World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:9, No:12, 2015

Clinical Signs of Neonatal Calves in Experimental Colisepticemia

Samad Lotfollahzadeh

Abstract*—Escherichia coli* (*E. coli*) is the most isolated bacteria from blood circulation of septicemic calves. Given the prevalence of septicemia in animals and its economic importance in veterinary practice, better understanding of changes in clinical signs following disease, may contribute to early detection of disorder. The present study has been carried out to detect changes of clinical signs in induced sepsis in calves with *E. coli*. Colisepticemia has been induced in 10 twenty-day old healthy Holstein- Frisian calves with intravenous injection of 1.5 X 10^9 colony forming units (cfu) of *O111:H8* strain of *E. coli*. Clinical signs including rectal temperature, heart rate, respiratory rate, shock, appetite, sucking reflex, feces consistency, general behavior, dehydration and standing ability were recorded in experimental calves during 24 hours after induction of colisepticemia. Blood culture was also carried out from calves four times during experiment. ANOVA with repeated measure is used to see changes of calves' clinical signs to experimental colisepticemia, and values of $P \leq 0.05$ was considered statistically significant. Mean values of rectal temperature and heart rate as well as median values of respiratory rate, appetite, suckling reflex, standing ability and feces consistency of experimental calves increased significantly during study ($P<0.05$). In the present study median value of shock score was not significantly increased in experimental calves ($P> 0.05$). The results of present study showed that total score of clinical signs in calves with experimental colisepticemia increased significantly, although score of some clinical signs such as shock did not change significantly.

***Keywords*—**Calves, Clinical signs scoring, *E. coli* O111:H8, Experimental colisepticemia,

I. Introduction

COLIBACILLOSIS occurs most commonly in newborn farm animals and is a significant cause of economic losses in raising livestock. It is a complex disease in which several different risk factors interact with certain pathogens, resulting in the disease. There are at least two different types of the disease: enteric colibacillosis is characterized by varying degrees of diarrhea, dehydration, acidosis, and death in a few days if not treated; coliform septicemia is characterized by severe illness and rapid death in several hours [1], [2]. Septicemic colibacillosis occurs in newborn animals that are agammaglobulinemic because they have not ingested sufficient colostrum early enough, or have absorbed insufficient colostral immunoglobulins, thus rendering them highly susceptible [2].

Many enteric pathogens can cause bacteremia and septicemia among dairy calves. Thirty-one percent of scouring calves were bacteremic and survival rates were low only 12% [2]. The common causes of calf septicemia are *E. coli* (*Escherichia coli*), and less frequently *Klebsiella*, *Salmonella*, other Gram-negative bacteria, and occasionally Gram-positive bacteria. It is thought that more than 50% of bacterial caused septicemia is *E. coli* [6]. In septicemia, the pathogen is present throughout the course of the disease and is directly responsible for initiation of the disease process. Sepsis manifests as localized infections without apparent systemic signs, or localized infections with signs of systemic illness without signs of localized infection [2].

Affected animals are depressed and weak, commonly recumbent, and dehydrated; tachycardia is present and, although the temperature may be high initially, it falls rapidly to subnormal levels when the calf becomes weak and moribund. The suckling reflex is weak or absent, the oral mucous membranes are dry and cool, and the capillary refill time may be prolonged. Cold extremities, weak peripheral pulse and prolonged capillary refill time and scleral injection are common. Diarrhea and dysentery may occur but are uncommon. In the absence of prompt treatment, this disease is rapidly progressive and commonly fatal.

In sepsis, a systemic inflammatory response to an infection occurs and leads to endothelial dysfunction, impairment of microcirculation, tissue hypoxia, apoptosis and finally multiple organ failure and death. Nowadays the role of inflammatory cytokines, oxidative stress and immune system in the pathogenesis of sepsis is obvious. Continuous elevated levels of various cytokines in severe sepsis could result in uncontrolled inflammation [3]. The systemic release of pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α) in response to bacterial derived immunogens causes vasodilation, increases vascular permeability and activates the clotting cascade all of which decrease perfusion of blood to many vital organs and can lead to irreversible organ damage and consequently death [4].

A clinical score intended to be used on the farm and a more complete scoring system intended to be used in patients on which ancillary tests is required. Given the prevalence of septicemia in animals and its economic importance in veterinary practice, better understanding of clinical signs and biochemical changes following disease, may contribute to early detection of disorder. The objection of present study was determining of accuracy of clinical score as a diagnostic tool to identify clinical signs of septicemia and blood cytokines levels in calves with experimental colisepticemia.

Samad Lotfollahzadeh is with the Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran (e-mail address: samadlzadeh@ut.ac.ir).

