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EVALUATION OF CARDIAC ENZYMES AND SERUM LIPID PROFILE IN GOATS INFECTED WITH*CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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ABSTRACT

The aim of the present study was to investigate the prognostic and diagnostic value of cardiac enzymes (creatine kinase-myocardial band (CK-MB), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)) along with a limited lipid profile, including triglycerides (TG), low density lipoproteins (LDL), cholesterol (TCHOL) and high density lipoproteins (HDL), for infection control in goats naturally infected with *Corynebacterium pseudotuberculosis (C. pseudotuberculosis)*. A total of 19 Turkish Hair goats, consisting of 9 healthy goats and 10 goats infected with *C. pseudotuberculosis*, were selected for the study; all goats were between the ages of 1.5-2 years and resided in a special dairy farm. The housing, nutrition and management conditions were the same for all goats. The selection of animals was based on microbiological analyses. A statistically significant difference was observed in the levels of creatine kinase (CK-MB), alanine aminotransferase, alkaline phosphatase, and low-density lipoprotein ($p<0.05$) in goats infected with *C. pseudotuberculosis* compared to the control group. In conclusion, this study has demonstrated that goats naturally infected with *C. pseudotuberculosis* have higher levels of cardiac enzymes compared with healthy goats. The prognostic significance of cardiac enzyme levels in goats infected with *C. pseudotuberculosis* deserves further study.

Key words: Aspartate aminotransferase, Cholesterol, Creatine kinase-myocardial band, Goat, *C. pseudotuberculosis*.

INTRODUCTION

Corynebacterium pseudotuberculosis (C. pseudotuberculosis) is the etiological agent of caseous lymphadenitis (CLA), which causes significant economic losses, including a decrease in hair, meat and milk production along with the culling of affected animals, carcasses and skin in slaughterhouses (Paton *et al*., 1988; Dorella *et al*., 2006). CLA in sheep and goats is usually associated with the development of large external abscesses in subcutaneous tissues, sub parotid and visceral organs such as the liver, spleen and kidneys (Abdullah *et al*., 2013; Alonso *et al*., 1993). CLA has an extraordinarily long incubation period ranging from 3 to 20 weeks. However, shorter incubation periods have been reported (Ashfaq and Campbell, 1979; Johnson *et al*., 1993) in which only a few animals develop distinct clinical signs; these signs include fever and other changes in vital signs such as heart and respiratory rate, inappetence and decreased food consumption, and a change in general health status.

Blood biochemical parameters, serum electrolytes and lipid profiles are very important in diagnosing the disease, monitoring the course of the disease, and following up on the treatment response. These parameters are essential to the diagnosis, treatment and follow-up prognosis. Data regarding the pathogenesis and prognosis of the disease can be obtained from these parameters (Dede *et al*., 2014).

Levels of circulating enzymes can be useful as an aid in the diagnosis of certain diseases (Eldirdiri *et al*., 1987). Biochemical analysis of serum can often provide valuable information regarding cardiovascular health and illness in animals (Coodley, 1970). When the myocardium is damaged, the level of enzymes, such as creatine kinase-myocardial band isoenzymes (CK-MB) (Kaneko, 2008), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Coodley, 1970), in the serum is elevated. Moreover, cardiac troponin I (cTnI) is a more specific marker than CK-MB for the diagnosis of myocardial

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necrosis (Basbugan *et al*., 2010); however, the myocardium is one of the richest sources of CK-MB. Therefore, CK-MB is the serum enzyme determinant most widely used for the diagnosis of cardiac diseases in large animals. AST and ALT are not organ-specific and may be of additional value for diagnosing cardiac issues (Coodley, 1970). Therefore, these diagnostic enzymes are valuable tools used in the early detection of cardiac abnormalities resulting from ischemia, injury, or inflammation (Sacher *et al*., 1991).

Lipoproteins are very large noncellular conglomerates (micelles) of lipids and proteins that are suspended in plasma or lymph. Their main function is to transport most lipids between tissues. Another function of lipoproteins is the esterification of cholesterol. Lipoproteins are classified by their density as determined by ultracentrifugation; the major classes are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

Although studies have evaluated the interrelationship among CK-MB, ALT and AST in animals (Kozat *et al*., 2011) and human beings (Polena *et al*., 2005), no reports have addressed such a probable relationship in goats infected with *C*. *pseudotuberculosis*. Therefore, the current study aimed to investigate the prognostic and diagnostic value of cardiac enzymes (CK-MB, ALT and AST) and a limited lipid profile, including cholesterol (TCHOL), triglycerides (TG), LDL and HDL, for the control of *C*. *pseudotuberculosis* infection in goats.

