



## Some Clinicopathological Changes in Buffalo Calves Infected with Enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> Detected by Direct ELISA

Israa Abdulghani AL-ROBAIEE<sup>1a</sup>, Maab Ibrahim AL-FARWACHI<sup>1b</sup>✉

1. University of Mosul, College of Veterinary Medicine, Department of Internal and Preventive Medicine, Mosul, IRAQ.  
ORCID: 0000-0003-1983-2491<sup>a</sup>, 0000-0002-2288-6274<sup>b</sup>

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**Abstract:** The objective of recurrent study was to determine the some clinicopathological (hematological and biochemical) changes in native buffalo calves (aged between 1-15 day) infected with enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> detected by direct ELISA, during the period between September 2018- April 2019. A total of 40 fecal samples (30 samples from infected animal and 10 samples from clinically healthy Buffalo calves) was examined by direct ELISA. Six samples form diarrheic animals (20%) were positive for *E. coli* (K99). The blood gas analysis of blood samples revealed significant decrease in the means of the blood pH, Partial pressure of oxygen concentration (PO<sub>2</sub>), Partial pressure of carbon dioxide concentration (PCO<sub>2</sub>), base excess (BE), as well as electrolytes: sodium (Na<sup>+</sup>), chloride (CL<sup>-</sup>), bicarbonate (HCO<sub>3</sub><sup>-</sup>), calcium (Ca<sup>++</sup>) and nonionized calcium (nCa<sup>++</sup>) ions concentrations, while a significant increase in the mean of hemoglobin concentration (tHB), packed cell volume (PCV) and anion gap (AG) in the buffalo calfs infected with enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> comparison with the control animals. The main clinicopathological changes in native buffalo calves infected with enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> were significantly decrease in electrolytes, blood gases, Calcium (Ca<sup>++</sup>) and nonionized Calcium (nCa<sup>++</sup>) ions concentrations.

**Keywords:** Blood Gases, Buffalo Calves, Clinical Pathology, Direct ELISA, *E. coli*.

### Direkt ELISA ile Tanısı Konmuş, Enterotoksijenik *E. coli* (ETEC) K99<sup>+</sup> ile Enfekte Manda Yavrularındaki (Malak) Bazı Klinikopatolojik Değişiklikler

**Öz:** Bu çalışmanın amacı, Eylül 2018 - Nisan 2019 tarihleri arasında, direkt ELISA ile Enterotoksijenik *E. coli* (ETEC) K99<sup>+</sup> tespit edilen doğal buffalo buzağlarındaki (1-15 gün arası) klinikopatolojik (hematolojik ve biyokimyasal) bazı değişiklikleri belirlemektir. Toplam 40 dışkı örneği (enfekte hayvandan 30 örnek ve klinik olarak sağlıklı Buffalo buzağlarından 10 örnek) direkt ELISA ile incelendi. Diyareik hayvanlardan alınan altı numune (%20), *E. coli* (ETEC) K99<sup>+</sup> için pozitif. Kontrol grubundaki hayvanlarla karşılaştırıldığında, Enterotoksijenik *E. coli* (ETEC) K99<sup>+</sup> ile enfekte olmuş hayvanların kan örneklerindeki kan gazı analizi kan pH'ı, kısmi oksijen konsantrasyonu basıncı (PO<sub>2</sub>), kısmi karbondioksit konsantrasyonu (PCO<sub>2</sub>), baz fazlalığı (BE) ve ayrıca elektrolitler: sodyum (Na<sup>+</sup>), klorür (CL<sup>-</sup>), bikarbonat (HCO<sub>3</sub><sup>-</sup>), Kalsiyum (Ca<sup>++</sup>) ve iyonlaştırılmamış Kalsiyum (nCa<sup>++</sup>) iyonları konsantrasyonlarında önemli azalmaya, hemoglobin konsantrasyonu (tHB), hematokrit (PCV) ve anyon açığı (AG) değerlerinde ise önemli bir artışa neden oldu. Enterotoksijenik *E. coli* (ETEC) K99<sup>+</sup> ile enfekte olmuş doğal buffalo buzağlarındaki ana klinikopatolojik değişikliklerden; elektrolitler, kangazları, Kalsiyum (Ca<sup>++</sup>) ve iyonize olmayan Kalsiyum (nCa<sup>++</sup>) iyonları konsantrasyonları önemli ölçüde azaldı.

