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Comparison of serum procalcitonin, haptoglobin and C-reactive protein levels in goats with *Corynebacterium pseudotuberculosis*

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ABSTRACT

This study was conducted to investigate the response of acute phase proteins, mainly Haptoglobin, C-reactive protein, Procalcitonin and the negative acute phase response, especially albumin in goats suffering from *C. pseudotuberculosis.* A total of 19 Turkish hair goats between the ages of 1.5 and 2 years in a special dairy farm was selected for the study, consisting of 9 healthy animals and 10 with *C. pseudotuberculosis*. There was a statistically significant difference in serum haptoglobin, C-reactive protein, procalcitonin and albumin levels in goats with *C. pseudotuberculosis*, compared to the control group ($p<0.05$). The results showed that Haptoglobin, C-reactive protein and Procalcitonin produce a higher, and Alb a lower, response in goats with *C. pseudotuberculosis* compared to the control. The presented study suggests that *C. pseudotuberculosis* can influence the level of acute phase proteins in goats. These results indicate that monitoring a number of acute phase proteins can increase the diagnostic information available for this disease.

Key words: *C. pseudotuberculosis*, C-reactive protein, Haptoglobin, Procalcitonin.

INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic disease of goats and sheep caused by *Corynebacterium pseudotuberculosis (C. pseudotuberculosis)* that has spread throughout almost the whole world (Dorella *et al.* 2006; Valli and Parry, 1993). CLA has a long incubation period ranging from 25 to 140 days. The spread of this facial cell organism through the lymphatic or blood system also leads to the formation of abscesses in the superficial and mediastinal lymph nodes, which are transmitted internally to the lymph nodes and organs (Fontaine and Baird, 2008). *C. pseudotuberculosis* has a potent exotoxin, phospholipase D, which is a key virulence factor in the development of CLA (Ceciliani *et al.* 2012). As lesions progress they become encased within fibrous capsules, the inflammatory immune response decreases, and continued slow expansion of the abscess may then occur.

Acute phase reactants to the traumatic, infective and inflammatory states are called acute phase proteins (APPs). It can be thought of as an early warning system that informs the condition of the body (Ceciliani *et al.* 2012; Gruys et al. 1994). The most common classification of APPs categorizes them as either "positive" or "negative". Positive APPs increase in response to challenge and include haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A (SAA), ceruloplasmin (CP), alph 1-acid glycoprotein (AGP) and fibrinogen; while negative APPs are albumin and transferrin (Ceciliani *et al.* 2012). Acute phase proteins are released not only in acute events but also in chronic processes. Serum Hp, a major acute phase protein in sheep and other ruminants, has several immune-modulatory functions (Sevgisunar and Şahinduran, 2014). CRP patterned elements in peripheral blood elements are part of the acute phase response and are frequently used as indirect diagnosis methods for infectious diseases (Sãez-Llorens and Lagrutta, 1993). Some studies have reported that CRP may be used to differentiate bacterial-viral or acute-chronic diseases (Alsemgeest *et al.* 1994).

A new diagnostic parameter to characterize the diagnosis of inflammatory diseases and to characterize the true immune response is procalcitonin (PCT). The production of PCT is provided by endotoxins or mediators produced in response to bacterial infections. Accordingly, it may be useful in differentiating viral and bacterial infections (Joo *et al.* 2011; Schuetz *et al.* 2011).

The aim of the present study is to determine the relationship between serum procalcitonin, haptoglobin, C-reactive protein and albumin levels in healthy goats and those suffering from *C. pseudotuberculosis*, and to evaluate the diagnostic value of Hp, PCT and CRP levels.

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MATERIALS AND METHODS

Animals and sample collection : The study was conducted in a single farm. Fluctuating viscous orange-sized lumps were observed in the right submandibular and/or prescapular lymph nodes of 10 animals; creamy, slightly greenish, malodorous content was extracted by puncture of the lump. After examination of the microscopic morphology of the colonies, biochemical tests were performed according to the suspected factors, and the bacteria isolate was identified as *C. pseudotuberculosis*. These animals formed the group *C. pseudotuberculosis* (n:10). The other 9 goats that had not lost appetite and performance, showed no significant loss of condition, and had normal clinical examination results, were evaluated as healthy. These formed the control (n:9).

