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Determining Serum Haptoglobin and Proinflammatory Cytokines Concentration in the Calves Clinically Diagnosed with Pneumonitis, Pneumoenteritis and Enteritis

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ABSTRACT

Background: This study aims to determine the concentration of serum haptoglobin (Hp), interleukin 1 (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) in cases of Pneumonia, Pneumoenteritis and Enteritis.

Methods: 60 calves were subjected to the study and they were divided into four groups. The study group consisted of the claves diagnosed with clinical pneumonia (Group P; n=15), pneumoenteritis (Group PE; n=15) and enteritis (Group E; n=15) while the control group included the healthy calves (Group C; n=15). The measurements of the concentration of serum haptoglobin (Hp), interleukin 1 (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), Total protein (TP) and Albumin (ALB) were made by using commercial kits.

Conclusion: In all infection groups (P, PE ve E), Haptoglobin concentration, serum cytokine (IL-1β, IL-6 and TNF-α) and Albumin values were found to have been higher than the control group (p≤0,005). However, there was no difference in total protein. In the light of these findings, it is suggested that routine controls for Haptoglobin and cytokine (IL-1β, IL-6 and TNF-α) concentrations would be rewarding to determine the severity of the infection, to choose the suitable treatment and to detect subclinical infections in veterinary medicine.

Key words: Calf, Haptoglobin (Hp), Interleukin 1(IL-1β), Interleukin 6 (IL-6), Tumor necrosis factor-α (TNF-α).

INTRODUCTION

Common cases of pneumonia, enteritis, pneumoenteritis in calves embody the majority of diseases encountered in calves. Calf fatalities due to these diseases cause significant economic losses all over the world (Güneş *et al*., 2013). Physiological response to infections and injuries including the onset of episodes that will emerge as inflammation and systemic response is also called acute phase reactions Include as after called as acute phase reactions (AFR) (Kumar *et al*. 2015). These alterations occurring in a remote location from the inflammation area also includes fever, leukocytosis and the qualitative and quantitative modifications of a specific group of proteins other than structural proteins functioning in blood and other body fluids. These proteins are known as Acute Phase Proteins (AFP). Proteomic studies on serum/plasma trailing natural or experimental infections proved that there were significant changes in AFPs and they were present in those fluids in high levels in various ways (Ceciliani *et al*., 2012, Anand Laxmi *et al*. 2013).

Proinflammatory cytokines such as Interleukin 1 and 6 (IL-1β, IL-6) and Tumor Necrosis Factor (TNF-α) are the main mediators of the AFPs which are synthesized from the liver. While IL-6 is more effective in hepatic acute phase response, IL-1β and TNF-α are effective in extrahepatic cases. Basically, these cytokines are released from macrophages, however, they are also released from other cells in case of internal or external stimuli. IL-1β is produced by activated monocyte and macrophages. TNF is a polypeptide released from macrophages stimulated by lipopolysaccharides (Murata *et al*. 2004). TNF is defined as the biological active mediator or primary cytokine that is effective in response related to gram-negative bacterial septicemia or endotoxemia. IL-6 can be synthesized from liver Kupfer cells, keratinocytes, hypophysis or mucosal epithelium. In case of inflammation, infection or tissue damage, the release of cytokines is stimulated by the cells that organize the defence. Hence, the synthesis of AFPs is also stimulated. It has been stated that physical and physiological stress increases plasma IL-6 and AFP levels in humans and experimental animals. Moreover, physical

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stress has been reported to have increased AFPs in calves (Nukina *et al*.,2001; Yoshioka *et al*., 2002; Murata *et al*., 2004; Dogra *et al.,* 2020). The conducted study has aimed to determine serum Haptoglobin concentration trailing clinical symptoms of pneumonia, enteritis and pneumoenteritis cases, which are common for the calves in veterinary medicine.

MATERIAL AND METHODS

Collecting animal material and blood samples

The calves subjected to the study were clinically examined (body temperature, heart and respiratory rate, examination of mucous membranes, lung auscultation). Healthy calves presenting no pathological findings in the clinical examination were assigned to the control (C) group; those with pathological sound in lung auscultation in clinical examination were placed in pneumonia (P) group; those with diarrhea in the clinical examination were placed in enteritis (E) group; those with pathological sound in lung auscultation and diarrhea in clinical examination were selected to the pneumoenteritis (PE) group In the study, 60 calves were used and they were divided into four groups. The calves clinically diagnosed with pneumonia (Group P; n=15), enteritis (Group E; n=15), pneumoenteritis (Group PE; n=15) formed the study groups while the healthy calves formed the control (Group C; n=15) group. Blood samples of all the calves in the study groups were taken from vena jugularis into dry biochemistry tubes. The study was approved by Afyon Kocatepe University Animal Experiments Local Ethics Committee (12/06/2014-49533702/85).