**Anahtar Kelimeler:** Direkt ELISA, *E. coli*, Kan Gazları, Klinik Patoloji, Manda Yavrusu (Malak).

✉Maab I. AL- Farwachi

University of Mosul, College of Veterinary Medicine, Department of Internal and Preventive Medicine Mosul, IRAQ.  
e-mail: maabalfwche@yahoo.com

## INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is a pathogenic serotype causes enteric form of colibacillosis in neonate buffalo calves (1,2). The main two virulence factors of ETEC are the K99 fimbrial adhesion antigen (F5), and the heat-stable (STa or STb) enterotoxin (3,4). The K99 antigen contributes in attaching of the bacteria to the intestinal epithelium; thus, the bacteria proliferate and produce the enterotoxin that results in excessive secretion of fluid causing severe watery diarrhea (5,6). Consequently, severe dehydration can occur rapidly ending to death within 24 hours (7). The mortality constitutes an economic burden to the producers (8,9).

Clinical symptoms associated with colibacillosis are usually accompanied with variety of metabolic disorders manifested by changes in biochemical indices and hematological parameters (10,11). Prevalence of *E. coli* infection in buffalo calves has been reported in different countries around the world. In Pakistan, the studies reported prevalence of *E. coli* (15.3%) in buffalo calves (12), In Egypt the infection rate of *E. coli* 73.3% (11), In England, Sherwood et al. (13) record ETEC in 7.51% of diarrheic calves, but not from clinically normal calves.

In Iraq, the prevalence of *E. coli* K99 was 80% of diarrheic and 20% of non-diarrheic buffalo calves aged (3-11 day) (14).

Up to our knowledge, there is no previous study published about ETEC K99<sup>+</sup> in buffalo calves in Mosul. The objective of recurrent study was to determine the some clinicopathological (hematological and biochemical) changes in native buffalo calves (aged between 1-15 day) infected with enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> detected by direct ELISA.

## MATERIALS and METHODS

### Fecal Sample

Thirty samples from infected calves and 10 samples from clinically healthy buffalo calves were examined by direct ELISA.

A total of 30 buffalo calves fed on whole milk (aged between 1-15 day) were randomly selected from the diarrheic cases came to the veterinary teaching hospital/college of veterinary medicine, University of Mosul, Mosul, Iraq, between September 2018 - April 2019. A ten fecal samples were collected from clinically healthy (negative by direct ELISA) buffalo calves as a control group. After collection of fecal samples and transportation to the laboratory, they would be storage at -20 °C until examined by ELISA test.

All fecal samples were examined using Safranin-Methylene blue technique (SMB) (15) to ensure absence of *Cryptosporidium* oocysts in the fecal samples. This study was conducted in accordance with ethical principles.

A commercial direct ELISA kit (BIO-X *E. COLI* F5 ELISA KIT, Bio-x Diagnostics, Belgium) was used for detection of enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> antigen in the fecal samples according to the manufacturing company.

Blood samples were collected from infected and clinically healthy animal for blood gas analysis. A total of 5 ml of venous blood was drawn from the jugular vein puncture into heparinized vials capped and placed on ice immediately to avoid alteration in blood gas tension. Blood pH, partial pressure of oxygen (pO<sub>2</sub>), partial pressure of carbon dioxide (pCO<sub>2</sub>), base excess (BE), electrolytes: sodium (Na<sup>+</sup>), chloride (CL<sup>-</sup>), bicarbonate (HCO<sub>3</sub><sup>-</sup>), Calcium (Ca<sup>++</sup>) and nonionized Calcium (nCa<sup>++</sup>) ion concentrations, hemoglobin concentration (tHB), packed cell volume (PCV) were determined by blood gas analyzer (OPTI Critical Care analyzer/OPTI Medical Systems) within 3 h after sampling at 37°C, commercial chloride test kit (Fortress diagnostic, British) used to determine chloride ion concentration.

### Statistical Analysis

The data represented as means (±SE) were statistically analyzed by T test using SPSS version 21.

Differences between affected and control groups were considered significant when the value of  $P \leq 0.05$ .

