Evaluation of acute phase response in cattle with naturally infected bovine ephemeral fever virus

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Received June 21, 2023
Accepted June 18, 2024

Abstract

Bovine ephemeral fever is an arthropod-borne viral disease that primarily affects cattle and causes economic losses mainly due to the sudden decrease in milk yield. We aimed to reveal the biochemical reflection of the acute phase response by detecting the changes in serum acute phase proteins in cattle naturally infected with the bovine ephemeral fever virus. The material of this study consisted of 21 Simmental dairy cows (4–6 years old) naturally infected with bovine ephemeral fever virus (infected group) and 10 clinically and serologically healthy Simmental dairy cows (control group). The prevalence of the disease in suspected cattle was 52 per 100. It was determined that in infected cows levels of serum haptoglobin, serum amyloid A ($P < 0.001$), and ceruloplasmin increased significantly ($P < 0.05$), whereas levels of albumin decreased significantly ($P < 0.05$). It is thought that the acute phase proteins variation pattern for bovine ephemeral fever would be beneficial both in better understanding the pathogenesis of the disease and in determining the diagnosis and prognosis correctly.

Haptoglobulin, serum amyloid A, ceruloplasmin, albumin, bovine ephemeral fever

Bovine ephemeral fever, also known by various local identifications such as bovine enzootic fever, three-day fever, three-day sickness, bovine influenza, or stiffseitke, is a viral disease that mainly affects cattle (Walker and Klement 2015). Bovine ephemeral fever (BEF) is an arthropod-borne disease, the etiologic agent of which is the bovine ephemeral fever virus. Bovine ephemeral fever virus (BEFV) is a member of the genus *Ephemerovirus* within the family *Rhabdoviridae* and has been reported to be responsible for an increasing number of outbreaks/epidemics (Hou et al. 2018; Lee 2019). The characteristic clinical findings of the disease (which can also progress subclinically) include acute high fever, appetite loss, increased salivation, nasal and eye discharge, muscle stiffness and difficulty standing in the following period, decreased male fertility, and sudden decrease in milk production in cattle. The disease has been reported in subtropical and tropical areas of Africa, Australia, and Asia (Hou et al. 2018; Camkerten et al 2019; Abdullah et al. 2020). Even though BEF mortality is often less than 1%, it results in economic loss since dairy cow's milk production suddenly drops and beef cattle's condition deteriorates (Walker and Klement 2015; Lee 2019).

A variety of stimuli, infectious or non-infectious, such as bacterial or viral infections, trauma, neoplasia, surgery, stress, ongoing inflammatory processes, or chronic disease, result in disruption of homeostasis. A response develops in the early defence system whose main task is to eliminate agents that disrupt the animal's homeostasis. This non-specific systemic reaction is called the acute phase response (APR) (Cray et al. 2009). In response

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to pro-inflammatory cytokines originating from activated natural defense cells arriving at the damaged tissue, there is a change in the synthesis of some proteins produced by the liver and an integral part of APR, called acute phase proteins (APPs). Cytokines could be used as biomarkers for infection, inflammation, or trauma. However, after the stimulus, cytokines are removed from circulation within a short time, while APP concentrations remain changed for a long time (Vargová et al. 2018; Saco and Bassols 2023).

Acute phase proteins with increasing serum concentrations during the APR are positive while decreasing APPs are negative APPs (Saco and Bassols 2023). Depending on the dynamics and fold of the altered, positive APPs are classified as major (× 10–100 increase), moderate (× 2–10 increase), or minor (mild increase) (Cray et al. 2009; Trela et al. 2022). The patterns of change of APPs in serum are used as biomarkers in monitoring general health status, diagnosis, prognosis, and treatment strategies of diseases (Schrödl et al. 2016). The degree of variation of APP is different in livestock, domestic animals, and humans. Thus, this pattern is species-specific (Trela et al. 2022). In ruminants, haptoglobin (Hp), serum amyloid A (SAA), and ceruloplasmin (Cp) are positive APPs, while albumin (Alb) is a negative APP (Tothova et al. 2014).

To prevent economic losses in farm animals, it is an effective strategy to be able to quickly identify infections in their subclinical/clinical state and isolate infected animals from other animals or eliminate them from production. According to reports, APPs can be employed as a supportive diagnostic tool in ruminant viral infections (Reczyńska et al. 2018). Additionally, there is insufficient scientific data about APPs levels and APR of the BEF. Therefore, this study was aimed to investigate serum Hp, SAA, Cp, and Alb concentrations of naturally BEFV-infected cattle.

