

## Journal of the Hellenic Veterinary Medical Society

Vol 69, No 4 (2018)



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doi: [10.12681/jhvms.15926](https://doi.org/10.12681/jhvms.15926)

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### To cite this article:

Aydogdu, U., Coskun, A., Yildiz, R., Guzelbektes, H., & Sen, I. (2019). Clinical importance of lipid profile in neonatal calves with sepsis. *Journal of the Hellenic Veterinary Medical Society*, 69(4), 1189–1194.  
<https://doi.org/10.12681/jhvms.15926>

## Clinical importance of lipid profile in neonatal calves with sepsis

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**ABSTRACT.** In this study, it was aimed to determine of diagnostic importance of blood lipid levels in neonatal calves with sepsis. The study was carried out on a total of 70 calves, 60 with sepsis and 10 healthy calves. The calves with sepsis were included in the study, according to clinical and hematological findings. The blood samples were taken from the V. jugularis for hematological, lipid profile and biochemical analyzes after the routine clinical examinations of the calves. There were significantly ( $P < 0.05$ ) decrease in body temperature, increase in respiration rate and capillary refill time in the calves with sepsis compared to control group. The levels of blood urea nitrogen, creatinine concentrations of calves with sepsis were significantly higher ( $P < 0.05$ ), however, levels of total cholesterol, HDL and LDL concentrations were significantly lower ( $P < 0.05$ ) than control group. In addition, blood triglyceride and VLDL concentrations of calves with sepsis were higher than control group, however there was no statistical difference.

In conclusion, serum total cholesterol, HDL and LDL in neonatal calves with sepsis could be used in evaluation of the sepsis in calves.

**Keywords:** Sepsis, calves, lipid profile

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*Date of initial submission: 9-2-2018*  
*Date of revised submission: 27-5-2018*  
*Date of acceptance: 15-6-2018*

## INTRODUCTION

The disease of calves are the most important causes of economic losses in the cattle industry (Ortiz-Pelaez et al., 2008). The important part of the calf morbidity and mortality is observed in the neonatal period (Guzelbektes et al., 2007; Radostits et al., 2007; Basoglu et al., 2014). Sepsis is defined as a combination of focal or generalized infection (suspicious infection) and systemic inflammatory response to the infections (Radostits et al., 2007). Sepsis is the most common cause of morbidity and mortality in newborn (House et al., 2011). Mortality rate at high levels in sepsis because the process is progressing fast. For this reason, early diagnosis and treatment have great importance in order to reduce sepsis mortality (Aldridge et al., 1993; Radostits et al., 2007; Fecteau et al., 2009; Basoglu et al., 2014).

Biomarkers play an important role in understanding the diagnosis, prognosis and pathogenesis of sepsis. For this reason, biomarkers such as lipid profile are still being an investigation for an early diagnose of sepsis. It has been reported that significant changes in plasma lipid and lipoprotein concentration, composition and function during inflammation and infections have been reported in humans (Wendel et al., 2007; Barati et al., 2011) and in calves (Civelek et al., 2007) and dogs (Yilmaz and Senturk 2007). These changes have been reported to be induced by released cytokines (Khovidhunkit et al., 2000; Murch et al., 2007; Lekkou et al., 2014). Lipoproteins in the circulation play very important role in the pathophysiology of infectious diseases. Many studies reported that the serum level of total cholesterol, LDL and HDL decreased, and the serum triglyceride level increased in patients with inflammatory response. These changes were reported to be independent of the underlying disease or infectious agents (Alvarez and Ramos 1986; Fraunberger et al., 1999; Wendel et al., 2007; Barati et al., 2011).

The purpose of the study was to determine of diagnostic importance of blood lipid profile levels in neonatal calves with sepsis.