II. Materials and Methods

Ten Holstein-Frisian bull calves (10 ± 1 d of age) were acquired from a local commercial dairy in February 2014 and transported to the Veterinary Research and Teaching Hospital of the University of Tehran (VRTH). The calves were given colostrum (10% BW) within the first 6 h of life. The calves were selected on the basis of their serum total proteins and zinc sulfate turbidity test on second days of birth. Peripheral blood samples were collected and physical examination was carried out on the calves immediately after arrival. Animals were individually housed in stainless steel pens (1 m × 1.5 m × 1m) with a chaff coated floor and these boxes had as height as 20 cm of the floor. Air flow was supplied in air condition system and windows. Ambient temperature ranged from 15 to 20°C. the calves were fed twice daily with whole milk at 7:30 and 17:30, and starter concentrate (composed of : barley 40%, corn 20%, bran 19%, soybean meal 18%, mineral 1.5% and vitamin supplement 1.5%) provided ad libitum. The calf nursery was illuminated with fluorescent lighting 24 h a day. The adaptation period of calves in this situation lasted 10 days. The calves were evaluated for vital signs (heart rate, respiratory rate, and temperature) on entrance day (day -10), 5 days after entrance (day -5), and 1 day before beginning the study (day -1). As long as the calves maintained there, to keep the biosecurity, getting into by others except the responders was prevented. To perform any examination or sampling and handling calves, sanitary principles was considered. The *E. coli* O111:H8 strain was chosen in the present study because of its availability to us and it was shown to be rapidly phagocytized and produce a robust oxidative burst [5].

The displaying of sickness was based on clinical scores. Procedures described for sepsis scoring in neonatal calves, previously [4]. Seven clinically assessed criteria included appetite, dehydration, fecal consistency, behavior, shock, standing ability, and sucking reflex which are listed in Table I. Heart rate, respiratory rate and temperature were measured, too. All findings in physical examination, from before challenge (day -10, day -5, day -1), 0h and after bacteria injection till the end of the study was recorded individually by one observer as schedules for every calf. After bacteria injection, clinical scores (Table I) were assessed at 30 min intervals through first 8 h, hourly till 12 h, then every 3 hours till 24h after injection.

III. Results

Clinical signs of experimental calves before and at challenge time were in normal standard range defined for calves. Of clinical scores, appetite, dehydration, fecal nature, behavior, standing ability, and sucking reflex changed during the septicemia procedure and reached the peak at 3, 3, 1.5, 3, 1, and 3.5h after challenge.

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 appetite score during 2 to 7.5 h after challenge was 2. In 22% of calves (2 of 9), anorexia was observed (score 3) at 0.5h after challenge and in 33% (3 of 9) anorexia appeared between 1 to 3.5h after challenge. Change proceeding in 28 times was significant ($p<0.001$).

Median value of suckling reflex at 2.5 to 12h after challenge was 3. 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 colicepticemia induction and signs progression, feces was observed semisolid, and liquid with solid particles (score 2 and 3, respectively). The minimum fecal consistency score was 2 (liquid with solid particles) and appeared in one calf between 2 to 6.5h and between 2.5 to 18h after challenge. Thirty three percent of calves experienced decrease in fecal consistency from 12h after challenge to end of study. However, the changes were not significant ($p = 0.067$).

Maximum dehydration score and median was 2 that 55% of calves manifested 3 to 3.5h after challenge. Twenty two percent of calves from 0.5h after challenge, had score 1, and 22% had no signs of dehydration. In present study score 2 was recorded as maximum score for dehydration and 78% of calves had various degree of dehydration during study. Changes were significant in Freidman test ($p<0.001$).

Median score for standing ability from beginning of challenge to end of the study was 1 and which defined as calves abled to stand but with difficulty. Twenty two percent of calves showed score 3 from 0.5 to 3.5h after challenge. Maximum weakness in standing was appeared in 78% of calves at 3 to 3.5h after challenge and changes proceeding were significant in Freidman test ($p<0.001$).

Thirty minutes after challenge, 44% of calves showed behavioral changes. Maximum behavioral median score was 2 which is occurred from 2.5h to 5h after challenge. Change proceeding was significant ($p<0.001$).

One out of 9 experimental calves showed mild shock signs at 0.5h after challenge and 33% at first hour after challenge got score 2. Sixty seven percent of calves had no signs of shock.

Total score ranged from 0 to 21(the dead calf) and its 11. Maximum total score was 17 in one calf at 1 and peak was observed at 3-3.5 h after challenge. Median total score at 3 and 3.5h after challenge was 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 Fig 1.