Materials and Methods

Ethical statement

The investigation was conducted with approval from the Harran University Local Animal Experiments Ethics Committee (HRU-HADYEK), under the authority of its permit number 2022/009/05.

Study design and population

The material of this study consisted of 21 Simmental dairy cows (4–6 years old) naturally infected with BEFV (infected group) and 10 clinically and serologically healthy Simmental dairy cows (control group). Samples were collected in August and October 2020 from individual cattle keepers in Şanlıurfa province of Türkiye. Bovine ephemeral fever virus was detected with the aid of reverse transcription-polymerase chain reaction (RT-PCR) in 21 of the samples taken from 40 suspected cows. The RT- PCR evaluation was also performed for the selection of the control group; cows that were confirmed both clinically and by PCR as uninfected, constituted the control group.

Blood sampling and laboratory analyses

Blood samples were collected from v. jugularis using tubes without anticoagulant for the assays of serum APPs. After that, all blood samples were centrifuged for 10 min at 2,000 × *g* and stored at −20 °C until analysis. Serum Hp concentrations were determined using Skinner et al. (1991) procedures, while serum Cp concentrations were determined using the spectrophotometric technique developed by Colombo and Ricterich (1964). A solid-phase sandwich commercial ELISA kit (Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland) was used by following the manufacturer's instructions to determine the level of SAA in serum. Serum Alb concentrations were measured using commercial kits (Biolabo®, Maizy, France) (Epoch, BioTek, Winooski, USA) by the colorimetric method.

For viral nucleic acid isolation, blood samples were taken into tubes with ethylenediaamine tetra acetic acid (EDTA) and centrifuged in a cooling centrifuge at +4 °C, at 8,000 × *g* for 10 min. Then, the leukocyte layer was transferred into sterile tubes containing 2 ml phosphate buffer solution (PBS, pH: 7.4; Invitrogen, Eugene, OR, USA) with the use of a Pasteur pipette and was centrifuged again. The leukocytes were stored at −20 °C until used in the study.

RNA extraction and RT-PCR for the G protein gene

Following the manufacturer's instructions, a high pure viral nucleic acid kit (Roche Diagnostic, Mannheim, Germany) was used to extract BEFV RNAs from clinical specimens. Before RT-PCR analysis, purified virus RNAs were kept at −80 °C. A High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Waltham,

MA, USA) was used to synthesize the cDNA. To detect the BEFV partial G gene (800 base pairs [bp]), PCR was carried out using the primers 420F (5' AGA GCT TGG TGT GAA TAC 3') and 420R (5' CCA ACC TAC AGC AGA TA 3'). The PCR reactions were done using an initial denaturation at 94 °C for 5', followed by 35 cycles at 94 °C for 40'', at 46 °C for 60'', at 72 °C for 40'', and a final extension at 72 °C for 10' (Zheng and Qiu 2012). After agarose gel (1%) electrophoresis, the visual of the amplified PCR products under UV light in the UPV GelSolo system (Analytik Jena, Jena, Germany) is presented in Fig. 1 (Plate I).

Statistical analyses

All analyses were performed using the Minitab program (2010). Levene's test for variance equality assumption and Shapiro-Wilk test for normality assumption was used to statistically assess the data. Independent sample *t*-test was used to compare Hp, SAA, Cp, and Alb levels between the control and infected groups. Pearson correlation analysis was applied to examine the relationships between measures within both groups. Data were presented as minimum, maximum, mean ± standard deviation (SD). Significance was calculated at $P < 0.05$ in all tests.

Results

In BEFV-infected cows, high fever (≥ 39.5 °C), anorexia, severe salivation, serous nasal and ocular discharge, lameness, muscle tremors, and a sudden decrease in milk yield of infected dairy cows were observed. Bovine ephemeral fever virus was identified in 21 out of 40 samples in suspected cows with the RT-PCR. Results of PCR identifications revealed 52% cows to be BEFV positive.

The mean serum concentrations of Hp, SAA, Cp, and Alb in control group and BEFV-infected cows are presented in Table 1 and Fig. 2 (Plate I). Accordingly, it was observed that the infected cow's serum levels of Hp, SAA, and Cp were significantly higher than those of the control group. The mean serum Alb concentrations of the infected and healthy cows were detected as 2.963 ± 0.496 and 3.491 ± 0.493 g/dl, respectively. The infected cow serum Alb concentrations were lower than those measured in the healthy cows, and this difference was significant ($P < 0.05$).