## MATERIALS AND METHODS

### Study design and animals

The study was carried out on a total of 70 calves, 60

with sepsis mean of age (days) was  $13.13 \pm 1.23$ ) at brought to Large Animal Clinic of Faculty of Veterinary Medicine of Selcuk University from different farms by owner and 10 Holstein healthy calves (mean of age (days) was  $12.60 \pm 2.25$ ) were belong to Faculty farm. Breeds of calves with sepsis were Holstein 45, Simmental 10, and Montofon 5. Routine clinical examinations of all the calves were performed. Laboratory and clinical findings as described by Fecteau et al. (2009) and Lofstedt et al. (1999) were used for the diagnosis of sepsis in the calves. Along with the presence or suspected of infection and the SIRS criteria were evaluated as sepsis. A diagnosis of SIRS was made if at least two of the following criteria were fulfilled: leukopenia or leukocytosis (reference value,  $4-12 \times 10^3/\mu\text{L}$ ), hypothermia and hyperthermia (reference value;  $38.5-39.5^\circ\text{C}$ ), bradycardia or tachycardia ( $< 90$  or  $> 120$  beats per minute), and tachypnea ( $> 36$  breaths per minute). Blood samples for leukocyte count (tubes with  $\text{K}_3\text{EDTA}$ ) and biochemical analyses (tubes without anticoagulant) were collected from the vena jugularis and the tubes without anticoagulant were kept at room temperature and coagulated. Serum was removed by centrifugation for 5 min at 2500 g. Serum samples were stored at  $-20^\circ\text{C}$  until analyzed. Leukocyte levels in blood with  $\text{K}_3\text{EDTA}$  of the calves were determined using a hematologic analyzer (Hemocell Counter MS4e, Melet Schloesing Laboratories, France). Serum samples were analysed for triglyceride, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN) and creatinine. The analyses were performed on an automated analyser (BS-200, Mindray, China) and VLDL levels were calculated by the following formula:  $\text{triglyceride}/5$  (Tietz 1995, Sevinc et al., 2003).

### Statistical analysis

All data were presented as mean and standard error of mean (Mean  $\pm$  SEM). Power analysis was performed and sample size of the groups was determined as statistically appropriate. Independent samples t-test was used to assess the significance of the differences between the groups. The level of statistical significance was at  $P < 0.05$ . Receiver Operating Characteristics (ROC) curves were used to determine the cut-off values of total cholesterol, HDL and LDL.

The likelihood ratio value for the cut-off threshold was calculated and the highest calculated value was considered as the optimum cut-off point. The SPSS software program (Version 18.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

## RESULTS

It was determined that hypothermia or hyperthermia, tachypnea, dehydration, tachycardia or bradycardia, depression, lack of suction reflex, cold in the mouth and cooling in the extremities, and capillar refill time was prolonged in calves with sepsis. Leukocyte count was significantly higher in calves with sepsis than in control group. There were significantly ( $P < 0.05$ ) decrease in body temperature, increased in respiration rate and capillary refill time in the calves with sepsis, compared to control group (Table 1). In the sepsis group, 48 of the calves had enteritis, 7 calves had pneumonia, but in 5 calves the origin of the sepsis could not be determined.

The changes in lipid profile and biochemical parameters of sepsis and healthy calves are presented in Table 1. The levels of blood urea nitrogen and creatinine concentrations of calves with sepsis were significantly higher ( $P < 0.05$ ) compared to control

group. However, levels of total cholesterol, HDL and LDL in calves with sepsis were significantly lower ( $P < 0.05$ ) than the control group. In addition, blood triglyceride and VLDL concentrations of calves with sepsis were higher than control group, but there was no statistical difference.

The results of ROC analysis of total cholesterol, HDL and LDL are given in Table 2 and Figure 1. The optimal cut-off values of total cholesterol, HDL and LDL were 67, 51.2, and 9.53 mg/dL, respectively. The specificity was 90% of all the parameters in these cut-off values and the sensitivities were 86.7, 88.3 and 66.7%, respectively.

## DISCUSSION

Until now, this is the first study to evaluate lipid profile parameters in calves with sepsis. In the present study, we demonstrated that the serum lipid profile has the potential use for diagnosis of sepsis in calves. An increase in the level of triglyceride and a decrease in the levels of total cholesterol, HDL and LDL have been observed in patients with sepsis and SIRS (Alvarez and Ramos 1986; Barati et al., 2011). Sepsis is usually accompanied by a significant decrease in cholesterol levels (Cirstea et al., 2017).

