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Clinicopathological changes associated with *Escherichia coli* infection in Calves

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Abstract

Virulent *E. coli* is potentially zoonotic pathogens play a contributing role in calf diarrhoea which is one of the most important problems of neonatal calves due to high morbidity and mortality rate of these animals which cause great economic loss to their industry, so the purpose of this work is to characterize pathogenic *E. coli* in apparently healthy and diarrheic neonatal calves with special reference to the *shiga* toxin producing *E. coli* strains and evaluate their clinical and haemato-biochemical consequences. A total of 160 samples (80 of each faecal swabs and blood samples) were collected from neonatal (1-30 days) suffered from diarrhea and subjected to the bacteriological and haematobiochemical investigation respectively, in addition 20 apparently healthy calves blood samples were collected as control. A complete clinical evaluation of calves cases history previous diseased conditions was performed before sampling. The results showed prevalence rate of positive *E. coli* was 52.5% (n=42) from 80 diarrhoeic calves and 15% (N=3) from 20 healthy calves, the total percent was (45%) from 100 calves. The O-H serotyping of cultural and biochemically positive isolates identified (45) isolates belonging to 5 serotypes O157:H7 (N=6), O26:H11 (N=6), O119:H4 (N=7), O63:H5 (N=5) and O125: H69 (N=11) and untypabl (N=10). Molecular characterization of *E. coli* isolates on *shiga toxin* type 1 and 2 (*stx*1 and *stx*2*)* revealed two well-known pathotypes with high frequency of *E. coli*. PCR results verified that *stx*1 and *stx*2 genes were amplified in 10 %, 30% of *E. coli* isolates from calves respectively, Clinical and haemato-biochemical studies revealed appreciable tachypnea, tachycardia, significant increase in value of haemoglobin (Hb), total erythrocyte count (TEC) and highly significant increase in packed cell volume (PCV); significant increase in total leukocytic count (TLC), neutrophilia, hypoproteinemia, hypoalbuminemia, hypoglobulinemia and hypoglycemia, increased hepatic enzyme activity level, compromised kidney function, while low A/G ratio, hyponatremia and hypochloremia were observed in calves affected with diarrhoea. These findings may be important in the diagnosis and assessment of animals affected with diarrheaic disease.

Keywords: *Calf, diarrhea, E. coli, Enterotoxins, Clinical-pathology, shiga toxin, antibiotic, PCR.*

Introduction

Bacterial organisms play an important role in diseases causing heavy morbidity and mortality as they are important etiological agents causing gastroenteritis in calves **Singh *et al.* (2014)**.

Diarrhea define as a complex syndrome as it involves interaction between environmental, nutritional factors and infectious agents, several pathogens have been incriminated in calf diarrhea including *E. coli*, *Salmonella*, *Clostridium*, bovine coronavirus, bovine rotavirus group A, bovine viral diarrhea virus and *cryptosporidium* species **Singla *et al*. (2013)**.

According to National Animal Health Monitoring System that follows (**USDA**), diarrhea is

clamed to cause the highest percentage of mortality between neonatal calves. In addition to high mortality rate of calves, diarrhea accounts also for non direct economic losses to breeders that result from reduction of animal weight and production due to developing of chronic illnesses and the high costs of medication and veterinary supervision **Tajik *et al*. (2012**).

The clinical signs include subnormal body temperature, cold clammy skin, pale mucosae, collapse of superficial veins and periodic apnoea. Because of the multifactorial nature of calf diarrhea, it is difficult to be controlled effectively, major cause of economic loss, due to inefficient feed conversion, reduced live body weight, delay marketing, costly preventive and therapeutic program, deaths, loss of genetic material for herd improvement and decrease in the number of neonates for herd replacement and expansion **Cho and Yoon, (2014**).

Diarrheagenic *E. coli* come on top of bacteria causing diarrhea, they can be classified into five main groups, among them only *shiga* toxin -producing *E. coli* (STEC) is considered of zoonotic importance **Fadel *et al.* (2017**). Cattle can be considered the main reservoir of STEC and from them they can be transferred to human. Man infection may occur through ingestion of contaminated undercooked beef or raw milk without sufficient heat treatment **Aidar-Ugrinovich *et al.* (2007**). Human can contract infection also by ingestion of vegetables and water contaminated with carrier faeces especially with the ability of these pathogens to survive for several months in the environment **Fremaux *et al.* (2008**).