MATERIALS AND METHODS

Animals:
The study was conducted on a single farm. Ten animals with fluctuating, viscous, orange-sized lumps palpable in the right submandibular and/or prescapular lymph nodes were identified, and a creamy, slightly green, malodorous substance was removed by aspiration of the lump. After microscopic examination of colony morphology, biochemical tests were performed according to the characteristics of the suspected bacteria. The isolate was identified as *C*. *pseudotuberculosis*. These 10 animals comprised the group of *C*. *pseudotuberculosis*-infected animals (n=10) in this study. The control group was composed of the other 9 goats (n=9), who demonstrated no loss of appetite, performance, or condition; and were evaluated as healthy by their normal clinical examination results. The examiners were in contact with the animals for approximately 4 hours a day on the goat farm. Ultimately, 19 Turkish Hair goats between the ages of 1.5-2 years were selected for the study. The housing, nutrition and management conditions were the same for all goats.

Samples collection and analysis:
Blood samples from the jugular vein of the animals were collected for serum samples into tubes without anticoagulant. After incubation for 2 hours at room temperature to allow clotting, and centrifugation (at 1500 rpm, for 10 minutes, in room temperature), sera were carefully harvested and stored at -20°C until analyzed. The levels of gamma-glutamyl transferase (GGT), creatinine (CRE), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose (GLU), triglycerides (TG), low-density lipoproteins (LDL), cholesterol (CHOL), high-density lipoproteins (HDL), total proteins (TP), and creatine kinase (CK-MB and CK-NAC) were measured by using an automatic analyzer (ADVIA 1800 Clinical Chemistry System).

Microbiological analysis:
The samples were transferred on dry ice in antibiotic-free transport medium (Stuart's medium, Copan, Italy) and submitted to the Microbiology laboratory within 18 hours of collection. Analysis of all samples was initiated immediately after transfer to the laboratory. Culture samples were inoculated onto 5% sheep blood agar (BBL; 297876), MacConkey agar (BBL; 211387) and Eosin Methylene-blue (EMB) Lactose Saccharose agar (BBL; 221355) and incubated at 37°C for 24-72 hours. Isolated colonies were evaluated according to colony morphology and Gram stain characteristics. For identification of the suspected bacterial colonies, the cultures were analyzed using commercial miniaturized identification systems (BBL Crystal Panel^{TM}).

Statistical analysis:
Descriptive statistics were calculated for each variable. Prior to hypothesis testing, data were analyzed with the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance to ensure that the parametric test assumptions were satisfied. Student's t-test was used to evaluate the difference in CRE, ALT, AST, GLU, TG, LDL, HDL, TP, CK-MB and CK-NAC levels between the healthy controls and the infected group, while the nonparametric Mann-Whitney U test was used for GGT, ALP and CHOL levels. The level of significance was determined as $p<0.05$. All statistical analyses were calculated using SPSS version 14.01 statistical software.

RESULTS

The results of the cardiac enzyme analysis are shown in Table 1. CK-MB and ALT levels were higher in the goats with *C*. *pseudotuberculosis* compared to the control group ($p<0.05$). The serum AST level was higher in the animals of the *C*. *pseudotuberculosis* group than in those of the control group, although there was no statistically significant difference between the groups.

The results of the serum biochemical analysis are shown in Table 2. The ALP level was higher in the goats infected with *C. pseudotuberculosis* compared to the control group ($p<0.05$). On the other hand, the levels of GGT, CRE and GLU did not differ between the two groups. The serum CK-NAC level was similar, but the concentration of CK-NAC was higher in the animals of the *C. pseudotuberculosis* group than in those of the control group, although there was no statistically significant difference between the groups.

The results of the serum lipid profile analysis are shown in Table 3. The LDL level was higher in the goats infected with *C. pseudotuberculosis* compared to the control group ($p<0.05$). The HDL and CHOL levels were similar, but the concentrations of HDL and CHOL were higher in the animals of the *C. pseudotuberculosis* group than in those of the control group, although there was no statistically significant difference between the groups.

Table 1: Cardiac enzyme levels in healthy goats (n = 9) and in goats infected with *C. pseudotuberculosis* (n = 10). The results are expressed as the mean ± standard error (Mean ± SE) and Median (Min-Max).

