JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Clinical and paraclinical changes in experimental colisepticemia in neonatal calves

Mohammad Reza Mokhber Dezfoulie[1], Samad Lotfollahzadeh[1*], Masoomeh Heidari[2], Gholamreza Nikbakht Boroujeni[3]

1.Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran**. 2.**Iran Veterinary Organization (IVO), Tehran, Iran **. 3.** Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Samad Lotfollahzadeh ORCID: 0000-0002-6549-7922.

Research Article

Volume: 3, Issue: 2
August 2019
Pages: 49-56

ABSTRACT

The present study aim was to assess changes in clinical signs and plasma cytokines in calves experimentally infected with *Escherchia coli* and use them as a tool to diagnose colisepticemia. Ten healthy Holstein-Frisian bull calves were used for this experimental study. Experimental colisepticemia was induced in calves with intravenous injection of 1.5 × 109 CFU of O111:H8 strain of E.coli. Clinical scores were recorded before induction of septicemia, every 30 minutes for 8 hours, every hour from 8 till 12 h, every 3 hours from 12 till 24 hours after bacterial challenge. Blood samples were collected to determine plasma concentration of tumor necrosis factor-α (TNF-α) and gamma interferon gamma (IFN-γ) before and after E.coli IV injection. Blood culture was performed before and after bacterial challenge to confirm colisepticemia. The present study showed that total clinical score of the calves increased with a simultaneous significant rise in plasma concentration of TNF-α and INF-γ during septicemia period (P< 0.05). Changes in the heart and respiratory rate during septicemia and using clinical scoring are not enough to assess the magnitude of infection and disease progress; therefore, it is recommended some laboratory tests be used for better evaluation of clinical status of the septic calves

Article History

Received: 24.05.2019

Accepted: 17.07.2019

Available online: 06.08.2019

Keywords: experimental colisepticemia, calf, clinical scoring, tumor necrosis factor-α, interferon gamma

DOI: 10.30704/http-www-jivs-net.569912

To cite this article: Dezfoulie, M. R. M., Lotfollahzadeh, S., Heidari, M., & Boroujeni, G. N. (2019). Clinical and paraclinical changes in experimental colisepticemia in neonatal calves. *Journal of Istanbul Veterinary Sciences. 3*(2), 49-56, Abbreviated Title: *J Ist Vet Sci*

Introduction

Septicemic colibacillosis is a highly fatal disease of calves less than 2 weeks of age and occurs mainly in newborn animals that are agammaglobulinemic due to not receiving enough colostrum on the first day of their life (Constable et al., 2017; Rezazadeh et al., 2004; Thomas et al., 2004) and inflicts significant loss on dairy farms. Calves are mostly infected from their early environment (Gay et al., 1994), during nursing attempts in heavily contaminated udder (Aldridge et al., 1993), and or through contact with infected feces (Rabbani et al., 2007). The disease is characterized by depression, weakness, listlessness, recumbency, poor response to external stimuli, cold extremities, and coma. Affected animals have a poor suckling reflex, an abnormal body temperature (low or high), cardiac dysfunction (tachycardia or bradycardia) and

*Corresponding Author: Samad Lotfollahzadeh1
E-mail: samadlzadeh@ut.ac.ir

Journal home page: www.jivs.net
http://dergipark.gov.tr/http-www-jivs-net

leukocyte abnormality. The sick animals will die due to hypovolemic shock (dehydration) and multiple organ insufficiencies soon after onset of clinical signs. Although bacteremia is the main cause of death in calves with colisepticemia, the early diagnosis is important for veterinarian and farm owners (Aldridge et al., 1993; Fecteau et al., 1997; Gay et al., 1994). Blood and postmortem tissue culture are sure methods to diagnose bacteremia but unfortunately this tool is time consuming, costly and needs technical skills all of which persuaded researchers to search for an easier method. Studies based on prospective and retrospective records developed a sepsis score to predict sepsis in human and equine neonate (Ballou et al., 2011; Brewer and Koterba, 1998; Fecteau et al., 1997). A clinical scoring based on the status of hydration, fecal consistency, scleral vessels appearance, general attitude and umbilical abnormality in calf to predict bacteremia with 75% sensitivity and 71% specificity was developed (Blackwell and Christman 1996). It has been shown that as well as changes of general status of calf during colisepticemia many inflammatory mediators such as tumor necrosis factor (TNF- α), interleukin-1 (IL-1), IL-6, alpha interferon (IFN- α), and eicosanoids are released in the blood (Annane and Bellissant, 2005). Adams et al. (1990) observed a rapid rise of TNF in the blood after IV administration of Escherichia coli lipopolysaccharide (LPS) (Adams et al., 1990). The blood elevation of TNF- α and IFN-ϒ after IV injection of LPS was also observed (Bieniek et al., 1998). Many deleterious and pathologic effects of LPS could be consequences of TNF- α release in the blood (Beutler et al., 1985), since TNF- α suppressors can inhibit some of the LPS effects such as fever (LeMay et al., 1990). The objective of this study was to use a clinical sepsis score in association with the serum TNF- α and IFN-ϒ to predict the status and early diagnosis of bacteremia in newborn calves.