**Table 1:** The levels of serum lipid profile and some biochemical parameters in calves with sepsis and healthy calves (Mean  $\pm$  SEM)

Parameters	Sepsis n = 60	Healthy n = 10	P levels
Total cholesterol (mg/dL)	43.37 $\pm$ 3.87	100.50 $\pm$ 11.00	< 0.001
Triglyceride (mg/dL)	21.12 $\pm$ 3.25	15.17 $\pm$ 2.51	0.155
HDL (mg/dL)	30.42 $\pm$ 3.00	71.86 $\pm$ 6.57	< 0.001
LDL (mg/dL)	6.82 $\pm$ 0.73	22.84 $\pm$ 5.44	0.017
VLDL (mg/dL)	4.22 $\pm$ 0.65	3.03 $\pm$ 0.50	0.155
BUN (mg/dL)	40.50 $\pm$ 2.60	10.20 $\pm$ 0.98	< 0.001
Creatinine (mg/dL)	3.33 $\pm$ 0.43	1.59 $\pm$ 0.06	< 0.001
Leukocyte count ( $10^3/\mu\text{L}$ )	21.58 $\pm$ 1.67	9.34 $\pm$ 0.88	0.003
Heart rate (min)	99.54 $\pm$ 4.78	93.33 $\pm$ 7.94	0.514
Respiratory rate (min)	41.96 $\pm$ 2.72	30.14 $\pm$ 2.01	0.001
Temperature ( $^{\circ}\text{C}$ )	37.74 $\pm$ 0.23	38.90 $\pm$ 0.13	0.023
CRT (sec)	4.13 $\pm$ 0.17	1.89 $\pm$ 0.11	< 0.001

HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low density lipoproteins, BUN: blood urea nitrogen, CRT; capillary refill time

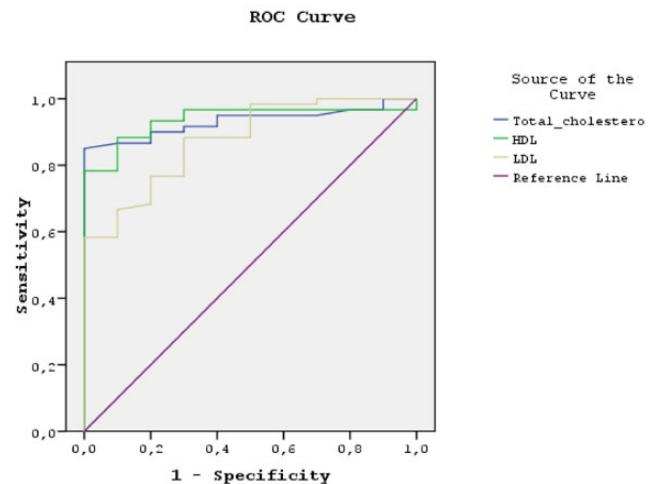
**Table 2.** Cut-off, sensitivity and specificity values of total cholesterol, HDL and LDL in calves with sepsis

Parameters	AUC	Cut-off values	Sensitivity (%)	Spesifty (%)	P value	SEM
<b>Total cholesterol (mg/dL)</b>	0.932	67	86.7	90	<0.001	0.030
<b>HDL (mg/dL)</b>	0.937	51.2	88.3	90	<0.001	0.031
<b>LDL (mg/dL)</b>	0.876	9.53	66.7	90	<0.001	0.052

HDL: high-density lipoprotein, LDL: low-density lipoprotein

The pathophysiological mechanisms associated with hypocholesterolemia during the sepsis process are not fully understood (Barati et al., 2011). Different mechanisms including the imbalance between the synthesis and use of plasma lipids, the use of lipids to replace damaged cell membranes, and the interaction of lipids with bacterial toxins and cytokines are still being discussing (Akgun et al., 1998; Levels et al., 2001; Levels et al., 2003; Esteve et al., 2005; Morin et al., 2015). Clinical and experimental studies have shown that high levels of circulating cytokines to reduce cholesterol levels in patients with severe infection (Murch et al., 2007; Lekkou et al., 2014; Morin et al., 2015). In contrast, lipoproteins have the ability to regulate cytokine production during the inflammatory response. Therefore, the reduction in circulating levels of cholesterol plays a crucial role in the pathophysiology of sepsis (Hardaróttir et al., 1994; Fraunberger et al., 1999). In the present study, total cholesterol level in calves with sepsis was significantly lower than the control group. The possible cause of low cholesterol in calves with sepsis is caused by cytokines release to circulation in response to inflammation (Hardaróttir et al., 1994; Fraunberger et al., 1999). In some studies (Akgun et al., 1998; Fraunberger et al., 1999; Gordon et al., 2001; Lekkou et al., 2014), have been reported that inflammation with high cytokine level may be associated with hypocholesterolemia. El-Bahr and El-Deep (2013) reported that cytokine levels in bronchopneumonic water buffalo calves were significantly higher than healthy calves while serum total cholesterol, HDL and LDL levels were significantly lower. It has also been reported that cytokine (TNF- $\alpha$  and IL-6) levels are increased while circulating levels of cholesterol are decreased in inflammatory conditions (Akgun et al., 1998; Gordon et al., 2001; Lekkou et al., 2014).

