Concentrations of serum amyloid A, haptoglobin and some cytokines in calves with Cryptosporidiosis in the pre- and post-treatment stage

Research Article

ABSTRACT

This study aims to determine serum amyloid A (SAA), haptoglobin (Hp), interleukin-1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) concentration in cases of Cryptosporidiosis that are frequently encountered in calves in veterinary medicine. Although many experimental studies have been conducted in this field, studies of naturally infected calves are quite a few. In this study, 10 neonatal Holstein calves diagnosed with *Cryptosporidium* were included. Stool samples were taken from calves with diarrhea using a rapid test kit. Blood samples were obtained from the jugular vein pre- and post-treatment for biochemical measurements. SAA, Hp, IL-1β, IL-6 and TNF-α concentration measurements were conducted with ELISA reader using commercial kits. Calves with Cryptosporidiosis presenting with diarrhea showed a statistical difference in SAA, Hp, IL-1β, IL-6, white blood cell and hemoglobin values before and after treatment, whereas hematocrit, red blood cell and TNF-α concentrations did not show any statistical difference before and after treatment. According to these findings, to follow up the treatment process of calves with Cryptosporidiosis, it is thought that measuring the concentration of SAA, Hp, IL-1β and IL-6 will be useful for determining disease severity, selecting appropriate treatment, following treatment efficacy and determining subclinical diseases.

Keywords: Haptoglobin, interleukin 1, interleukin 6, serum amyloid A, tumor necrosis factor-α

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INTRODUCTION

Cryptosporidiosis causes severe diarrhea in humans and farm animals (Cho et al., 2013; Kotloff et al. 2013). Cryptosporidium species commonly occur in ruminants and include *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium ryanae* and *Cryptosporidium andersoni* (Ryan et al., 2014). Among these, *C. parvum* is zoonotic and leads to *Cryptosporidium* infection in humans (Chalmers & Giles, 2010; Taylan-Özkan et al., 2016; Xiao 2010). Suler et al., (2016) reported that gastrointestinal diseases with diarrhea are highly common in calves, and the major cause is *Cryptosporidium*, which is transmitted to humans through the fecal–oral route. Physiological responses to infections and injuries, including the onset of episodes that will emerge as inflammation and systemic response, are also known as acute phase reactions (APR). These changes, occurring in a remote location from the inflammation area, are also characterized by fever, leukocytosis, and qualitative and quantitative modifications of a specific group of proteins, excluding structural proteins, functioning in blood and other body fluids. These proteins are called acute phase proteins (APPs) and are promising biomarkers in veterinary medicine (Ceciliani et al., 2012; Zhang et al., 2019).

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Proteomic research on serum/plasma trailing natural or experimental infections found that APPs underwent significant changes and were found in very high levels in these fluids in a wide variety of ways (Ceciliani et al., 2012; Zhang et al., 2019). Today, APPs are routinely used for the diagnosis and prognosis of many diseases (Eckersall & Bell, 2010; Petersen et al., 2004; Zhang et al., 2019). APPs have been demonstrated to have a different level of importance for each animal species; however, due to lack of sufficient research in this area, these proteins have not been fully utilized in the field of veterinary medicine (Eckersall & Bell, 2010; Gruys et al., 2005). SAA is used to determine the activity and prevalence of inflammatory cases, to distinguish inflammatory diseases from non-inflammatory diseases, to monitor the course of diseases and to evaluate the success of the treatment applied (Petersen et al., 2004; Sack, 2018). In addition, it has been reported that high C-reactive protein (CRP) CRP and SAA levels can be considered indicators of latent infection or malignancy even in the absence of fever and neutrophilia (Ceciliani et al., 2012; Eckersall & Bell 2010; Germolec et al., 2018; Sack, 2018).