Haptoglobin (Hp), interleukin 1(IL-1β), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) Measurements

Anticoagulant-free blood samples taken for biochemical parameters were centrifuged at 5000 rpm and room temperature. Serum samples were stored at-20°C until the measurement time.

Include in the serum sample in between measured and the concentrations of Haptoglobin (Hp) (Life Diagnostics Inc. Bovine Haptoglobin Test Kit), interleukin 1(IL-1β) (Cusabio Biotech CO.LTD, China), interleukin 6 (IL-6) (Cusabio Biotech CO.LTD, China), tumor necrosis factor-α (TNF-α) (Cusabio Biotech CO. LTD, China) were measured via the ELISA Reader. Measurements for Albumin (ALB) and Total protein (TP) were done by an autoanalyzer.

Statistical analysis

The data were tested by Shapiro-Wilk and it was seen that the data were heterogeneous. Considering the number of the subjects (n=15) in each group and the heterogeneous distribution, in order to determine whether there was a significant difference among the K, P, E and PE groups in terms of measured parameters, Kruskal-Wallis test was applied. The Mann-Whitney U test was used for paired comparisons in groups with differences. Significance level was set as p<0,05. In order to avoid Type 1 alfa error, Bonferroni correction was used in paired comparisons, and the significance level was set as 0,05/4=0,0125 (p<0,0125) for The Mann-Whitney U test now that there were four groups. Table values were given as mean ± standard error.

RESULTS AND DISCUSSION

In groups of Pneumonia (P), Pneumoenteritis (PE) and Enteritis (E), the concentrations of haptoglobin (Hp), interleukin 1(IL-1β), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) (p≤0, 05) were measured higher than the control group (p≤0,05). (Table 1)

The highest concentration levels of Hp (167,48±47,08) and of TNF-α (0,28±0,03) were in Group P (p≤0,05), of IL-1β (55,69±9,82) was in Group E (p≤0,05) and of IL-6 (16,26±1,14) was in Group PE (p≤0,05). The lowest concentration levels of Hp, TNF-α, IL-1β, IL-6 were determined in the control group. (Table 1)

The highest level of Total protein (TP) concentration was found in Group P (61,51±10,26) (p≤0,05) and of Albumin (ALB) concentration in Group E (31,24±0,97) (p≤0,05). The lowest concentration of TP (50, 13±3,51) and of ALB (22,72±0,75) were found in the control group (Table 2).

Our study was conducted on the calves and the study group consisted of calves with diarrhea, those with respiratory system problems and those with both.

Haptoglobin (Hp) is one of the most important acute phase proteins specific to calves. It has been reported that serum or plasma Hp concentration increases in calves following a natural or experimentally imposed infection or inflammation (Alsemgeest, 1994; Heegard *et al*., 2000; Fisher *et al*., 2001). Alsemgeest (1993) and Nazifi *et al*. (2008) reported that they did not detect haptoglobin in the blood of healthy calves; nevertheless, Hp level was high during inflammatory infections (enteritis, pneumonia,

Table 1: Serum haptoglobin (Hp), interleukin 1(IL-1β), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) concentrations (mean ± SE) in the control and study groups.

	Hp (µg/ml)	TNF (ng/ml)	IL -1β (pg/ml)	IL-6 (pg/ml)
Control	5,24±0,16^{a}	0,11±0,01^{a}	15,53±1,19^{a}	5,30±1,08^{a}
P	167,48±470.,08^{b}	0,28±0,03^{b}	40,30±7,11^{b}	10,78±1,17^{b}
PE	114,60±33,93^{b}	0,24±0,02^{b}	55,32±5,75^{b}	16,26±1,14^{c}
E	163,16±44,58^{b}	0,26±0,07^{b}	55,69±9,82^{b}	14,72±4,34^{abc}
p	0,000**	0,000**	0,000**	0,000**

*****Significant at P>0.05; NS- Non Significant at P>0.05.

Table 2: Serum total protein (TP) and albumin (ALB) concentrations (mean ± SE) of the control and study groups.