Material and methods

Study design: Ten Holstein-Frisian bull calves between 8 and 10 days of age with body weight ranging from 53 to 60 kg were studied. The calves were fed colostrum (10% BW) within six hours of birth and housed in individual metal pens (1 m × 1.5 m × 1m) with a chaff coated floor and were fed twice daily with whole milk at the rate of 10% of their body weight per day divided into 2 feedings at 7:0 and 16:0. Water and starter (composed of: barley 40%, corn 20%, bran 19%, soybean meal 18%, mineral 1.5% and vitamin supplement 1.5%) were provided ad libitum. The calves' vital signs (temperature, heart and respiratory rate) and clinical scores were checked at the arrival day, 5 days after that and the day before start of experiment. The E.coli strain of O111:H8 was chosen in the present study because of its availability and being rapidly phagocytized, producing a robust oxidative burst (Hulbert et al., 2011). This strain was cultured and after incubation at 37°C for 24h, DNA was extracted in boiling method and the bacteria were checked for expressing Intimin (eae) and Shiga-like toxin (STX1 and STX2). Antibiogram test was performed for choosing an appropriate antibiotic to treat the septicemia. All experimental procedures followed the guidelines on ethical standards for experimental processes in animals according to a protocol approved by the Animal Ethics Committee, University of Tehran, Iran. To induce septicemia experimentally, a catheter was then inserted in the jugular vein, an extension set was attached to the catheter, and the catheter and extension set were secured to the calf's neck. A suspension of E-coli (1.5 × 109 CFU) in 5 ml isotonic saline was administered as a bolus. The challenge dose was prepared about 1 hour before the start of experiment and checked with spectrophotometer for its concentration. For ethical reasons, an untreated control group in this study was thought to be unnecessary. Therefore, all calves were treated with a suitable antibiotic selected by antibiogram. Treatment began 24h after bacteria administration with ceftazidime (ZACZIDIM 1g vial by DAANA Pharma Co) at dose of 10 mg/kg IV every 8h for 3 days.

Clinical assessment: The clinical score procedure was adapted from a system previously described for scoring sepsis in neonatal calves (Fecteau et al., 1997). Seven clinically assessed criteria including appetite, dehydration, fecal nature, behavior, shock, standing ability, and suckling reflex were considered (Table1). Rectal temperature, the heart and respiratory rate were also measured. All the findings in physical examination were individually recorded by the same observer in each calf. After injection of bacteria, clinical scores were assessed at 30 min intervals for 8 h, hourly till 12 h, then every 3 hours till 24h. Each of the seven scores was classified as 0, 1, 2, and 3 so that 0 is normal state and 3 is the worst condition (Table 1). Respiratory and heart rate was examined with auscultation of the trachea and heart region in one minute, respectively, by 2 persons. Rectal temperature was taken with a digital thermometer.

Laboratory examination: Blood samples were collected into 6-mL tubes containing EDTA for determination of plasma TNF-α and IFN-ϒ before (day -10, day -5, and day -1) and after (0, 2, 8, and 24 hour) challenge and centrifuged at 2000 g for 15 minutes. Four mL of peripheral blood was collected into a sterile syringe and injected to a two-phasic media and

Table1. Clinical scoring system for calf neonatal sepsis used in the study (29)

Parameter	Score	Criteria
Appetite	0	Normal
	1	Slightly decreased (<50%)
	2	Decreased (>50%)
	3	Anorexic
Dehydration	0	Normal hydration: a skin fold tented and twisted 90 ° for 1 sec. returns immediately to original position when released
	1	Slight (<5%): skin fold remains tented for up to 4 sec.
	2	Mild (5 to 10 %): skin fold remains tented for 4 to 8 sec.
	3	Severe(>10%): skin fold tented for more than 8 sec.
Nature of feces	0	Normal
	1	Semi-solid
	2	Liquid with solid particles
	3	Liquid
General behavior	0	Normal: calf is vigorous, alert, and responsive
	1	Dull: calf quiet, slow to respond, and/or move
	2	Depressed: calf is dull and markedly slow to respond
	3	Prostrated or coma
Shock	0	Absent
	1	Slight or early: dull, heart rate decreased
	2	Mild or advanced: weakness, pale/dry mucous membranes, oliguria, cold extremities
	3	Severe: weak rapid pulse, diminished heart sounds, coma
Ability to stand	0	Normal
	1	Able to stand but with difficulty
	2	Unable to stand without assistance
	3	Recumbent and unable to stand
Suckling reflex	0	Present
	3	Absent