Proinflammatory cytokines such as IL-6, TNF-α and IL-1β are the main mediators for APPs 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 can also be released from other cells as a result of internal or external stimuli. IL-1β is produced by activated monocytes and macrophages. TNF-α is a polypeptide released from macrophages stimulated by lipopolysaccharides (Murata et al., 2004). It is defined as an effective biologically active mediator or primary cytokine in response to gram-negative bacterial septicemia or endotoxemia. IL-6 can be synthesized from the liver's Kupffer cells, from keratinocytes, from hypophysis or from mucosal epithelia. In the event of inflammation, infection or tissue damage, the release of cytokines is stimulated by the cells that organize the defence. Hence, the synthesis of APPs is also stimulated (Murata et al., 2004; Nukina et al., 2001; Slaats et al., 2016; Yoshioka et al., 2002).

In this study, it was aimed to determine the concentrations of SAA, Hp, IL-1β and IL-6 and TNF-α before and after treatment in calves with Cryptosporidiosis. It also aims to reveal the changes in acute phase proteins (APPs) and cytokines of the applied treatment protocol.

MATERIALS AND METHODS

Calves aged between 1-30 days with clinical complaints of diarrhea constitute the study. Agent isolation was performed with rapid test kits in calves with diarrhea complaints. Cryptosporidium positive animals were included in the study routine clinical examinations were performed on calves with the disease to eliminate other diseases. Care was taken that the calves included in the study had never been treated before. Thus, the interaction of different treatment protocols and their effect on values were eliminated. Calves constituting the patient group were selected to be included in the study at the latest two days after the onset of symptoms. Calves with signs of disease for three days or more were not included in the study. For the treatment of Cryptosporidium, 100 µg halofuginone base/kg ca/day was applied to the calves. The current study was performed Afyon Kocatepe University, Türkiye after the approval of the Local Ethics Committee of Faculty of Veterinary Medicine under approval No: AKÜHADYEK-121-16, on 08.11.2016.

Detecting Cryptosporidium by clinical examination and rapid test kits

In the study, stool samples were taken from calves with diarrhea using a measuring spoon; the sample was diluted with the liquid mixture in the test kit (BIO-K 313 rapid test kit), and then the test kit was soaked in the mixture for 10

minutes. A total of 10 calves diagnosed with *Cryptosporidium* were included in the study.

Treatment procedure

The calves with Cryptosporidiosis diarrhea were treated with 100 µg halofuginon base/kg ca/day administered orally for 7 days. In the pre- and post-treatment stages, blood samples were collected from the jugular vein in tubes without anticoagulant and transferred to EDTA tubes for plasma and hematological measurements.

Hematological procedure

For hematological examination, blood samples were measured for white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB) and hematocrit (HCT) (Compteur Analyseur d'Hematologie MS9-3).

SAA, Hp, IL-1, IL-6 and TNF-α measurements

Anticoagulant-free blood samples taken for biochemical parameters were centrifuged at 5000 rpm for 5 min at room temperature. Serums were stored at –20°C until measurement. In the serums obtained from the blood samples, the concentrations of SAA (Tridelta Development LTD, Ireland), Hp (Life Diagnostics Inc. Bovine Haptoglobin Test Kit), IL-1β (Cusabio Biotech Co. LTD, China), IL-6 (Cusabio Biotech Co. LTD, China), TNF-α (Cusabio Biotech Co. LTD, China) were measured via ELISA reader.

Statistical analysis

After applying ANOVA using SPSS software for Windows (version 18.0) in an electronic environment, the statistical difference was determined via the Tukey's test and the significance of the difference between pre- and post-treatment was determined via Duncan's test. Differences were considered significant when p values were less than 0.05; table values are given as mean ± standard error.

RESULTS

SAA and Hp concentrations in calves with Cryptosporidiosis presenting with diarrhea were higher in the pre-treatment stage at a statistically significant level ($P<0.05$) in comparison to the post-treatment stage (Table 1). IL-1β and IL-6 levels were higher in pre-treatment compared to post-treatment ($P<0.05$), TNF-α concentrations were not found to be different before and after treatment in calves (Table 1).