Variable	Group	n	Mean ± SE	Median (Min - Max.)	p
ALT (µkat/L)	Control	9	0.23 ± 0.01	0.21 (0.18 – 0.33)	<0.001✣
	C. pseudotuberculosis	10	0.34 ± 0.01	0.34 (0.3 – 0.38)	
AST (µkat/L)	Control	9	1.08 ± 0.05	1.08 (0.85 – 1.3)	0.214 *
	C. pseudotuberculosis	10	1.17 ± 0.04	1.21 (0.98 – 1.36)	
CK-MB (µkat/L)	Control	9	2.78 ± 0.43	3.03 (0.92 – 4.57)	<0.001 *
	C. pseudotuberculosis	10	5.43 ± 0.36	5.99 (3.4 – 6.66)	

✣*According to the Mann-Whitney U test*

**According to Student's t-test*

(alanine aminotransferase, ALT; aspartate aminotransferase, AST; creatine kinase-myocardial band, CK-MB)

Table 2: Serum biochemical profiles in healthy goats (n = 9) and in goats infected with *C. pseudotuberculosis* (n = 10). The results are expressed as the mean ± standard error (Mean ± SE) and Median (Min-Max).

Variable	Group	n	Mean ± SE	Median (Min - Max.)	p
GGT (µkat/L)	Control	9	0.79 ± 0.14	0.7 (0.08 – 1.43)	0.986*
	C. pseudotuberculosis	10	0.79 ± 0.04	0.77 (0.56 - 1)	
CRE (mg/dL)	Control	9	0.82 ± 0.03	0.85 (0.68 – 0.93)	0.037*
	C. pseudotuberculosis	10	0.72 ± 0.03	0.71 (0.6 – 0.95)	
ALP (µkat/L)	Control	9	1.69 ± 0.31	1.46 (0.83 – 3.71)	0.004 *
	C. pseudotuberculosis	10	2.91 ± 0.09	2.85 (2.4 – 3.5)	
GLU (mg/dL)	Control	9	56.56 ± 2.29	58 (46 - 64)	0.898*
	C. pseudotuberculosis	10	56.1 ± 2.61	55.5 (46 - 70)	
CK-NAC (µkat/L)	Control	9	2.22 ± 0.39	2 (0.96 – 4.81)	0.271 *
	C. pseudotuberculosis	10	2.68 ± 0.16	2.61 (2.01 – 3.48)	
TP (g/dL)	Control	9	7.11 ± 0.07	7.1 (6.8 – 7.5)	0.02*
	C. pseudotuberculosis	10	7.38 ± 0.08	7.45 (7 – 7.7)	

✣*According to the Mann-Whitney U test*

**According to Student's t-test*

(gamma-glutamyl transferase, GGT; creatinine, CRE; alkaline phosphatase, ALP; glucose, GLU; total proteins, TP; creatine kinase, CK-NAC)

Table 3: Serum lipid levels in healthy goats and in goats infected with *C. pseudotuberculosis*. The results are expressed as the mean ± standard error (Mean ± SE) and Median (Min-Max).

Variable	Group	n	Mean ± SE	Median (Min - Max.)	p
TG (mg/dL)	Control	9	30.22 ± 3.86	31 (15 - 45)	0.566*
	C. pseudotuberculosis	10	27.7 ± 2.16	29.5 (17 - 37)	
LDL (mg/dL)	Control	9	215.33 ± 2.38	215 (204 - 225)	<0.001*
	C. pseudotuberculosis	10	245.3 ± 2.55	242.5 (236 - 259)	
CHOL (mg/dL)	Control	9	45.33 ± 2.79	48 (34 - 56)	0.205✣
	C. pseudotuberculosis	10	53.9 ± 3.18	50 (45 - 75)	
HDL (mg/dL)	Control	9	26.14 ± 1	26.4 (21.2 – 29.6)	0.012*
	C. pseudotuberculosis	10	29.34 ± 0.6	29.2 (26.3 – 32.2)	

✣*According to the Mann-Whitney U test*

**According to Student's t-test*

(triglycerides, TG; low density lipoprotein, LDL; cholesterol, CHOL, high density lipoproteins, HDL)

DISCUSSION

The measurement and evaluation of the biochemical profiles may be helpful in elucidating the pathogenesis and prognosis of certain diseases (Şevik *et al*., 2016). Therefore, this study was designed to investigate the prognostic and diagnostic value of cardiac enzymes (CK-MB, ALT and AST) and a limited lipid profile, including TCHOL, TG, LDL and HDL, for the control of *C. pseudotuberculosis* infection in goats.