	TP (g/L)	ALB (g/L)
Control	50,13±3,51	22,72±0,75[a]
P	61,51±10,26	28,20±1,05[b]
PE	58,58±2,46	28,90±1,42[b]
E	59,59±8,63	31,24±0,97[c]
p	0,051	0,019*

*Significant at P>0.05; NS- Non Significant at P>0.05.

pleuropneumonia, peritonitis, reticuloperitonitis traumatica, endocarditis, abscess, abomasal ulcer, trauma, endometritis, myocarditis, digestive tract diseases). Ganheim *et al*. (2007), in a study where acute phase proteins were used to determine calf herd health, reported the haptoglobin concentration in healthy calves as 60–123 µg/ml.

In our study, a statistical increase was found in serum Hp concentration in the Pneumonia (P), Pneumoenteritis (PE) and Enteritis (E) groups compared to the control group. While serum Hp concentration was detected as 5,24±0,16 µg/ml in the control group, in Pneumonia (P), Pneumoenteritis (PE) and Enteritis (E) groups, it was as P:167,48±47,08, PE:114,60±33,93, E: 163,16±44,58 µg/ml, respectively. Similar to the studies reporting an increase in serum Hp concentration during infectious or inflammatory diseases, in our study Hp level was found to be high, as well. Infectious agents were considered as the reason of this situation (Makimura and Usui 1990; Alsemgeest, 1994; Skinner and Roberts, 1994; Risalde *et al*., 2011).

It has been reported that IL-6 is the most important of the cytokines mediating the hepatocytic secretion of AFPs (Le and Vilcek, 1989; Sehgal *et al*., 1989; Heinrich *et al*., 1998). It has also been reported that the synthesis of AFPs from liver cells is initiated by pro-inflammatory cytokines (TNFα, IL-1β and IL-6) released from monocytes and macrophages during inflammation (Baumann and Gauldie, 1994).

In the presented study, serum IL-1β (pg/ml), IL-6 (pg/ml) and TNFα (ng/ml) concentrations in calves were found as statistically significant in Pneumonia (P), Pneumoenteritis (PE) and Enteritis (E) groups compared to the control group. In their study, Molina *et al*., (2012) found that serum IL-1β concentration in the calves induced with experimental Bovine viral diarrhea (BVD) was higher than the control group on the nineth day of the induced disease. In the same study, it was determined that serum TNFá concentration decreased in the study group compared to the control group on the nineth day of the disease. In another study, the calves were experimentally administered i.v. endotoxin; following the administration, serum IL-1β, IL-6 and TNF α concentrations were found to be higher in comparison to the control group (Carroll *et al*., 2009). El-bahr and El-deeb (2013) reported that IL-1β concentration could increase six times more in the calves diagnosed with pneumonia than the healthy calves. There are other studies that IL-1β, TNFα concentration was found higher in the calves with diarrhea than the control group (Risalde *et al*, 2011). In our study, higher serum IL-1β, IL-6 and TNFα concentrations detected in the groups of P, PE and E compared to the control group are compatible with the literature.

In a study conducted on healthy calves, serum TP concentration was reported to be within 4, 71-6,35 g/dL range (Piccione *et al*., 2010). In our study, the average values of calves in all groups (K:50,13±3,51, P:61,51±10,26, PE:58,58±2,46, E:59,59±8,63) was not statistically significant. According to the research conducted, it was reported that the increase in serum TP and ALB concentrations in calves with diarrhea was related to the hemoconcentration having formed as a result of extracellular fluid loss due to diarrhea (Groutides and Michell, 1990; Turgut *et al*., 1992). In our study, even though serum ALB concentrations of calves were found to be statistically significant in infection groups, they remained within the reference ranges. It was determined that the increase in TP concentrations in all groups was not statistically significant.

CONCLUSION

Considering the findings of the present study, it was concluded that the rise in serum haptoglobin (Hp), interleukin 1(IL-1β), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) concentrations in the study groups was secondary to the clinical symptoms of the calves with pneumonia, pneumoenteritis and enteritis, and Hp, IL-1β, IL-6 and TNF-α were in very low concentrations in the healthy animals in the control group. In the light of these findings, it is suggested that routine measurements of serum Hp, IL-1β, IL-6 and TNF-α concentrations would be rewarding to determine the severity of the infection, to choose the suitable treatment, to monitor the efficiency of the selected treatment method and would be highly beneficial for detecting animals that show no clinical symptoms and have a subclinical course during the herd health screening in terms of veterinary medicine. Further research is needed to determine the Hp, IL-1β, IL-6 and TNF-α concentrations for the diagnosis of viral, bacterial, parasitic, diseases etc., in animals as well as to understand the efficiency of this parameter in the control as selection of treatment protocols.

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Conflict of Interest declaration

The authors declare that they have no competing interests.

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