WBC and HGB concentrations in calves with Cryptosporidiosis presenting with diarrhea showed statistically significant differences ($P<0.05$) before and after treatment. RBC and HCT values were lower in the pre-treatment stage than in the post-treatment stage (Table 2).

Table 1. SAA, Hp, IL-1β, IL-6, TNF-α concentrations (mean ± SE) in pre- and post-treatment stages

Parameters	Pre-treatment	Post-treatment	P value
SAA (mg/L)	2.43 ± 1.11	1.69 ± 0.83	0.045
Hp (µg/mL)	1.01 ± 0.52	0.09 ± 0.02	<0.001
IL-1 (pg/mL)	2.83 ± 0.42	2.38 ± 0.34	0.030
IL-6 (pg/mL)	3.89 ± 2.15	3.07 ± 2.67	0.019
TNF-α (ng/mL)	1.01 ± 0.14	1.03 ± 0.18	0.597

SAA: Serum Amyloid A; Hp: Haptoglobulin; IL-1: Interleukin 1; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor- α

Table 2. WBC, RBC, HGB, HCT concentrations (mean ± SE) in pre- and post-treatment stages.

Parameters	Pre-treatment	Post-treatment	P value
WBC x10^9/L	13.97 ± 3.77	9.91 ± 1.04	0.007
RBC x10^{12}/L	7.02 ± 0.87	7.70 ± 1.23	0.139
HGB g/dL	8.81 ± 1.07	9.98 ± 1.25	0.033
HCT %	27.54 ± 3.72	30.08 ± 4.05	0.636

WBC: White Blood Cell; RBC: Red Blood Cell; HGB: Hemoglobin; HCT: Hematocrit

DISCUSSION

Cryptosporidium is an obligate intracellular protozoan parasite that infects a wide variety of vertebrate hosts including humans and poses a significant threat to public health (Bouzid et al., 2013; Santin, 2020). *Cryptosporidium* infections are noted as an important problem for animal health, mostly in newborn livestock, and lead to economic losses associated with reduced growth rate and increased mortality in infected animals. Moreover, Cryptosporidiosis increases the costs of animal health maintenance and veterinary services (Santin, 2020). Our study was conducted on calves and pre- and post-treatment stage APPs and cytokines, and several hematological parameters were monitored in calves with Cryptosporidiosis presenting with diarrhea. Thus, while determining the effect of Cryptosporidiosis on APPs and cytokines in calves, it was possible to evaluate the efficacy of the applied treatment through the changes in APPs and cytokines.

SAA and Hp concentrations in calves with Cryptosporidiosis presenting with diarrhea were higher in the pre-treatment stage at a statistically significant level ($P<0.05$) in comparison to the post-treatment stage (Table 1). In their study of calves, Kabu et al. (2016) found that SAA concentrations in calves with enteritis were higher than in the control group. In some studies, SAA and Hp concentrations were reported to have remained within normal ranges in experimentally created bacterial and aseptic infections (Abdallah et al., 2016; Chae et al., 2019; Horadagoda et al., 1999). In addition, SAA and Hp concentrations have been reported to increase after natural or experimentally induced infection or inflammation in cattle (Abdallah et al., 2016; Alsemgeest et al., 1994; Fisher et al., 2001; Heegard et al., 2000). Alsemgeest et al., (1993) and Nazifi et al., (2008) reported that they could not detect Hp in the blood of healthy cattle; however, Hp levels were found to be high during inflammatory infections (enteritis, pneumonia, pleuropneumonia, peritonitis, traumatic reticuloperitonitis, endocarditis, abscess, abomasal ulcer, trauma, endometritis, myocarditis, digestive tract diseases). Ganheim et al., (2007) experimentally administered endotoxin lipopolysaccharide intravenously to calves and reported that serum Hp concentrations were higher than in healthy calves after administration. In their study, Albayrak & Kabu, (2016) reported that there was a statistical increase in serum Hp concentration in calves with enteritis compared to the control group. In our study, serum Hp concentration in calves with Cryptosporidiosis presenting with diarrhea was higher in the pre-treatment stage and was statistically significant ($P<0.05$) compared to the post-treatment stage (Table 1). Similar to the studies reporting that SAA and Hp concentration increased during infectious and inflammatory diseases (Abdallah et al., 2016; Alsemgeest et al., 1994; Risalde et al., 2011; Skinner & Roberts 1994), in our study SAA and Hp levels were also noted to be high in calves with enteritis presenting with diarrhea. It is known that Hp and SAA are the major APPs in ruminants and circulating concentrations of these APPs have generally been associated with severity of inflammation and degree of tissue damage (Ceciliani et al., 2012; Murata et al.,