It has been reported that lipopolysaccharide (LPS)



**Figure 1.** Plot of receiver operating characteristic (ROC) curve for total cholesterol, HDL and LDL variables

is neutralized by lipoproteins. It is stated that an important mechanism causing the decrease in HDL is consumed by LPS and other endotoxins (Levels et al., 2001; Levels et al., 2003; Wu et al., 2004; Esteve et al., 2005; Barati et al., 2011; Morin et al., 2015). Thus, it is thought that HDL and LDL are important regulators of the host immune response during endotoxemia and have the potential to treat patients with gram-negative sepsis (Wendel et al., 2007; Barati et al., 2011). In addition, it has been reported that HDL induces various anti-atherogenic, anti-inflammatory and anti-oxidative effects, independent of changes in cholesterol metabolism (Khovidhunkit et al., 2000; Gordon et al., 2001; Barter et al., 2004; Murch et al., 2007; Barati et al., 2011). It has been reported that total cholesterol and HDL levels are significantly decreased in calves with bronchopneumonia (Civelek et al., 2007; Joshi et al., 2015) and dogs with parvoviral enteritis (Yilmaz and Senturk 2007). It is reported that low HDL levels in septic patients are significant-

ly associated with mortality and the development of adverse clinical outcomes (Chien et al., 2005; Lekkou et al., 2014; Cirstea et al., 2017). In this study, it was observed that serum HDL and LDL levels in calves with sepsis were significantly lower than control group ( $P < 0.001$ ,  $P < 0.05$ , respectively). It has been showed that the low level of HDL and LDL may be used as diagnostic criterias in evaluation of sepsis. Nassaji and Ghorbani (2012) reported that acute bacterial infections are associated with low serum total cholesterol and HDL levels and they indicate that changes in plasma lipid levels may be an important indicator of acute bacterial infections.

Sepsis causes hypertriglyceridemia in humans and animals, and this increase is due to the induction of hepatic and adipose tissue lipolysis and the increase in VLDL production (Alvarez and Ramos 1986; Civelek et al., 2007). In another study, have been suggested that as the cause of hypertriglyceridemia is to diminished conversion of VLDL to LDL by inhibition of lipoprotein lipase activity (Feingold et al., 1992; Gouni et al., 1993). Civelek et al. (2007) reported that VLDL and triglycerides levels of calves with bacterial lower respiratory tract infections were significantly higher than healthy ones. Another study reported that triglyceride concentration was higher in children with bacterial pharyngitis than in healthy children but this difference was not statistically significant (Iskan et al., 1998). In this presented study, similar to current studies, serum TG and VLDL levels were increased in calves with sepsis but this increase was not statistically significant (Table 1).

In humans, studies are being conducted on the diagnostic and prognostic value of dislipidemia in critical diseases such as sepsis and SIRS (Lüthold et al., 2007; Lekkou et al., 2014; Zou et al., 2016). However, our study has limitation. Unfortunately, the prognostic significance of this study has not been established, as there is insufficient information about whether or not the calves survived. The missing part

of this study is that the prognostic follow-up of the calves with sepsis is not performed and the parameters are not considered as a mortality factor. However, we think that changes in the lipid profile may give an idea of the diagnostic value. For this purpose, ROC analysis for total cholesterol, HDL and LDL was performed to determine the optimal cut-off value and sensitivity and specificity of the relevant parameters according to this cut-off value. According to ROC analysis results, the cut-off values of total cholesterol, HDL and LDL were 67, 51.2, and 9.53 mg/dL, respectively. Despite the high specificity (90%) of all the parameters in these cut-off values, the sensitivities were 86.7, 88.3 and 66.7% respectively. According to these results, it has been assumed that total cholesterol and HDL can be used as parameters for diagnosis inflammatory response of sepsis in the calves. However, LDL is not a suitable parameter because it has low sensitivity.

## CONCLUSIONS

In conclusion, it has been shown that the decrease in serum total cholesterol and HDL levels may be a sign of intense inflammatory response and that these changes in lipid levels (especially total cholesterol and HDL) can be used to detect inflammatory response in calves with sepsis. We could be said that serum total cholesterol and HDL may be used as a diagnostic indicator for sepsis in calves. However, further studies are needed to evaluate serum total cholesterol and HDL as prognostic and mortality indicators in calves with sepsis.

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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