Table 1. The serum haptoglobin, serum amyloid A, ceruloplasmin, and albumin concentrations of healthy and bovine ephemeral fever virus infected cattle.

APPs	Groups	n	Minimum	Maximum	Mean ± SD	*P*
Hp (g/l)	Control	10	0.07	0.14	0.10 ± 0.02[a]	***
	Infected	21	0.28	0.97	0.48 ± 0.20[b]	
SAA (µg/ml)	Control	10	3.94	7.72	5.68 ± 1.15[a]	***
	Infected	21	22.65	88.79	42.65 ± 16.97[b]	
Cp (mg/dl)	Control	21	3.17	9.68	6.76 ± 2.24[a]	*
	Infected	10	3.07	18.2	9.83 ± 4.41[b]	
Alb (g/dl)	Control	10	2.61	4.21	3.49 ± 0.49[a]	*
	Infected	21	2.11	3.72	2.96 ± 0.49[b]	

[a-b] Different letters in the same row indicate significant differences (***: $P < 0.001$, *: $P < 0.05$).

APPs - Acute phase proteins; Hp - haptoglobin; SAA - serum amyloid A; Cp - ceruloplasmin; Alb - albumin; SD - standard deviation

Figure 3 (Plate II) represents the Pearson correlations coefficient between Hp, SAA, Cp, and Alb concentrations of control (a) and BEFV-infected cows. A correlation was detected between Hp with Alb (r = 0.66) ($P < 0.05$) and a negative correlation between SAA with Cp (r = −0.662) ($P < 0.05$) concentrations of control group samples. There was no significant correlation between APP concentrations in infected group samples.

Discussion

Outbreaks of BEF have been recorded in various countries around the world (Hsieh et al. 2005; Tonbak et al. 2013; Zheng and Qiu 2012). Although the morbidity and mortality rates of the disease differ according to these reports, high morbidity and low mortality are generally reported as the characteristic attribute of the disease (Walker and Klement 2015). It could be said that the most important effect of BEF on livestock is economic because it has been reported that while in affected dairy cattle lactation is interrupted, in beef cattle condition loss occurs (Walker and Klement 2015; Lee 2019).

In the current study, in consistence with the literature, clinical examination of naturally infected cattle with BEFV revealed high fever, anorexia, excessive salivation, serous nasal and ocular discharge, lameness, shivering, and a decrease in milk yield (Camkerten et al. 2019; Abdullah et al. 2020). The prevalence of BEF disease in our study was 52%. The prevalence of infection reported in previous studies was mostly higher compared to our results (Zaher and Ahmed 2011; Finlaison et al. 2014; Abdullah et al. 2020). The diversity of the methods and the geological-climatic conditions of the regions may cause differences.

Serum Hp, SAA, Cp, and Alb, which can be evaluated as a biochemical reflection of the APR, are part of the innate immune system that occurs with contact with pathogens such as viruses or in other conditions unrelated to pathogens (Gómez-Laguna et al. 2011). These proteins represent a heterogeneous group of APR-related polypeptides and proteins. The roles, modes of action, and structures of APPs in the organism can differ significantly (Eckersall and Bell 2010). These proteins are regarded as biomarkers in cattle due to the variations in APP concentration that occur with different diseases. APPs are helpful, particularly in the early detection of clinical-subclinical diseases or changes in health status, in determining treatment strategies, despite the lack of specific APP information linked to any one disease (Trela et al. 2022).

For effective infection prevention and treatment, it is crucial to comprehend how an organism fights a pathogen. Since APPs are one of the primary immune defense mechanisms, it is critical to comprehend how they act. In most widespread ruminant viral diseases, increased blood levels of Hp and SAA have been observed (Reczyńska et al. 2018). In our study, a significant increase was observed in serum Hp and SAA levels of the infected group compared to the control group. It was determined that the increase in serum Hp in infected cows was × 4 higher than in the control group, and the increase in SAA was more than 8-fold. Both these APPs are considered major APP in cattle. However, the stage of the inflammatory process against the pathogen may cause a difference in the increase of the coefficient. The fact that the SAA increase coefficient is higher than the Hp increase coefficient in infected cows suggests that SAA may be more sensitive than Hp in the evaluation of APR in cattle. In the literature review, scarce information was achieved about APPs levels in cattle infected with BEFV. According to the evaluations of a study performed by Nazifi et al. (2010) on five BEFV-infected cattle, serum Hp and SAA concentrations were reported as 0.33 g/l and 26.51 μg/ml, respectively. Bovine respiratory disease, bovine viral diarrhoea, and foot and mouth disease are among the common viral diseases of cattle. In studies performed in cattle infected with these diseases, serum Hp and SAA concentrations have been reported as 8–10 mg/ml and 60–80 μg/ml, respectively, for bovine respiratory disease (Heegaard et al. 2000), 0.89–1.87 g/l and 77.7–375 mg/ml, respectively, for bovine viral diarrhoea (Ganheim et al. 2003), and 0.308–0.41 g/l and 28.8–45.44 μg/ml, respectively, for foot and mouth disease (Nazifi et al. 2012; Merhan et al. 2017). These findings show that the concentration of APP can vary between infections and between phases of the same infection, making it significant to understand not only which proteins change but also at what concentrations.