2004). Therefore, measuring serum Hp and SAA concentrations in ruminants can provide diagnostic and prognostic information and evaluation of the response to the trigger event (Ceciliani et al., 2012; Gruys et al., 2005; Iliev & Georgieva, 2019; Murata et al., 2004; Tothova et al., 2014). In their study of lambs, Carroll et al., (2009) reported that with Cryptosporidiosis, SAA and Hp concentrations were high; there was a correlation between oocyte excretion and Hp; however, that was not the case for SAA. Al-Zubaidi, (2015) reported higher SAA and Hp values in calves with Cryptosporidiosis than in healthy ones. The fact that the Cryptosporidiosis agent damages the intestinal lumen and causes microvillus atrophy and mononuclear cell infiltration in the lamina propria has been suggested as the reason APPs increase (Al-Zubaidi, 2015). In our study, serum SAA and Hp concentration were high in the pre-treatment stage, and these values demonstrated a tendency to decrease during treatment, which signalled the cellular response to treatment efficacy.

IL-1β, IL-6 and TNF-α are known to play a key role in APRs (Ceciliani et al., 2012). It has been reported that they initiate the production of APPs by activating and modifying hepatocyte receptors. IL-6 is reported to be the most important of the cytokines that mediate the hepatocytic secretion of APPs (Heinrich et al., 1998). Furthermore, it has been reported that the synthesis of APPs from liver cells is initiated by pro-inflammatory cytokines (TNFα, IL-1β and IL-6) released from monocytes and macrophages during inflammation (Baumann & Gauldie, 1994).

In the presented study, while IL-1β and IL-6 levels were higher in pre- compared to post-treatment ($P<0.05$), TNF-α concentrations were not found to be different before and after treatment in calves (Table 1). In several studies of calves with diarrhea that were diagnosed with enteritis, IL-1β, IL-6 and TNF-α concentrations were found to be statistically significantly higher ($P<0.001$) in comparison to the control group (Albayrak & Kabu, 2016; Kabu et al., 2016). In another study, calves were experimentally administered intravenous endotoxin; following 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). Other studies have demonstrated that IL-1β and TNF-α concentrations were higher in calves with diarrhea than in control groups (Risalde et al., 2011). Al-Zubaidi, (2015) reported no difference in TNF-α concentrations between calves with Cryptosporidiosis and healthy ones. In our study, whereas serum IL-1β and IL-6 and their concentrations were higher in the pre-treatment stage than the post-treatment stage, TNF-α concentrations did not present a difference. Our study results are consistent with the literature.

WBC and HGB concentrations in calves with Cryptosporidiosis presenting with diarrhea showed statistically significant differences ($P<0.05$) before and after) treatment. RBC and HCT values were lower in the pre-treatment stage than in the post-treatment stage (Table 2). WBC count has been reported to increase significantly in calves with diarrhea, and leukocytosis caused by the relative increase of neutrophil granulocytes occurs as a result of the body's reaction to gastrointestinal infection (Merk Manual, 2013). In our study, the increase in WBC values pre-treatment can be explained as such. In calves with diarrhea, the HCT value would be high due to plasma fluid loss; however, RBC and HGB values may increase relatively. In our study, HGB values were found to be higher in the post-treatment stage than in the pre-treatment stage, and it was concluded that this situation could be associated with excessive fluid loss. Although changes in WBC, RBC, HGB, HCT values were detected in calves with diarrhea due to Cryptosporidiosis, all

hematological findings were within the reference ranges (Aiello, 2016; Brun-Hansen et al., 2006).