Minimum possible score = 0, Maximum possible score = 21

incubated at 37°C for 24 h for septicemia confirmation. Then pure culture was provided to detect the isolated bacteria and serotyping was performed to confirm the E-coli strain O111:H8. The plasma concentration of IFN-Ɣ and TNF-α was measured with commercial kits (AbD-serotec kit, Bio-Rad Company, UK and Vet Set kit, Kingfisher Biotech INC, USA), respectively. Statistical analysis of data was performed using SPSS version 13.0. Ordinal data were expressed as median values and changes occurring in different times were analyzed by non-parametric Friedman test. Descriptive data were expressed as mean and standard deviation (SD). Illness procedure and changes in cytokines levels were done with repeated measures ANOVA and significance level was considered as P less than 0.05.

Results

Clinical examination performed on calves was normal before and at challenge time. Appetite, dehydration, behavior, and standing ability changed after septicemia induction and reached a peak at 3.5, 3, 3, 3 to 3.5h after challenge, respectively. Appetite in all calves during the adaptation period and at challenge time was normal (Score 0). As disease progressed, the calves became reluctant to eat and median score was 2 between 3 and 7.5 h post challenge. In 22% of calves (2 of 9), anorexia was observed (score 3) at 0.5h after challenge and in 33% (3 of 9) it appeared between 1 to 3.5h after challenge. Change proceeding in these times was significant ($P < 0.001$). Suckling reflex had median score 3 between 2.5 and 12h after challenge. Seventy-eight percent of calves (7 of 9) showed lack of suckling reflex at 3.5h after challenge. The changes were not significant ($P = 0.166$).

After colisepticemia induction and signs progression, feces became semisolid, and liquid with solid particles (score 2 and 3, respectively). The minimum fecal nature was score 2 (liquid with solid particles) and appeared in one calf between 2 and 6.5h and between 2.5 and 18h after challenge. Thirty-three percent of the calves had decrease in fecal nature from 12h after challenge to end of study, changing the score from 1 to 2. However, the changes were not significant ($P = 0.067$). Maximum dehydration score was 2 in 55% of the calves to 5h

after challenge. Twenty-two percent of calves from 0.5h after challenge had score 1, and 22% had no signs of dehydration. Also, 67% of the calves showed score 1 at hour 24. In the present study maximum score for dehydration was 2 and 78% of the calves had various degree of dehydration during study. Changes were significant in Freidman test ($P < 0.001$).

Median score for standing ability from first hour to end of the study was 1 and calves were able to stand, but with difficulty. Twenty-two percent of the calves showed score from 0.5 to 3.5h after challenge. Maximum weakness in standing appeared in 78% of the calves at 3 to 3.5h after challenge and changes proceedings were significant in Freidman test ($P < 0.001$). Thirty minutes after challenge, 44% of the calves showed behavioral changes. Maximum median score was 2 from 2.5h to 5h after challenge. Maximum score (score 3) for behavior was only observed in one calf (11%) at 3 and 3.5h. Change proceeding was significant ($P < 0.001$).

One out of 9 calves showed signs of a mild shock at 0.5h; on the other hand, 33% of the calves showed a score of 2 for shock at first hour after microbial challenge. Sixty-seven percent of calves had no signs of shock. Changes pertaining to shock were not significant ($P > 0.05$). Total score ranged from 0 to 21 (the dead calf) and its peak was observed at 3-3.5h after challenge. Median total score at 3 and 3.5h after challenge was 11. Maximum total score was 17 in one calf at 1 and 1.5h after challenge. Median total score was 5 at 24h. Changing of total score was significant ($P < 0.001$) and details are shown in Figure 1.

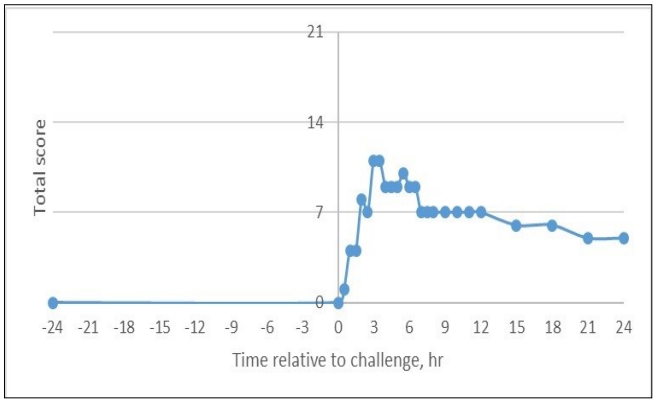

Figure 1. Effects of intravenous E.coli challenge dose on total score (Median).

