. Emerg Infect Dis 1997; 3: 213–221.

5. Refai M. Incidence and control of brucellosis in the Near East region. Vet Microbiol 2002; 90: 81–110.
6. Corbel MJ. Brucellosis in Humans and Animals. Geneva, Switzerland: World Health Organization; 2006.
7. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis 2006; 6: 91–99.
8. OIE. Manual of Standards for Diagnostic Tests and Vaccines. Bovine Brucellosis. Paris, France: Office International des Epizooties; 2008.
9. McDermott JJ, Arimi SM. Brucellosis in Sub-Saharan Africa: epidemiology, control and impact. Vet Microbiol 2002; 90: 111–134.
10. Singh G, Sharma DR, Sandhu KS, Dhand NK. Economic losses occurring due to bovine abortions in Punjab. In: 10th International Congress of Asian-Australasian Indian Association of Animal Production and World Buffalo Trust, New Delhi, India, 2002. pp. 23–27.
11. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. Lancet Infect Dis 2007; 7: 775–786.
12. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary Medicine, A Text Book of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th ed. London, UK: W. B. Saunders; 2007.
13. Gonzalez M, Andrews E, Folch H, Saez D, Cabrera A, Salgado P, Onate A. Cloning, expression and immunogenicity of the translation initiation factor 3 homologue of *Brucella abortus*. Immunobiology 2009; 214: 113–120.
14. Alton GG, Jones LM, Angus RD, Verger JM. Technique for the Brucellosis Laboratory. Versailles, France: IRNA Publication; 1988.
15. Samaha H, Mohamed TR, Khoudair RM, Ashour HM. Serodiagnosis of brucellosis in cattle and humans in Egypt. Immunobiology 2009; 214: 223–226.
16. Carvalho Neta AV, Mol JP, Xavier MN, Paixão TA, Lage AP, Santos RL. Pathogenesis of bovine brucellosis. Vet J 2010; 184: 146–155.
17. Poester FP, Samartino LE, Santos RL. Pathogenesis and pathobiology of brucellosis in livestock. Rev Sci Tech Off Int Epiz 2013; 32: 105–115.
18. Nath R, Das S, Sarma S, Devi M. Comparison of blood profiles between healthy and *Brucella* affected cattle. Veterinary World 2014; 7: 668–670.
19. El-Boshy M, Abbas M, El-Khoderyl H, Osman S. Cytokine response and clinicopathological findings in *Brucella* infected camels (*Camelus dromedarius*). Vet Med 2009; 54: 25–32.
20. Hoffman WE, Solter PF. Diagnostic enzymology of domestic animals. In: Kaneko JJ Harvey JW, Bruss ML, editors. Clinical Biochemistry of Domestic Animals. 6th ed. San Diego, CA, USA: Academic Press; 2008. pp. 351–378.
21. Bain PJ. Liver. In: Latimer KS, Mahaffey EA, Prasse KW, editors. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology. 4th ed. Ames, IA, USA: Iowa State Press; 2003. pp. 193–214.
22. Stockham SL, Scott MA. Fundamentals of Veterinary Clinical Pathology. 2nd ed. Ames, IA, USA: Blackwell Publishing; 2008. pp. 639–674.
23. Allison RW. Laboratory evaluation of the liver. In: Thrall MA, Weiser G, Allison RW, Campbell TW, editors. Veterinary Hematology and Clinical Chemistry. 2nd ed. Ames, IA, USA: Wiley-Blackwell Inc.; 2012. pp. 401–424.
24. Sattler M, Furll H. Creatine kinase and aspartate aminotransferase in cows as indicators for endometritis. J Vet Med A 2004; 51: 132–137.
25. Azawi OI, Omran SN, Hadad JJ. A study of endometritis causing repeat breeding of cycling Iraqi buffalo cows. Reprod Domest Anim 2008; 43: 735–743.
26. Dutta JC, Dugwekar YG. Serum alkaline phosphatase activity and lactic dehydrogenase activity in cows with retained fetal membranes. Theriogenology 1982; 18: 423–429.
27. Ali AT, Penny CB, Paiker JE, van Niekerk C, Smit A, Ferris WF, Crowther NJ. Alkaline phosphatase is involved in the control of adipogenesis in the murine preadipocyte cell line, 3T3-L1. Clin Chim Acta 2005; 354: 101–109.
28. Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB Jr, Haffner SM. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. Diabetes 2005; 54: 3140–3147.
29. Webber M, Krishnan A, Thomas NG, Cheung BMY. Association between serum alkaline phosphatase and c-reactive protein in the United States National Health and Nutrition Examination Survey 2005-2006. Clin Chem Lab Med 2010; 48: 167–173.
30. Fernandez NJ, Kidney BA. Alkaline phosphatase: beyond the liver. Vet Clin Pathol 2007; 36: 223–233.
31. Hamada DM, Mohamed AH, Mabrouk A, Emad M, Ah ME. Seroprevalence of abortion causing agents in Egyptian sheep and goat breeds and their effects on the animal's performance. J Agric Sci 2013; 5: 92–101.
32. Morag GK. Veterinary Laboratory Medicine, Clinical Biochemistry and Haematology. 2nd ed. Oxford, UK: Blackwell Science Ltd.; 2002.