Efficacy of Paromomycin Sulphate for the Treatment of Cryptosporidiosis in Goat Kids

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Abstract

Background: This study aimed to evaluate the therapeutic efficacy of paromomycin sulphate on serum amyloid A (SAA) and haptoglobin (Hp) in Cryptosporidiosis in goat kids.

Methods: In the study, stool samples were taken from the kids and Cryptosporidium was detected via the rapid test kit. Then, by examining the same stool samples microscopically, we included 9 kids in the study and 50 mg/kg of paromomycin sulphate was administered to the kids in which Cryptosporidium spp. was detected for five days as a treatment. For the SAA and Hp estimation, blood samples were drawn from the kids in the pre and post treatment stages.

Result: In the kids with Cryptosporidium, the concentrations of SAA and Hp were measured as (SAA; 17.10±4.18 ng/mL Hp; 2.68±1.08 µg/mL) in pre-treatment and as (SAA; 9.71±4.13 ng/mL Hp; 1.23±0.64 µg/mL) in the post-treatment stage (p<0.05). In accordance with these findings, we suggest that routine measurements of SAA and Hp concentrations of the goat kids with diarrhea due to cryptosporidiosis could be of value in follow up of treatment, determining the severity of the infection, selection of therapy and monitoring the efficacy of the preferred treatment protocol and in detection of subclinical infections.

Key words: Cryptosporidiosis, Goat kids, Haptoglobin, Serum amyloid A.

Introduction

Neonatal Diarrhea Syndrome is a common issue in sheep and goat herds and Cryptosporidiosis, which also contributes to that syndrome, has been reported to be one of the reasons of the enteric disease in humans and farm animals (Kabu et al., 2023; Feng and Xiao, 2017; Giadinis et al., 2012). Among the Cryptosporidium species, Cryptosporidium parvum, C. ubiquitum and C. xiaoai are the dominant species in the sheep and goats. C. parvum is one of the most pathogenic species while, C. ubiquitum is less common in regions where C. parvum is endemic. C. xiaoai has a narrower host range owing to its non-pathogenic character and nature (Guo et al., 2021). Besides this, some of Cryptosporidium isolates are zoonotic (Giadinis et al., 2015; Cacciò et al., 2013). Due to the high mortality and morbidity rate associated with its infection, this protozoan parasite has been reported to cause economic losses (Giadinis et al., 2015). It has also been reported that Cryptosporidium transmits both directly and indirectly via fecal-oral path; direct transmission occurs when fecal oocysts are swallowed whereas indirect transmission involves the consumption of oocysts contaminated food and water (Santin, 2020). It has been stated that Cryptosporidium spp. has a significant impact on the health of 4-15 days old kids; although the animals have a normal appetite at the beginning of the infection, they may die after developing diarrhea, dehydration and electrolyte imbalance (Giadinis et al., 2015; Giadinis et al., 2012). Moreover, it has been asserted that when other enteropathogens become more complicated with Cryptosporidium infection, the course of infection gets shorter, ending in death within 2 to 3 days (Wright and Coop, 2007).

Acute phase response (APR) is defined as a non-specific and complex reaction which occurs shortly after the tissue damage caused by various conditions such as infection, trauma, neoplasia, inflammation and stress (Criscitelli et al., 2019). APR has been reported to include countless symptoms like edema, erythema and fever, which develop due to a complex interaction between cytokines, chemokines and acute phase proteins (APPs), leukocytosis, increased secretion of various hormones along with the regulation of plasma protein synthesis (Criscitelli et al., 2019; Cray, 2012). It has been ascertained that although APPs are mainly synthesized by hepatocytes that are stimulated by cytokines or endogenous glucocorticoids, they can be produced by other tissues as well (Criscitelli et al., 2019; Ceciliani et al., 2012; Cray, 2012). In response to bacterial and parasitic infections, the host may also cause acute phase response, which causes many systemic effects.
such as stimulation of fever, cachexies and acute phase proteins (Ngure et al., 1997). In ruminants, Hp and SAA are seen as the main source of acute phase response in a positive direction (Lecchi et al., 2012). It has been reported that SAA, which is a major APP, has three distinct aspects including the binding of cholesterol, immunomodulator activity and opsonization while Hp is an indicator of chaperone activity owing to binding free hemoglobin, anti-inflammatory characteristic, bacteriostatic impact and its role in angiogenesis (Cray, 2012). It has been reported that SAA and Hp concentrations in goats increased in helminth infections, pregnancy toxemia, pneumonia toxemia (Albay et al., 2023), SAA and Hp concentrations were found high in lamb on pneumonic goats that SAA concentration was higher in pneumonic group than the healthy goats. Kabu et al. (2021). Demirbaş and Kabu (2021) determined in their study on pneumonic goats that SAA concentration was higher in the pneumonic group than the healthy goats. Kabu et al., (2023), SAA and Hp concentrations were found high in lamb with diarrhea due to Cryptosporidium. Additionally, SAA and Hp concentrations were found high in goats with pregnancy toxemia (Albay et al., 2014). In a study on the impacts of temperature and transport stress on SAA and Hp concentrations in goats, SAA and Hp concentrations were

The purpose of this study was to determine the changes caused by cryptosporidiosis in SAA and Hp concentrations in neonatal goat kids after treatment with paromomycin sulphate and to evaluate the therapeutic efficacy of the treatment.