Ceruloplasmin is a metalloprotein that binds up to 95% of the copper ions in blood serum and is mostly produced in the liver. Ceruloplasmin facilitates the oxidation of Fe^{2+} to Fe^{3+} and is also in charge of reducing oxygen substrates without generating H_2O_2 or free oxide radicals. In addition, Cp indicates oxidative activity against organic complexes. Cp, which is evaluated as a moderate APP in cattle, makes a significant contribution to plasma antioxidant capacity (Healy and Tipton 2007; Reczyńska et al. 2018; Camkerten et al. 2019). In this study, Cp which is both an actor of the APR and an extracellular antioxidant, increased approximately 1.5-fold in infected cows compared to the control group. Similar to our findings, a signifcant difference was reported between serum Cp concentrations of the control and infected cattle in a study by Camkerten et al. (2019) to determine the oxidant-antioxidant status of BEFV-infected cattle in Türkiye. Additionally, this increase is approximately 1.5-fold.

Albumin, one of the negative APPs whose concentration decreases during inflammatory processes, takes part in the transport of organic and inorganic substances. It also acts as an amino acid reservoir for Alb, positive APP synthesis. In a pathological process, Alb synthesis is reduced as amino acids are diverted to positive APP production instead of Alb (Ceciliani et al. 2012; Tothova et al. 2014). While positive APP levels increased in this study, the decrease in Alb concentrations could be considered a reflection of the APR.

In conclusion, it was determined that serum Hp, SAA, and Cp levels increased significantly, while Alb levels decreased in cowse that were suspected due to clinical signs and confirmed to be infected with BEFV by RT-PCR. The changing pattern of APPs in cattle infected with BEFV was revealed and information was obtained about the biochemical reflection of the APR caused by the disease. It was concluded that these data would contribute to the diagnosis of the disease and a better understanding of its pathogenesis.

Conflict of Interest

The authors declare they have no conflicts of interest.

Acknowledgements

The authors thank Dr. Hamza Yalçın for his contribution to the statistical analysis.

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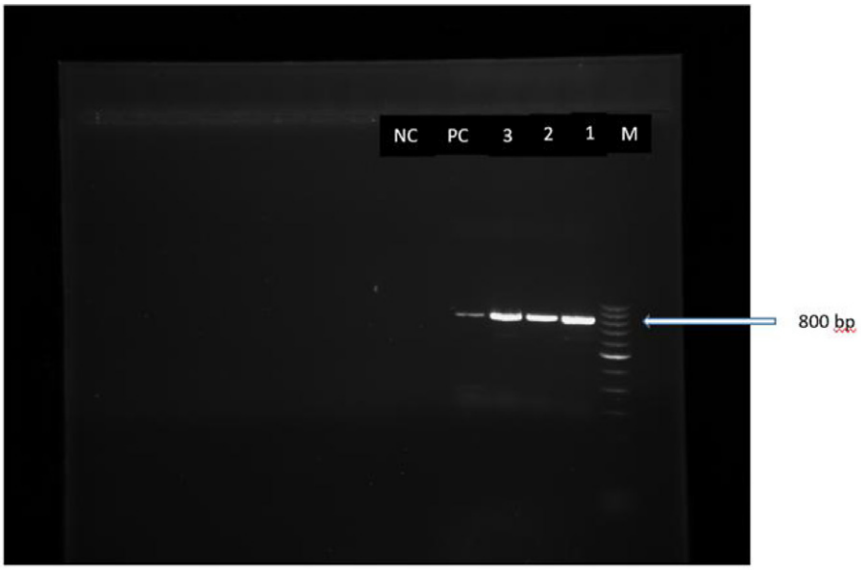