CONCLUSION

In light of these findings, it is thought that measuring the concentrations of SAA, Hp, IL-1β and IL-6 routinely would be beneficial for determining infection, for choosing the most appropriate treatment and for monitoring the efficacy of the selected treatment. In addition, it would be useful for detecting animals that show no clinical symptoms and have a subclinical course during herd health screening in terms of veterinary medicine. Further research is needed to determine the SAA, Hp, IL-1β, IL-6 and TNF-α concentrations for the diagnosis of viral, bacterial, parasitic, and other diseases in animals and to better understand the efficiency of this parameter in the control of treatment protocols.

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REFERENCES

Abdallah, A., Hewson, J., Francoz, D., Selim, H., & Buczinski, S. (2016). Systematic review of the diagnostic accuracy of haptoglobin, serum amyloid A, and fibrinogen versus clinical reference standards for the diagnosis of bovine respiratory disease. *Journal of Veterinary Internal Medicine*, *30*(4), 1356-1368. https://doi.org/10.1111/jvim.13975

Aiello, S.E. (2016). The Merck Veterinary Manual, 11th Edition, Merck&Co., Inc., New Jersey.

Albayrak, H., & Kabu, M. (2016). Determining serum haptoglobin and cytokine concentrations in diarrheic calves. *Fırat Universitesi Sağlık Bilimleri Veteriner Dergisi*, *30*(2), 113-117.

Alsemgeest, S.P.M., Kalsbeek, H.C., Wensing, T.H., Koeman, J.P., Ederen, A.M., & Gruys, E. (1994). Concentrations of serum amyloid A (SAA) and haptoglobin (Hp) as parameters of inflammatory disease in cattle. *Veterinary Quarterly*, *16*(1), 21-23. https://doi.org/10.1080/01652176.1994.9694410

Alsemgeest, S.P.M., Taverne, M.A.M., Boosman, R., Van Der Weyden, B.C., & Gruys E. (1993). Peripartum acute-phase protein serum amyloid- A concentration in plasma of cows and fetuses. *American Journal of Veterinary Research*, *54*, 164-167.

Al-Zubaidi, S. (2015). Acute phase proteins in calves naturally infected with cryptosporidium. *AL-Qadisiya Journal of Veterinary Medicine Sciences. 14*(2), 19-22.

Baumann, H., & Gauldie, J. (1994). The acute phase response. *Immunol Today*, *15*, 74-80. https://doi.org/10.1016/0167-5699(94)90137-6

Bouzid, M., Hunter, P.R. Chalmers RM, & Tyler KM. (2013). Cryptosporidium pathogenicity and virulence. *Clinical Microbiology Reviews*, *26*(1), 115-34. https://doi.org/10.1128/cmr.00076-12

Brun-Hansen, H.C., Kampen, A.H., & Lund, A. (2006). Hematologic values in calves during the first 6 months of life. *Veterinary Clinical Pathology*, *35*(2), 182-187. https://doi.org/10.1111/j.1939-165X.2006.tb00111.x

Carroll, J.A., Arthington, J.D., & Chase, C.C. (2009). Early weaning alters the acute-phase reaction to an endotoxin challenge in beef calves. *Journal of Animal Science*, *87*, 4167-4172. https://doi.org/10.2527/jas.2009-2016

Ceciliani, F., Ceron, J.J., Eckersall, P.D., & Sauerweın, H. (2012). Acute phase proteins in ruminant. *Journal of Proteomics*, *75*(14), 4207-31. https://doi.org/10.1016/j.jprot.2012.04.004

Chae, J.B., Park, J., Jung, S.H., Kang, J.H., Chae, J.S., & Choi, K.S. (2019). Acute phase response in bovine coronavirus positive post-weaned calves with diarrhea. *Acta Veterinaria Scandinavica*, *61*, 36. https://doi.org/10.1186/s13028-019-0471-3