Mean temperature during adaptation period and at the time of challenge was 38.6 and 38.7°C, respectively. Thirty minutes after colisepticemia, the temperature reached 39.2°C. In the current study, increase in body temperature after the challenge was significant at -6 h and maximum temperature was 39.7°C at hour six. In that time, 78% of calves had temperature more than 39.5°C. At the end of study, mean temperature was 39.5°C and 33% of the calves had rectal temperature more than 39.5°C. Maximum temperature recorded in this study was 40.9°C in one calf at hour 12 post challenge. In general, fever occurred in all the calves mostly between 3 and 12h after the challenge and showed reduction to normal after that (Figure 2). Repeated measures of ANOVA showed significant temperature changes ($P < 0.001$).

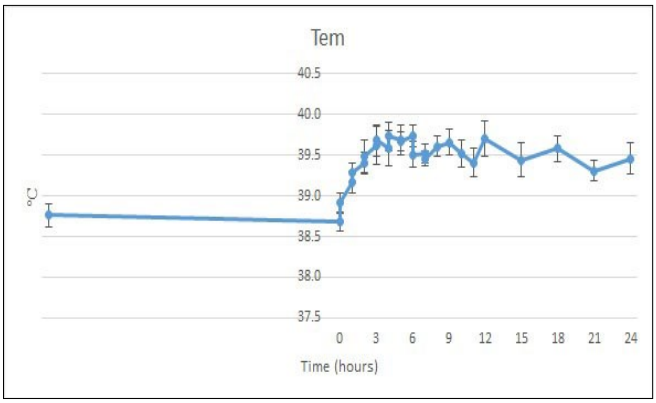

Figure 2. Effects of intravenous E.coli challenge dose on rectal temperature (Mean ± SE).

The highest and lowest mean heart rate was at 4h (116 bpm) and 1h (83 bpm) after challenge, respectively. Analysis of these changes showed significances ($P = 0.040$). Increasing respiratory rate was observed from challenge time to 2.5h later on and also at 6.5 and 12h after the challenge (Figure 3). Respiratory rate reached 48 rpm at 2.5h after challenge (respiratory rate before challenge was 27.4 rpm). In general, respiratory rate variations were significant ($P = 0.009$).

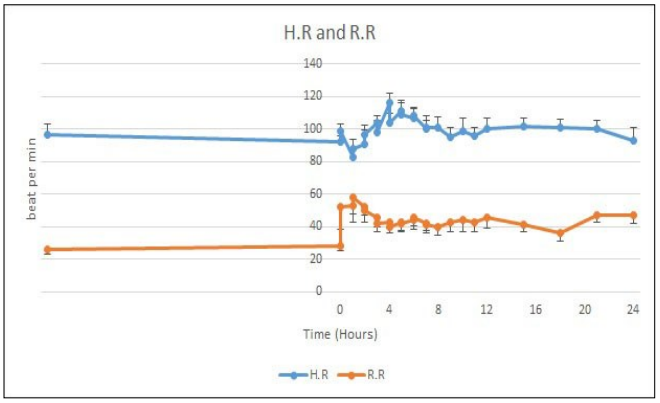

Figure 3. Effects of intravenous E.coli challenge dose on heart rate (H.R) and respiratory rate (R.R). (Mean ± SE).

Mean concentration of TNF-α increased 2h after the challenge from 484.9 to 682.6 pg/ml but no significant changes were observed after that. Mean plasma concentration of IFN-ϒ in adaptation period and 8 hours after the challenge time was 427.4 and 451.1 pg/ml respectively and reached pretreatment value after 24 hours. However, these changes were not significant (Table 2).

Table 2. Changes of plasma cytokines during experimental septicemia with E-coli O111:H8.

	TNFα (pg/ml)	IFN- ϒ (pg/ml)
Hour -24	487 ± 18	426 ± 18
Hour 0	449 ± 29	433 ± 16
Hour 2	598 ± 32	448 ± 32
Hour 8	487 ± 39	451 ± 33
Hour 24	521 ± 13	428 ± 18

The blood cultures for all calves at the time of challenge (0h) were negative, and at 8, 15, and 24h later were positive for E.coli. One of 10 calves showed severe signs of shock and died 25 minutes after the challenge. Bacterial culture of tissue samples of the dead calf included lung, liver, heart, spleen, intestine, cerebrospinal fluid, and brain was positive for E.coli. No bacteria were isolated from bone marrow culture. Microscopic and macroscopic pathological examination revealed septic shock as the cause of death. Serotyping of isolated bacteria from blood and necropsy confirmed their strain O111:H8. The mortality rate in present study was 10%.