Clinical examination of all animals (body temperature, pulsation, number of respirations, lymph nodes, tracheal palpation, lung auscultation and percussion) was carried out in detail. At the end, 19 Turkish hair goats between the ages of 1.5 and 2 years were selected for the study. Housing, nutrition and management conditions were the same for all the goats.

Blood samples for C-reactive proteins (CRP), procalcitonin (PCT), haptoglobin (Hp) and albumin (Alb) were collected from the jugular vein into plain tubes (Kheir and Ahmedi 2008). After clotting, the samples were centrifuged at 1000 rpm for 15 min and sera were immediately separated and stored at -20°C until analysis.

Biochemical analysis: Serum CRP and Alb levels were measured using an automatic analyzer (Advia 1800 chemistry system). Serum Hp and PCT levels were measured according to manufacturers' instructions using commercial ELISA kits.

Microbiological examination: The suspected lymph nodes were sampled. Creamy, slightly greenish, malodorous content was extracted by puncture of the lump and swapped. The swab was cultured on 7% sheep blood agar for 48 h at 37ºC. After obtaining a pure culture from suspicious colonies, Gram-positive coryneform bacteria were detected by staining, bacterial colonies were characterized and suspected colonies tested with API systems.

Statistical analysis: Descriptive statistics for each variable were calculated. Prior to hypothesis testing, data were examined with Shapiro-Wilk test for normality and Levene test for homogeneity of variances as parametric test assumptions. Student t test was used to evaluate the difference between healthy controls and the study group for Alb levels, while the non-parametric Mann Whitney U test was performed for CPR, Hp and PCT levels. Level of significance was set at $p<0.05$. All statistical analyses were calculated using SPSS 14.01 statistical software.

RESULTS AND DISCUSSION

Serum procalcitonin, haptoglobin, C-reactive protein and albumin levels in goats with Corynebacterium pseudotuberculosis is illustrated in Table 1. Clinically, the studied goats demonstrated common clinical findings including fluctuating viscous orange-sized lumps were observed in the right submandibular and/or prescapular lymph nodes. Values of serum CRP and Hp showed a statistically significant higher values ($P\leq0.05$) in goats with *C. pseudotuberculosis* compared with the control. For serum PCT, its values were significantly increased ($P\leq0.05$) in the group of goats with C. pseudotuberculosis compared with the control. The mean concentration of serum Alb showed significant decrease ($p<0.05$) in goats with *C. pseudotuberculosis* compared with the control.

C. pseudotuberculosis is the causative agent of caseous lymphadenitis (CLA), a disease characterized by the formation of suppurative abscesses particularly in superficial and internal lymph nodes, and in internal organs in small ruminants (Alonso *et al.* 1993).

Hp is a group of blood proteins whose concentrations in animals can be influenced by infection, inflammation, surgical trauma or stress, is an important APP that also exists in bivalent animals and is primer-specific in various situations (Gruys *et al.* 1994). The present study showed significant increase ($p < 0.05$) in mean serum Hp concentration (Table I) in the C. pseudotuberculosis group, which could be interpreted as the primary action of Hp to restore the homeostasis in the body. This finding is similar

Table 1: Mean (Mean ± SE) and Median (Min-Max) values in *C. pseudotuberculosis* group and in control group.

Variable	Groups	n	Mean ± Standard Error of Mean	Median (Min - Max)	P
CRP	Control	9	0.87 ± 0.1	0.8 (0.5 – 1.5)	0.001❖
	C.pseudotuberculosis	10	3.47 ± 0.78	1.85 (1.3 – 6.6)	
Hp	Control	9	0.13 ± 0.01	0.12 (0.11 – 0.18)	<0.001❖
	C.pseudotuberculosis	10	0.27 ± 0.01	0.28 (0.21 – 0.31)	
PCT	Control	9	0.08 ± 0.01	0.1 (0.02 – 0.11)	0.001❖
	C.pseudotuberculosis	10	0.2 ± 0.02	0.2 (0.1 – 0.3)	
Alb	Control	9	2.76 ± 0.06	2.8 (2.5 - 3)	0.005✳
	C.pseudotuberculosis	10	1.91 ± 0.19	1.85 (1.1 - 3)	