Chalmers, R.M., & Giles, M. (2010). Zoonotic cryptosporidiosis in the UK - challenges for control. *Journal of Applied Microbiology*, *109*, 1487-1497. https://doi.org/10.1111/j.1365-2672.2010.04764.x

Cho, Y.I., Han, J.I., Wang, C., Cooper, V., Schwartz, K., Engelken, T., & Yoon, K.J. (2013). Case-control study of microbiological etiology associated with calf diarrhea. *Veterinary Microbiology*, *166*, 375-385. https://doi.org/10.1016/j.vetmic.2013.07.001

Eckersall, P.D., & Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Veterinary Journal*, *185*, 23-27. https://doi.org/10.1016/j.tvjl.2010.04.009

Fisher, A.D., Knight, T.W., Cosgrove, G.P., & Death, A.F. (2001). Effects of surgical or banding castration on stres responses and behaviour of bulls. *Australian Veterinary Journal*, *79*, 279-84. https://doi.org/10.1111/j.1751-0813.2001.tb11981.x

Ganheim, C., Alenius, S., & Waller, K. (2007). Acute phase proteins as indicators of calf herd health. *Veterinary Journal*, *173*(3), 645-651. https://doi.org/10.1016/j.tvjl.2006.01.011

Germolec, D.R., Shipkowski, K.A., Frawley, R.P. & Evans E. **(2018).** Markers of ınflammation. *Methods in Molecular Biology*, *1803*, 57-79. https://doi.org/10.1007/978-1-4939-8549-45

Gruys, E., Toussaint, M.J.M., Niewold, T.A., & Koopmans, S.J. (2005). Acute phase reaction and acute phase proteins. *Journal of Zhejiang University Science*, *6B*(11), 1045-1056. https://doi.org/10.1016/j.tvjl.2006.01.011

Heegard, P.M.H., Godson, D.L., Toussaint, M.J.M., Tjornehoj, K., Larsen, L.E., Viuff B., & Ronsholt, L. (2000). The acute phase response of haptoglobin and Serum Amyloid A in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Veterinary Immunology and Immunopathology*, *77*, 151-159. https://doi.org/10.1016/S0165-2427(00)00226-9

Heinrich, P.C., Behrmann, I., Muller-Neven, G., Schaper, F., & Graeve, L. (1998). Interlukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *The Biochemical Journal*. *334*, 297-314. https://doi.org/10.1042/bj3340297

Horadagoda, N.U., Knox, K.G., Gibbs, H.A., Reid, S.W.J., Horadagoda, A., Edwards S.E.R., & Eckersall, P.D. (1999). Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *The Veterinary Record*, *144*, 437-441. https://doi.org/10.1136/vr.144.16.437

Iliev, P.T., & Georgieva, T.M. (2019). Acute phase biomarkers of diseases in small ruminants: an overview. *Bulgarian Journal of Veterinary Medicine*, *22*(1), 1-12. https://doi.org/10.15547/bjvm.1051

Kabu, M., Elitok, B., & Küçükkurt, İ. (2016). Detection of serum amyloid-A concentration in the calf clinically diagnosed with pneumonia, enteritis and pneumoenteritis. *Scielo Brazil Journal*. *46*(2), 293-299. https://doi.org/10.1590/0103-8478cr20150571

Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S., Wu, Y., Sow, S.O., Sur, D., & Levine MM. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. *Lancet*, *382*, 209-222. https://doi.org/10.1016/ S0140-6736(13)60844-2

Murata, H., Shımada, N., & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis. *The Veterinary Journal*, *168*, 28-40. https://doi.org/10.1016/S1090-0233(03)00119-9

Nazifi, S., Rezakhani, A., Koohimoghadam, M., Ansari-Lari, M., & Esmailnezhad, Z. (2008). Evaluation of serum haptoglobin in clinically healthy cattle and cattle with inflammatory diseases in shiraz, a tropical area in Southern Iran. *Bulgarian Journal of Veterinary Medicine*, *11*(2), 95−101.