Discussion

Two models for prediction and diagnosis of septicemia and bacteremia have been described by Fecteau et al. (1997) and Lofstedt (1999). These models are used on farm to assist in early and better diagnosis of sepsis. Clinical evaluation and experience are the major tools available for veterinarians to start treatment in a prompt but rational manner. An association between an objective clinical evaluation and bacteremia has been reported for bovine neonates, as it has for human infants and foals (Brewer and Koterba, 1988; Tollner 1982). Fecteau et al. (1997) suggested a model to predict bacteremia in ill calves based on clinical sepsis score (Fecteau et al., 1997). A clinical illness score system is commonly used in the clinical assessment of animals to measure the probability of a specific outcome (Amrine et al., 2013). In the present study, as disease progressed, the calves became reluctant to eat and median score for appetite was 2 at 3 to 7.5 h after the challenge. These findings are similar to Thomas et al. (2004) study which showed loss of appetite was a common sign in all septicemic calves and anorexia was reported in 38% of them (32 of 84). In another study on experimental endotoxemia in calves, severe inappetence was observed at 3h after endotoxin injection which is in accordance with our study (Gerros et al., 1993). Thomas et al. (2004) stated that lack of suckling reflex was observed in 54% of septicemic calves which is similar to our observations at 2.5, 4 to 5.5 and 8 to 12h after the challenge (Thomas et al., 2004). Gerros et al. (1993) reported maximum lack of the suckling reflex at 3h after E.coli LPS challenge. In another study, Lofstedt et al. (1999) reported that lack of suckling reflex or weak reflex in septic calves was 91.8% compared with non-septic calves (Lofstedt et al., 1999). In septicemic colibacillosis, diarrhea occurs in the final stages of disease in which the volume of feces increases, but it is not liquid excess (Fecteau et al., 2009). Thomas reported that 79% of septicemic calves had liquid feces which could be due to disease progression before the beginning of treatment (Thomas et al., 2004). The calves in the present study did not show significant fecal changes, which could be due to intravenous rout of infection. The best assessment of dehydration in calves is eyeball recession into the orbit and skin tent duration in the neck (normally < 2 sec). In the present study, score 2 was recorded as maximum score for dehydration and it was observed that, with this score 78% of the calves had various degree of dehydration. Using the same score, Thomas et al. (2004) reported 89% of calves were dehydrated and Lofstedt et al. (1999) stated 7.7% dehydration in 92% of septicemic calves.

Thirty minutes after the challenge, 44% of the calves showed behavioral changes and median score was 2 from 2.5h to 5h after challenge. Ballou et al. (2011) studied pathophysiological response in calves infused E.coli intravenously and stated that recumbency and lack of response to stimuli occurred in 67% of the calves at the time of the peak behavioral changes (Ballou et al., 2011). Thomas et al. (2004). also reported 83% incidence of depression in septic calves. In the present study, all calves had behavioral changes and 78% showed depression at 3 and 3.5h after challenge. In calves with experimental endotoxemia, maximum scores were observed in the first and second hours after endotoxin injection. Lofstedt et al. (1999) proposed a model to predict septicemia in calves with diarrhea and reported 68.8% depression, 29.9% coma, and 84.6% recumbency in septicemic calves (Lofstedt et al., 1999). Basoglu et al. (2004) studied the serum concentration of TNF-α in septic calves and reported depression in 70% of TNF-positive calves and 74% of TNF-negative calves similar to the present results. Basoglu et al. (2004), reported 30% of TNF-positive and 13% of TNF-negative calves were comatose (Basoglu et al., 2004). Smith and Halls (1968) reported rapid changes in behavior which lasted for 1-5h after disease induction in calves which received E.coli O78:K80

intravenously (Smith and Halls, 1968).