Fig.1. Reverse transcription-polymerase chain reaction gel image of bovine ephemeral fever virus. Lane M: 100 bp DNA ladder. Lane 1-3: (+) polymerase chain reaction products, negative control (NC), positive control (PC) (800 bp)

NC - negative control; PC - positive control

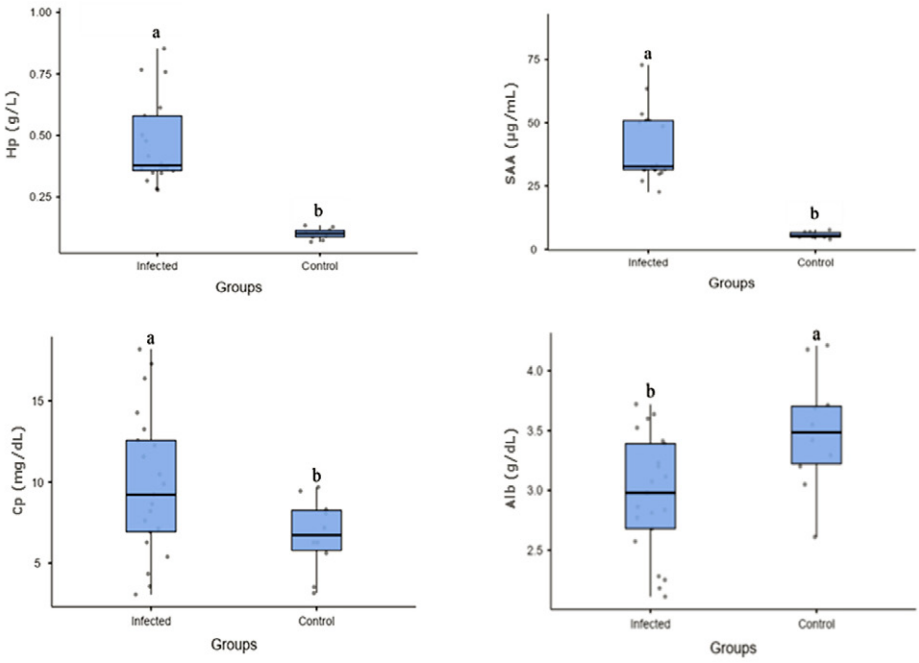

Fig. 2. Box plot of the bovine ephemeral fever virus infected and control group's haptoglobin, serum amyloid A, ceruloplasmin, and albumin concentrations.

Hp - haptoglobin; SAA - serum amyloid A; Cp - ceruloplasmin; Alb - albumin

Plate II

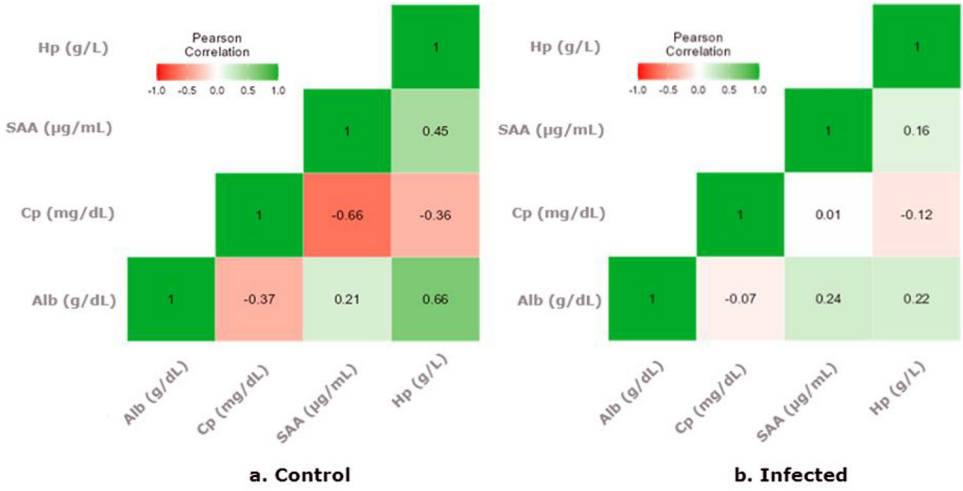

Fig. 3. Pearson correlation heatmap between serum concentrations of haptoglobin, serum amyloid A, ceruloplasmin, and albumin in (a) control, and (b) bovine ephemeral fever virus infected group.

Hp - haptoglobin; SAA - serum amyloid A; Cp - ceruloplasmin; Alb - albumin