Nukina, H., Sudo, N., Aiba, Y., Oyama, N., Koga, Y., & Kubo, C. (2001). Restraint stress elevates the plasma interleukin-6 levels in germ-free mice. *Journal of Neuroimmunology*, *115*(1-2), 46-52. https://doi.org/10.1016/S0165-5728(01)00260-0

Özkan, T.A., Yasa, D.S., Usluca, S., Lysen, C., Ye, J., Roellig, D.M., Feng, Y., & Xiao L. (2016). Cryptosporidium species and Cryptosporidium parvum subtypes in dairy calves and goat kids reared under traditional farming systems in Turkey. *Experimental parasitology*, *170*, 16-20. https://doi.org/10.1016/j.exppara.2016.06.014

Petersen, H.H., Neılsen, J.P., & Heegard, P.M.H. (2004). Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research*, *35*, 163-187. https://doi.org/10.1051/vetres:2004002

Risalde, M.A., Molinaa, V., Sánchez-Cordóna, P.J., Pedreraa, M., Panadero, R., Romero-Palomoa, F., & Gómez-Villamandos, J.C. (2011). Response of proinflammatory and anti-inflammatory cytokines in calves with subclinical bovine viral diarrhea challenged with bovine herpesvirus-1. *Veterinary Immunology and Immunopathology*, *144*, 135–143. https://doi.org/10.1016/j.vetimm.2011.07.022

Ryan, U., Fayer, R., & Xiao, L. (2014). Cryptosporidium species in humans and animals: current understanding and research needs. *Parasitology*, *141*(13), 1667-1685. https://doi.org/10.1017/S0031182014001085

Sack, Jr.G.H. (2018). Serum amyloid A - a review. *Molecular Medicine*. *24*, 46. https://doi.org/10.1186/s10020-018-0047-0

Santin M. (2020). Cryptosporidium and Giardia in ruminants. *The Veterinary Clinics of North America Food Animal Practice*, 36(1), 223-238.

Shaw, H.J., Innesa, E.A., Morrison, L.J., Katzera, F., & Wellsa, B. (2020). Long-term production effects of clinical cryptosporidiosis in neonatal calves. *International Journal for Parasitology*, *50* (5), 371-376. https://doi.org/10.1016/j.ijpara.2020.03.002

Skinner, J.G., & Roberts, L. (1994). Haptoglobin as an indicator of infection in sheep. *The Veterinary Record*, *134*, 33- 36. https://doi.org/10.1136/vr.134.2.33

Slaats, J., Oever, J., Veerdonk, F.L., & Netea, N.G. (2016). IL-1β/IL-6/CRP and IL-18/ferritin: Distinct inflammatory programs in infections. *PLoS Pathogens*. *12*(12), 1-13. https://doi.org/10.1371/journal.ppat.1005973

Suler, D., Mullins, D., Rudge, T., & Ashurst, J. (2016). Cryptosporidium parvum ınfection following contact with livestock. *North American Journal of Medical Sciences*, *8*(7), 323-325.

Tothova, C., Nagy, O., & Kovac, G. (2014). Serum proteins and their diagnostic utility in veterinary medicine: a review. *Veterinární Medicína*, *61*, 475-496. https://doi.org/10.17221/19/2016-VETMED

Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: an update. *Experimental Parasitology*, *124*, 80-89. https://doi.org/10.1016/j.exppara.2009.03.018

Yoshioka, M., Watanabe, A., Shimada, N., Murata, H., Yokomizo, Y., & Nakajima, Y. (2002). Regulation of haptoglobin secretion by recombinant bovine cytokines in primary cultured bovine hepatocytes. *Domestic Animal Endocrinology*, *23*(3), 425–433. https://doi.org/10.1016/S0739-7240(02)00174-1

Zhang, Y., Zhang, J., Sheng, H., Li, H., & Wang, R. (2019). Acute phase reactant serum amyloid A in inflammation and other diseases. *Advances in Clinical Chemistry*, *90*, 25-80. https://doi.org/10.1016/bs.acc.2019.01.002