In our study, 67% of the calves had no signs of shock. However, Thomas et al (2004) reported shock in all calves using similar scoring system, but only 5% had marked signs. In the present study an increase in body temperature 3-6 h after the bacteria injection with maximum rise (39.7°C) at 6 hours after challenge occurred. In another study, Thomas showed 32% of septic calves were febrile (Thomas et al., 2004). Ballou et al. (2011) reported rise in body temperature in calves with experimental septicemia was 40.3 °C and 39.7°C 12 and 24 hours respectively after the challenge (Ballou et al., 2011). The calves in the present study had milder fever as compared with calves in the Ballou study. Mean rectal temperature of 39°C in 28 septic calves was also reported (Fecteau et al., 1997). Lofstedt et al (1999) stated mean rectal temperatures in septic calves and non-septic calves were 37.6 and 38.2°C, respectively. Hypothermia in calves in the Lofstedt study was not unexpected because only 29.5% of septic calves survived in their research. Mean temperatures of 37°C and 39.8°C, respectively were observed in TNF-positive and TNF-negative septic calves (Basoglu et al., 2004). Peripheral inflammation during septicemia and endotoxemia can directly stimulate central nervous system and affect the thermoregulatory center in the brain (Givalois et al., 1994). According to the literature, normal heart rate in calf is between 72 and 120 bpm (Constable et al., 2017). No significant changes in the heart rate of calves were observed after bacterial challenge in the present study. Fecteau (1997) reported mean heart rate in bacteremic and non-bacteremic calves in their study was 104.8 and 111.8 bpm, respectively (Fecteau et al., 1997). Kinsbergen and Bruckmaier (1994) observed increased heart rate in calves (145bpm) till 3 to 4h after endotoxin administration. Respiratory rate reached 48 rpm at 2.5h after the challenge. Mean respiratory rate of 58.2 and 57 rpm, respectively in bacteremic and non-bacteremic calves was reported. Basoglu et al (2004) stated that mean respiratory rate in TNF-positive and TNF-negative calves was 48 and 35 rpm, respectively. Kinsbergen and Bruckmaier(1994) observed an increased respiratory rate lasting for 3 to 4 hours in calves after IV endotoxin injection (Kinsbergen and Bruckmaier, 1994). Lofstedt (1999) reported 44rpm as a predictor of septicemia in diarrheic calves (Lofsted et al., 1999). In another study no relationship of the vital signs (heart rate, respiratory rate, and rectal temperature) as marker to differentiate bacteremic from non bacteremic calves was observed (Fecteau et al., 1997). The autonomic nervous system controls heart rate, body temperature, respiratory rate, and other physiological adjustments to maintain a stable internal environment. This system, as well as immune system, is influenced by inflammatory responses. Their main interaction is through hypothalamus-pituitary-adrenergic axis (Chen et al., 2011).

TNF-α is a primary mediator of inflammation, and has been implicated in a large number of infectious and non-infectious inflammatory diseases. TNF-α plays various roles in acute phase of inflammation induced by the negative gram bacteria. A significant increase in the TNF-α of calves blood was observed 2 hours after bacterial challenge in the current research. In a study using chronically awake instrumented sheep, infusion of TNF-α resulted in pulmonary hypertension, decreased lung compliance, hypoxemia, and increases in pulmonary micro vascular permeability (Blackwell and Christman, 1996). Carroll et al in 2009 studied the profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge and found that the mean serum concentration of IFN-ϒ and TNF-α before challenge was 27±13 pg/ml and 85±49 pg/ml, respectively. Basoglu (2004) stated that TNF-α in healthy calves was 234±115 pg/ml. Ballou et al. (2011) used various doses of E.coli in calves to induce septicemia and only calves with injection of 1.5 × 109 CFU and more showed acute increase in TNF-α concentration 2-3h after challenge. However, this increase was not statistically significant. The lower doses of bacteria used in that study could be the reason for no enhancement in cytokines in the plasma. In a study a close relationionship between severity of disease and serum concentration of TNF-α has been shown (Basoglu et al., 2004). In the present study, the highest level of TNF-α was measured in 2-6h post challenge concurrently associated with marked increase in body temperature (3-6h post challenge). It has also been shown that some other clinical criteria including weakness or lack of suckling reflex, recumbency, no response to stimuli and comatose attitude, and mortality rate could be observed in calves with higher TNF-α level (Carrol et al., 2009; Kinsbergen and Bruckmaier, 1994). E.coli endotoxin infused to healthy volunteers significantly increased TNF-α with a peak 1h after infusion which is in agreement with the results of present study in the calves (Michie et al., 1988).

It has been shown that IFN-ϒ increases in the blood during septic shock. In the current study IFN-ϒ reached its peak at 8h after bacterial challenge and returned to baseline within 24h and changes of plasma IFN-ϒ were not significant. Other researchers showed a rise in peripheral IFN-ϒ with a peak at 4h post bacterial challenge (Ballou et al., 2011; Carrol et al., 2009). Carroll et al. (2009) reported that intravenous LPS

administration in beef calves caused a peak of IFN-Ɣ after 3-4h, returning to base level within 12h.

In the present investigation 100 percent of the calves showed bacteremia and one of them died 25 minutes after the challenge. The mortality rate in the present study was 10%. Fecteau et al in two different experiments in 2011 and 1997 observed that 24 and 31 percent of severely ill calves were bacteremic and the mortality of blood culture positive calves was greater than 57.4% versus 15.1 % for blood culture negative calves. In another study, the same researchers reported that of the 51 bacterial isolates, 51% were Escherichia coli, 25.5% from other gram negative enteric, 5.9% gram negative anaerobes, 11.8% gram positive cocci and 5.9% gram positive rods (Fecteau et al., 2001). Although presumptive diagnosis can be made in a significant proportion of cases on the basis of clinical signs, particularly if quantified by a standardized clinical scoring system, blood culture remains the definitive diagnostic test for septicemia.