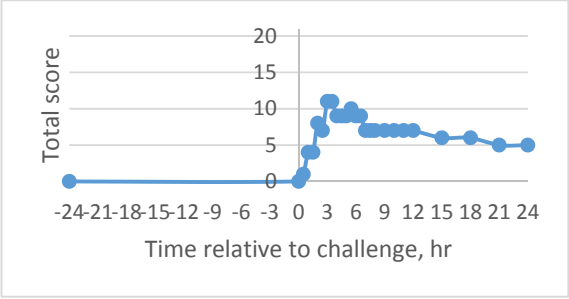

Fig. 1 Effects of intravenous *E.coli* challenge dose on total score (median)

Mean value of rectal temperature during adaptation period and at the time of challenge was 38.6 and 38.7°C, respectively. Just thirty minutes after E.coli injection, mean value of rectal temperature increased to 39.2°C. In the present study, increasing body temperature after challenge was marked at 3-6 h after challenge and maximum temperature was 39.7°C at 6h after challenge. In that time, 78% of calves had temperature more than 39.5°C. At the end of study, mean rectal temperature was 39.5 and 33% of calves had rectal temperature more than 39.5. Maximum temperature recorded in present study was 40.9°C which has been occurred in one calf at 12h after challenge. In general, fever occurred in all calves particularly between 3 to 12h after challenge. Rectal temperature decreased from 12h after challenge. Using ANOVA with repeated measures test showed significant changes in rectal temperature during different times in the study ($p< 0.001$).

The highest and lowest mean heart rate were at 4h (116bpm) and 1h (83bpm) after challenge, respectively and statistical analysis of heart rate changes revealed significant difference ($p = 0.040$). Increasing of respiratory rate was observed from challenge time to 2.5h later on and also at 6.5 and 12h after challenge. Respiratory rate reached to 48 rpm at 2.5 h after challenge. In general, respiratory rate changes were significant ($p = 0.009$).

TABLE I
CLINICAL SCORING SYSTEM FOR CALF NEONATAL SEPSIS USED IN THE

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 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		

IV. DISCUSSION

Two models for prediction and diagnosis of septicemia and bacteremia have been expressed by [6] and [7]. These models are used on farm and to assist for early and better diagnosis of sepsis. Clinical evaluation and experience are the major tools available for veterinarian to start treatment in a prompt but rational manner. An association between an objective clinical evaluation and bacteremia has been reported for the bovine neonate, as it has for human infants and foals. So, [6] suggested a model could be useful to predict the bacteremia in ill calves based on clinical sepsis score. A clinical illness score system is commonly used in the clinical assessment of animals and quantification or assignment of calves that correspond to the probability of a specific outcome. In present study, as disease progressed, the calves became reluctant to eat and median score was 2 between 3 to 7.5 h after challenge. These findings are similar to Thomas et al (2004) study that revealed inappetence was a common sign in all septicemic calves and reported anorexia in 38% of them (32 of 84) [8]. Another study showed in calves with experimental endotoxemia, most inappetence was at 3h after endotoxin injection. These results agreed with present study, regardless of scoring method in Gerros study (appetite and suckling reflex was assessed together). Thomas et al. stated lack of suckling reflex were in 54% of septicemic calves that agreed with present observation at hours of 2.5, 4 to 5.5 and 8 to 12h after challenge. In another study it is reported that maximum of lack of the suckling reflex occurred at 3h after *E.coli* LPS injection [8].

Thirty minutes after challenge, 44% of calves showed behavioral changes and median score was 2 from 2.5h to 5h after challenge. Ballou et al. studied pathophysiological response in calves infused *E.coli* intravenously, and stated recumbency and lack of response to stimuli occurred in 67% of calve at the time of the peak changes [4]. Also, Thomas et al. suggested 83% incidence of depression in septic calves. In present study, all of calves had behavioral changes and 78% showed depression at 3 and 3.5h after challenge [8]. In calves with experimental endotoxemia, maximum score was observed in first and second hours after endotoxin injection. Lofstedt suggested a model to predicting septicemia in diarrheic calves and reported 68.8% depression, 29.9% comatose, and 84.6% recumbency in septicemic calves [7]. In present study, 67% of calves had no signs of shock. But Thomas et al. in similar scoring system reported shock in all calves but only 5% had marked signs [8].

The total score ranged from 0 to 21(in dead calf) and its peak was observed at 3-3.5 h after challenge. Ballou et al. [4] achieved the calves which received O111:H8 (as suspension of 1.5×10^9 CFU) returned to normal condition, 32h after challenge and maximum was between 3 to 4 hours after challenge. In the study of Thomas et al (2004) [8], the mean of total score of 2 groups of septicemic calves under different treatment at beginning of study and first day was 12 and 5, respectively.

In present study, the results of clinical scoring, laboratory test and statistical analysis showed total score in colisepticemic calves increased. However, scores of some

criteria such as shock may have no change and no marked increase. Also, it seems heart rate and respiratory rate can be undergo easily and even in significant changes, and it's not appropriate to assess the infection magnitude and disease progress. In clinical scoring system, using observation and various examination as an appropriate clinical tool leads to early diagnosis, but have moderate sensitivity and ability to prediction the disease procedures and prognosis. It appears more laboratory tests associated with using clinical scores will provide more value for clinical scoring system to use in farm, and have sensitivity and specificity.

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