**MATERIALS AND METHODS**

In order to determine the changes in SAA and Hp values in goat kids in the pre and post treatment stages, nine 3-15 days old goat kids with clinical diarrhea were included in the present study. In fresh stool samples taken from diarrhea kids, Cryptosporidium spp. was detected by using antigen-sensitive fast test kits (Rota-Corona-E. Coli- Cryptosporidium) (Bio-X Diagnostics S.A. RoheFort/Belgium). The stool samples in which Cryptosporidium spp. was detected by using test kits in the field were transported to the laboratory within the same day. To detect the oocysts of Cryptosporidium spp., stool samples were stained by using Kinyoun Acid Fast staining method (Turgay, 2011). Stained samples were examined under Olympus CX31 trinocular research microscope at magnifications of 10x and 40x. The oocysts detected in the samples were photographed by using the Olympus LC30 digital camera system (Fig 1).

Blood samples (5 ml) taken from vena jugularis of kids with diarrhea drawn in vacuum with clot activator serum tubes before treatment were centrifuged at room temperature at 5000 rpm and serum was collected. For treatment of cryptosporidiosis 50 mg/kg of paromomycin sulphate (Paraför, Huvepharma®) was administered orally for five days. After the treatment, the blood samples taken from the jugular veins of the kids into vacuum tubes with clot activator were centrifuged at 5000 rpm at room temperature to obtain serum. Serum samples were stored at -20°C until the measurement. Serum Amyloid A (Cusabio Biontech CO., LTD. China) and Haptoglobin (Cusabio Biontech CO., LTD. China) were estimated using made in commercial sandwich ELISA kits.

For statistical analysis, the data was analysed with Wilcoxon test and the pre and posttreatment stage SAA and Hp values of the kids were compared. The significance level was set as p<0.05.

**RESULTS AND DISCUSSION**

In our study, SAA concentrations in goat kids with Cryptosporidium spp. infection were determined in pre (17.10±4.18 ng/mL) and post (9.71±4.13 ng/mL) treatment stages. In the light of these results, the comparison of SAA measurements of kids revealed a statistically significant difference (p<0.05) between pre-treatment values and post-treatment (9.71±4.13 ng/mL). SAA value was much higher in pre-treatment stage than in post-treatment phase (Table 1).

Similarly, the comparison of Hp concentration revealed values as in pre-treatment (2.68±1.08 µg/mL) and in post-treatment (1.23±0.64 µg/mL). According to these findings, there was a statistically significant difference between the pre- and post-treatment stages Hp value was higher in pre-treatment than in the post-treatment phase. When the estimated values are considered, it is obvious that the applied treatment apparently and positively affected both SAA and Hp concentrations, which indirectly proves the efficacy of the treatment (Table 1).

Although there are many studies on the values of APPs in Neonatal Diarrhoea Syndrome in calves and lambs, but no literature is available on the level APPs in goat kids affected with Neonatal Diarrhoea Syndrome (Kabu et al., 2023; Kabu and Uyarlar, 2022; Dinler et al., 2017; Albayrak and Kabu, 2016). Thus, results of our study indicate that APPs can be used as a potential diagnostic tool in veterinary medicine. Showing a faster response to an experimental inflammatory stimulus than other APPs in goats, SAA can be a major APP (increase up to 22 folds) indicating an acute condition (González et al., 2008). In a study on goats naturally infected with paratuberculosis, SAA concentration was reported to increase (Sevgisunar and Şahinduran, 2021). Demirbas and Kabu (2021) determined in their study on pneumatic goats that SAA concentration was higher in the pneumonic group than the healthy goats. Kabu et al., (2023), SAA and Hp concentrations were found high in lamb with diarrhea due to Cryptosporidium. Additionally, SAA and Hp concentrations were found high in goats with pregnancy toxemia (Albay et al., 2014). In a study on the impacts of temperature and transport stress on SAA and Hp concentrations in goats, SAA and Hp concentrations were

![Fig 1: Cryptosporidium spp. oocyst in faecal sample stained by using Kinyoun acid fast staining method.](image-url)
Acute phase proteins have been used to effectively evaluate the diagnosis, treatment and control mechanisms of various diseases in veterinary as well as in human medicine. Unfortunately, there is only limited information available on acute phase response in small ruminants. The present study, Hp concentration was determined to be statistically higher in pretreatment than in posttreatment in neonatal kids with Cryptosporidium spp. (p<0.05). We are of the opinion that the decreased level of post treatment SAA concentration is indicative of the efficacy of the treatment.

CONCLUSION
Acute phase proteins have been used to effectively evaluate the diagnosis, treatment and control mechanisms of various diseases in veterinary as well as in human medicine. Unfortunately, there is only limited information available on acute phase response in small ruminants. The present study is of vital importance as it is the first study that provides firsthand information on acute phase response in small ruminants. The present study

ACKNOWLEDGEMENT
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Conflict of interest
The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES


Table 1: Pre and Post treatment concentrations of SAA and Hp in goat kids with Cryptosporidium spp.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA (ng/mL)</td>
<td>17.10±4.18*</td>
<td>9.71±4.13*</td>
<td>0.021</td>
</tr>
<tr>
<td>Hp (μg/mL)</td>
<td>2.68±1.08*</td>
<td>1.23±0.64*</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*p<0.05 **p<0.001 (Differences in pre and post treatment stages were indicated by lower cases (a and b).