Conclusion

It should be considered that unnecessary treatment or treatment of every ill calf with systemic antimicrobials may result in unnecessary expenses, treatment side effects, drug residues, and increased resistance to antimicrobials. Prediction of sepsis allows for a more accurate prognosis and estimates the treatment costs required for a successful treatment.

In the present study, the results of clinical scoring, laboratory tests, and statistical analysis showed that the total score in the colisepticemic calves increased. However, scores of some criterion such as shock may have no marked increase. Also, it seems that the heart and respiratory rates may show insignificant changes and are not appropriate to assess the infection magnitude and disease progression. Using observation and various examinations as an appropriate clinical tool leads to early diagnosis, but has moderate sensitivity and ability to predict the disease procedures and prognosis. It seems that additional laboratory tests combined with clinical scores will increase sensitivity and specificity of scoring systems for application on farms.

Compliance with ethical standards: All experimental procedures performed in this study involving animals were followed the guidelines on ethical standards for experimental processes in animals according to a protocol approved by the Animal Ethics Committee, University of Tehran, Iran.

Author contribution: Conceptualization: Samad Lotfollahzadeh, Mohammad Reza Mokhber Dezfoulie, Data curation: Gholamreza Nikbakht Boroujeni, Masoomeh Heidari, Supervision: Samad Lotfollahzadeh, Gholamreza Nikbakht Boroujeni, Writing - original draft: Masoomeh Heidari, Writing - review & editing: Samad Lotfollahzadeh.

Conflict of interests: Author, Samad Lotfollahzadeh declares that he has no conflict of interest. Author, Author Mohammad Reza Mokhber Dezfouli declares that he has no conflict of interest. Author Masoomeh Heidari declares that she has no conflict of interest, Author Gholamreza Nikbakht Boroujeni declares that he has no conflict of interest.

References

Adams, J. L., Semrad, S. D.,& Czuprynski, C. J. (1990). Administration of bacterial lipopolysaccharide elicits circulating tumor necrosis factor-alpha in neonatal calves. *Journal of Clinical Microbiology, 28*(5), 998-1001.

Aldridge, B. M., Garry, F. B., & Adams, R. (1993). Neonatal septicemia in calves: 25 cases (1985-1990). *Journal of American Veterinary Medicine Association*, *203*(9), 1324-1329.

Amrine, D, E., White, B. J., Larson, R., Anderson D. E., Mosier D. A., & Cernicchiaro N. (2013). Precision and accuracy of clinical illness scores, compared with pulmonary consolidation scores, in Holstein calves with experimentally induced *Mycoplasma bovis* pneumonia. *Americam Journal of Veterinary Research, 74*(2), 310-315.

Annane, D., Bellissant, E., & Cavaillon, J. M. (2005). Septic shock. *Lancet, 365*(9453), 63-78.

Ballou M. A, Cobba C. J, Hulberta, L. E, & Carroll J. A. (2011). Effects of intravenous Escherichia coli dose on the pathophysiological response of colostrum-fed Jersey calves. *Veterinary Immunology and Immunopathology*, *141*(1-2), 76-83.

Basoglu, A., Sen, I., Sevinc, M., & Simsek, A. (2004). Serum Concentrations of Tumor Necrosis Factor-α in Neonatal Calves with Presumed Septicemia. *Journal of Veterinary Internal Medicine, 18*(2), 238-241.

Beutler, B., Greenwald, D., Hulmes, J. D., Chang, M., Pan, Y-C. E., Mathison, J., Ulevitch, R., & Cerami, A. (1985). Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature, 316* (6028), 552-554.

Bieniek, K., Szuster-Ciesielska, A., Kamin´ska, T., Kondracki, M., Witek, M., & Kandefer-Szerszeń, M. (1998). Tumor necrosis factor and interferon activity in the circulation of calves after repeated injection of low doses of lipopolysaccharide. *Veterinary Immunology and Immunopathology, 62*(4), 297-307.

Blackwell, T. S., &Christman, J. W. (1996). Sepsis and cytokines: current status. *British Journal of Anaesthiology, 77*(1), 110-117.

Brewer, B. D., & Koterba, A. M., (1988). Development of a scoring system for early diagnosis of equine neonatal sepsis. *Equine Veterinary Journal, 20*(1), 18-22.

Carroll, J. A., Reuter, R. R., Chase, C. C. Jr., Coleman, S. W., Riley, D. G., Spiers, D. E., Arthington, J. D., & Galyean, M. L. (2009). Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. *Innate Immunology, 15*(2), 81-89.