❖According to Mann Whitney U test
✳According to Student t test
(C-reactive protein; CRP, procalcitonin; PCT, haptoglobin; Hp, albumin; Alb)

to the studies conducted by (Cray *et al.* 2009). In the works of (Ozkanlar *et al.* 2012) they found that bacterial infection triggers cytokine production by antigen-presenting cells which further increases the rate of APPs production in general and Haptoglobin in particular. Immunological processes, infections, tissue damage and inflammatory events cause a systemic event over hours or days in the organism. The acute phase response to this table is the production of acute phase reactors (Khera *et al*. 2005). APPs are defined as those showing a plasma level increase of at least 25%, while negative APPs are defined as those showing a decrease of at least 25%. (Sãez-Llorens and Lagrutta, 1993). These findings concurred with the findings of the current study where the concentrations of Hp was upregulated in the presence of *C. pseudotuberculosis.*

Another important finding in our study was a difference in the CRP concentration of the C. pseudotuberculosis group compared to the control was found to be statistically significant. In the present study, the level of CRP in goats with C. pseudotuberculosis was significantly higher than in the control (Table I).In this study, the mean concentration of CRP in goat with Corynebacterium pseudotuberculosis was 3.47 ± 0.78. Literature about the CRP concentration in goats is limited. It was found to be 1.13 mg/dl in goats and 0.80 mg/dl in goat kids at 6 weeks of age. (He *et al.* 2014). Studies have reported that CRP levels are associated with body condition score, lactation status and animal health, and they rapidly increase in the case of infection. For this reason, it is reported that CRP may be a marker for herd health evaluation (When-Chuan *et al.* 2003). Ambalavanan *et al.* (2009) demonstrated that CRP modulated inflammatory and immune responses. The measurement of APPs is particularly important for differentiating bacterial from viral involvement in the infection and administering the appropriate treatment. However, APPs cannot form a reagent between bacterial and viral diseases in CRP production due to the wide range of individual varieties (Petersan *et al.* 2004). When used for this purpose, APP analysis strengthens the diagnosis and provides more accurate information on the prognosis of infected animals (Gruys *et al.* 1994; Gruys *et al.* 2005).

In our literature review, we did not find any studies that examine serum PCT levels in goats with *C. pseudotuberculosis.* In the present study, we investigated whether serum PCT levels could be of benefit in determining chronic inflammation in *C. pseudotuberculosis.* We found that the level of PCT in goats with *C. pseudotuberculosis* was significantly higher than in the control, and the *C. pseudotuberculosis* group compared to the control was found to be statistically significant ($p<0,05$) (Table I).Studies have shown that procalcitonin levels are indicative of acute systemic inflammation associated with infection, particularly bacterial infections (Reingardiene, 2004). In addition, increased interferon gamma (INF-γ) during viral infections suppresses PCT production. It has been suggested that this may be useful in differentiating viral and bacterial infections. Procalcitonin has been reported to have more specificity than CRP in the detection of certain bacterial diseases in human medicine (Elsammak *et al.* 2006). Procalcitonin (PCT) is selectively induced in bacterial inflammation, sepsis and multi organ failure syndrome. The primary trigger is the bacterial endotoxin. The production of PCT is stimulated by endotoxins or mediators produced in response to bacterial infections (TNF-a, IL-1, IL-6). Serum level is strongly associated with the prevalence and severity of bacterial infections (Schuetz *et al.* 2011).

This study showed that serum albumin concentration was significantly lower in goats with *C. pseudotuberculosis* compared to the control (Table I). We can say that the cellular mechanism of positive APP production is always associated with a decrease in negative APPs, especially Alb. The current study has confirmed this, with positive acute proteins, particularly Hp and CRP, significantly increased. Albumin, considered a negative APP, is a major component, representing approximately 60%, of all plasma proteins, and exhibits a reduced level of response upon infection (Fontaine *et al.* 2006).

CONCLUSION

In conclusion, an acute phase reaction that varied between APPs has been identified in CLA. The results showed higher levels of Hp, CRP, PCT and lower levels of Alb in the goats with *C. pseudotuberculosis* compared to the control. This suggests that *C. pseudotuberculosis* can influence the level of APPs in goats. These results indicate that monitoring Hp,CRP ve PCT can increase the diagnostic information available for this disease.

Ethical considerations: During the period of study, goats animal husbandry was adhered to and no animals were mistreated, starved or subjected to unorthodox procedures. Researchers and farm staff strictly observed animal welfare and public health guidelines.

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