## RESULTS

This study showed that six samples of total 30 examined buffalo calves were positive to *E. coli* (K99) with total infection rate 20%. The infected calves revealed significant variations in the means of following parameter: blood pH,  $PO_2$ ,  $PCO_2$ , BE, electrolytes:  $Na^+$ ,  $Cl^-$ , K,  $HCO_3^-$ ,  $Ca^{++}$  and  $nCa^{++}$  ions, tHB, PCV) concentration compared with control animals (Tables 1 and 2).

**Table 1.** Hematological and blood gas analysis in six infected buffalo calves with enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> detected by direct ELISA.

**Tablo 1.** Direkt ELISA ile tespit edilen Enterotoksijenik *E. coli* (ETEC) K99<sup>+</sup> ile enfekte altı bufalo buzağısında hematolojik ve kan gazı analizi.

Parameter	Groups	
	Control	Infected
pH	7.39 ± 0.06	7.02 ± 0.02*
$PO_2$ (mmHg)	43 ± 0.82	37.4 ± 0.63*
$PCO_2$ (mmHg)	50 ± 0.9	39 ± 0.72*
BE (mmol/L)	2.91±0.2	-1.8±0.35*
tHB (g/100 ml)	9.69±0.23	12.08±0.34*
PCV (%)	32.20±0.71	39.8±0.89*

\*Means significant change from control at  $P \leq 0.05$ .  $PO_2$ : Partial pressure of oxygen,  $PCO_2$ : Partial pressure of carbon dioxide, BE: Base excess, tHB: Total hemoglobin concentration, PCV: Packed cell volume.

**Table 2.** Electrolytes changes in six infected buffalo calves with enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> detected by direct ELISA.

**Tablo 2.** Direkt ELISA ile tespit edilen Enterotoksijenik *E. coli* (ETEC) K99<sup>+</sup> ile enfekte altı bufalo buzağısında elektrolit değişiklikler.

Parameter	Groups	
	Control	Infected
Na (mmol/L)	129±1.03	117±1.18*
CL (mmol/L)	89.9±0.6	80.2±1.08*
K (mmol/L)	3.8±0.09	3.4±0.08
$HCO_3^-$ (mmol/L)	29.2± 0.4	19.1±1.13*
$Ca^{++}$ (mmol/L)	0.80±0.01	0.60±0.01*
$nCa^{++}$ (mmol/L)	0.81±0.01	0.59±0.03*

\*Means significant change from control at  $P \leq 0.05$ . Na: Sodium, CL: Chloride, K: Potassium,  $HCO_3^-$ : Bicarbonate, Ca: Calcium, nCa: non-ionized calcium.

## DISCUSSION and CONCLUSION

*E. coli* is considered predominant agent of enteric disease in domestic animals, particularly in cow calves, buffalo calves, piglets and lambs, as well as diarrhoea in humans (2,16,17).

*Escherichia coli* is a dominant enteropathogen among diarrheic buffalo calves (18). In the current study, six samples were positive for *E. coli* K99 (infection rate 20%). This rate is lower than those documented in Babil (58.3%) (14) and in Egypt 73.6% (11) while higher to what was reported in Pakistan (15.3%) (12) and lower than those documented in Babil (58.3%) (2014) and in Egypt 73.6% (11).

The infected buffalo calves showed hematological and biochemical changes due to dehydration and *E. coli* infection. The result revealed significant increase of tHB and PCV level which may be due to decrease in plasma fluid or release of epinephrine as a result of stress in the infected animal, causing a compensatory mechanism leading to an increase in the level of tHB and PCV (19,20). The increase in hemoglobin concentration may also be attributed to increase the body demands for hemoglobin in order to ward off changes in the blood pH. The hemoglobin accepts the hydrogen ion from carbonic acid in the case of acidosis (19,21).

The results of the study indicated a significant decrease in the values of blood pH level,  $PO_2$ ,  $PCO_2$ , value of BE and concentration rates of sodium ion, bicarbonate, Chloride ion, calcium ion and nonionized calcium. The above changes indicate the occurrence of dehydration and acidosis in calves with diarrhea (11,22).

In conclusion, the main clinicopathological changes in native buffalo calves infected with enterotoxigenic *E. coli* (ETEC) K99<sup>+</sup> were significant decrease in electrolytes, blood gases, Calcium ( $Ca^{++}$ ) and nonionized Calcium ( $nCa^{++}$ ) ions concentrations.

## Conflict of interest

The authors declare that they have no conflict of interest.

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