Chen, X., Yin, Y., & Zhang, J. (2011). Sepsis and immune response. *World Journal of Emergency Medicine, 2*(2), 88-92.

Constable, P. D., Hinchcliff, K. W., Done, S., & Gruenberg, W. (2017). *Veterinary Medicine, A textbook of the diseases of cattle, horses, sheep, pigs and goats. 11th ed*. Philadelphia, USA: W. B. Saunders Ltd.

Fecteau, G., Fairbrother, J. M., Higgins, R., Van Metre, D. C., Paré, J., Smith, B. P., Holmberg, C.A., & Jang, S. (2001). Virulence factors in *E-coli* isolated from blood of bacteremic neonatal calves. *Veterinary Microbiology, 78*(3), 241-249.

Fecteau, G., Pare, J., Van Metre, D. C., Smith, B. P., Holmberg, C. A., Guterbock, W., & Jang, S. (1997). Use of a clinical sepsis score for predicting bacteremia in neonatal dairy calves on a calf rearing farm. *Canadian Veterinary Journal, 38*(2), 101-104.

Fecteau, G., Smith, B. P., & George, L. W. (2009). Septicemia and Meningitis in the Newborn Calf. *Veterinary Clinics of North America: Food Animal Practice, 25*(1), 195-208.

Gay, C. C., & Besser, T. E. (1994). Escherichia coli septicaemia in calves. In C. L. Gyles, (Ed.). *Escherichia coli in domestic animals and humans*. Wallingford, USA: CAB International.

Gerros, T. C., Semrad, S. D., Proctor R. A., & LaBorde A. (1993). Effect of dose and method of administration of endotoxin on cell mediator release in neonatal calves. *American Journal of Veterinary Research, 54* (12), 2121-2127.

Givalois, L., Dornand, J., Mekaouche, M., Solier, M. D., Bristow, A.F., Ixart, G., Siaud, P., Assenmacher, I., & Barbanel, G. (1994). Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. American Journal of Physiology, 267(1), R164-R170.

Hulbert, L. E., Cobb, C. J., Carroll, J. A., & Ballou, M. A. (2011). Effects of changing feeding milk replacer from twice to once daily on Holstein calf innate immune responses before and after weaning. *Journal of Dairy Science, 94*(5), 2557-2565.

Kinsbergen, M., & Bruckmaier, R. M. (1994). Metabolic, endocrine and haematological responses to intravenous E.coli endotoxin administration in 1-week- old calves. *Zentralblatt für Veterinärmedizin . A, 41*(7), 530-547.

LeMay, L. G., Vander, A. J., & Kluger, M. J. (1990). The effects of pentoxifylline on lipopolysaccharide (LPS) fever, plasma interleukin 6 (IL 6), and tumor necrosis factor (TNF) in the rat. *Cytokine, 2*(4), 300-306.

Lofstedt, J., Dohoo, I. R., & Duizer, G. (1999). Model to predict septicemia in diarrheic calves. *Journal of Veterinary Internal Medicine, 13*(2), 81-88.

Michie, H. R., Manogue, K. R., Spriggs, D. R., Revhaug, A., O'Dwyer, S., Dinarello, C. A., Cerami, A., Wolff, S.M., & Wilmore, D. W. (1988). Detection of circulating tumor necrosis factor after endotoxin administration. *New England Journal of Medicine, 318*(23), 1481-1486.

Rabbani, M., Zahraie-Salehi, T., Dezfouli, M. R., Rezazadeh, F., Yoosefi Ramandi, F., & Bahonar, A. R. (2007). Detection of anti-E.coli, rotavirus and corona virus antibodies in sera samples of diarrheic and normal calves under 1 month of age. J*ournal of Veterinary Research, 62*(3), 145-149.

Rezazadeh, F., Zahraei-Salehi, T., Mokhber Dezfouli, M.R., Rabani, M., Morshedi, A., Khaki, Z., Nabian, S., Rahbari, S., & Bahonar, A. (2004). Clinincal, biochemical and microbiological findings of calves diarrhea in a dairy herd in Suburb of Tehran. *Journal of Veterinary Research, 59*(4), 301-308.

Smith, H. W., & Halls, S. (1968). The experimental infection of calves with bacteriaemia-producing strains of Escherichia coli: the influence of colostrum. *Journal of Medical Microbiology, 1*(1), 61-78.

Thomas, E., Roy, O., Skowronski, V., Zschiesche, E., Martin, G., & Böttner, A. (2004). Comparative field efficacy study between cefquinome and gentamicin in neonatal calves with clinical signs of septicaemia. *Revue Médicine Véterinair, 155*(10), 489-493.

Tollner, U. (1982). Early diagnosis of septicemia in the newborn. *European Journal of Pediatrics, 138*(4), 331-337.