The identity of the tissues or organs that are mainly involved when the level of total CK is elevated in the skeletal muscle, heart, brain, and other organs and tissues, to a lesser degree, is not known. However, CK exists in 3 principal forms, differentiated by the tissue or organ in which it is found. One of these forms, CK-MB, is found predominantly in the heart. CK-MB levels increase under conditions of damage to the heart (Mehran *et al*.,2000). The other specific cardiac biomarker is cardiac troponin I (Basbugan *et al*., 2010). Unfortunately, cardiac troponin I was not included in this study.

In the current study, the elevated levels of cardiac enzymes in goats infected with *C. pseudotuberculosis* compared to healthy control animals indicated possible myocardial injury in the infected animals. CK-MB, AST and ALT are cardiac enzymes used in the diagnosis of heart conditions (Coodley, 1970). Levels of these enzymes were also increased in the serum of goats infected with *C. pseudotuberculosis* compared to healthy control animals, supporting the possibility of cardiac injury. A similar increase in these parameters was reported in lambs affected with white muscle disease (Tunca *et al*., 2009; Ataollahi *et al*., 2013). The actual mechanism of cardiac injury in goats infected with *C. pseudotuberculosis* is uncertain. However, to the best of the authors' knowledge, this is the first study addressing the diagnostic and prognostic accuracy of cardiac enzymes (CK-MB, AST and ALT) in cases of *C. pseudotuberculosis* infection in goats.

In addition to being present in hepatocytes, AST is also present in skeletal muscle and cardiac muscle cells. The observed increase in AST level in the infected goats can be related to cardiac injury because the CK-MB level was also increased. However, ALP is an enzyme found in the cells lining the intrahepatic bile ducts, and its concentration increases in biliary disease, intrahepatic cholestasis, and infiltrative diseases of the liver (Lim *et al*., 2014).We observed a significant increase in ALP concentration in the infected goats. The levels of both ALP and GGT have been reported to be elevated in cholestasis (Whitfield *et al*., 1972). In the current study, no significant difference in GGT level between the infected and control groups was observed. Therefore, the increase in the level of ALP in goats infected with *C. pseudotuberculosis* can be related to hepatic injury.

The liver is the most important organ in the metabolism of lipids and lipoproteins, and under normal conditions, most of the lipids and lipoproteins in the plasma are synthesized through the cellular functions of the liver and are released into the bloodstream (Bell, 1979; Tietge *et al*.,1998). From this point of view, the presence of certain lipids and lipoproteins in the plasma could be expected to contribute to the development of hepatic damage (Wang et al 2008). The results presented in the current study showed that the LDL level was

significantly increased in the goats infected with *C. pseudotuberculosis* compared to healthy controls (Table 3). Although the levels of HDL and CHOL were high, no statistically significant difference was found for either enzyme between the groups. In addition, TG levels were normal. The increased LDL, HDL and cholesterol levels found in the current study may be due to impaired lipid metabolism in the infected goats. Lipoproteins are the subject of extensive research in human medicine, especially in relation to the diagnosis of diseases of the cardiovascular system, but lipoprotein analysis is infrequently requested in domestic animals (Kaneko *et al*., 2008). Increased levels of LDL and decreased levels of HDL are also indicative of an increased incidence of cardiac events and are considered cardiovascular risk factors. Therefore, in this study, the relationship between lipid profile parameters and cardiac events was evaluated. The levels of both CK-MB and LDL in goats infected with *C. pseudotuberculosis* were higher compared to the control group ($p<0.05$).

CONCLUSION

In conclusion, these results suggest that goats naturally infected with *C. pseudotuberculosis* have higher levels of cardiac enzymes compared with healthy goats. To the authors' knowledge, no study has been performed previously using cardiac enzyme levels for the diagnosis of cardiac injury in goats infected with *C. pseudotuberculosis*. However, further studies, especially long-term or histological evaluations, are required to characterize more accurately the putative cardiotoxicity in goats naturally infected with *C. pseudotuberculosis*.

ANIMAL RIGHTS STATEMENT

This study was approved by the Siirt University Animal Research Local Ethics Committee.

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