Identification of the possible causative agent in outbreaks of diarrhea is important to allow targeted preventative measures, such as vaccination, and identification of possible risk factors or sources of infection **Izzo *et al.* (2011**). Ruminants, especially cattle and sheep, have been implicated as a principal reservoir of one of the entero-virulent *Escherichia coli* pathotypes, large and small ruminants could be a potential source of infection in humans with *E. coli* **Osman *et al.* (2012).** Strain of *Escherichia coli* have long been recognized as a major enteropathogen in the first 28 days of calf's life. *E. coli* normally inhabits the intestinal tract of man and animals with a potential to produce from mild to severe pathological conditions and it is considered one of the major causes of diarrhea in cattle. STEC is an important group of *E. coli* that can cause severe diarrhoea and responsible for a number of food borne outbreaks worldwide. The STEC family is diverse and more than 200 serotypes of STEC have been identified so far and more than 160 of these have been recovered from humans with haemorrhagic colitis or haemolytic uremic syndrome **Abassi and Elahe, (2015**). STEC strains produce cytotoxins known as *shiga toxins* (*stx*) which have been classified into two major classes they are *shiga toxin1* (*stx*1) *and shiga* toxin2*(stx*2*)* and coded by *stx*1 and *stx*2 genes respectively. **Bakhshi *et al.* (2014**). Serological and molecular techniques are essential for detection and characterization of pathogenic *E. coli* and are based on O-H antigens and virulence markers, respectively **Shams, *et al*. (2012**). **Khalifa *et al.* (2019)** reported that, the application of PCR assay was important to develop a highly sensitive and specific diagnostic method for rapid detection of *E. coli* in diarrheic calves and humans. Analysis of blood and serum samples of the diarrhoeic calves revealed significant increase in Hb, PCV, TEC, TLC, K and chloride while significant decrease in serum Na and glucose level However, no significant changes were observed in ALT and AST were recorded **Shekhar *et al.* (2017).**

Haematological and biochemical analyses in *E. coli* diarrhea are complex in nature comprising serious imbalances of fluid, electrolyte and acid base status threatening the life of the calf **Singh *et al*. (2014**). The evaluation of such alterations is important for determination of the proper medical intervention. Haematological and biochemical analyses of blood are very useful to get an insight in metabolic and health status of animal. During diagnostic procedure

it is very useful to compare the values obtained from ill animal with normal values in healthy animals **Ghanem *et al.* (2012).**

Therefore specific reference intervals are needed for each animal species for appropriate interpretation of results of haematological and biochemical analysis so aimed of this work was designed to evaluate the characterize pathogenic *E. coli* with special reference to the *shiga toxins* in diarrheic neonatal calves and evaluate their clinical and haemato-biochemical associated changes.

Materials and Methods

1-Animals:-

One-hundred of neonatal Friesian calves of both sexes aged from one day to 30 days from different farms belonging to Ismailia Governorate, Egypt, during the period from October 2018 to January 2019 were clinically examined, According to the clinical examination, calves were classified into two groups: The 1st group consisted of (20) apparently healthy calves that didn't show any diseased condition and didn't expose to any treatment; they were kept as a control calves. The 2nd group consisted of (80) calves suffered from diarrhea.

2. Clinical examination:-

A complete clinical evaluation was performed before collection of samples for investigation of various parameters. These evaluations were carried out carefully using the methods described by **Radostits *et al.* (2007),** through Skin fold (tent) test, Capillary refill time (CRT) and sinking eye**.** Data concerned with the case history, clinical findings, and medical record for each calf were illustrated in**.**

3.1. Collection of samples for bacteriological examination:-

Fecal samples were collected directly from the rectum of examined calves (20 apparently healthy and 80 diarrheic calves) from different farms in Ismailia Governorate in Egypt. Samples were individually collected using sterile swabs with nutrient broth as a transport medium then tube, labeled, placed into a small Coleman cooler and transferred immediately to the laboratory for bacteriological examination of *E. coli.* prevalence.

3.2. Isolation and identification of *E. coli*:-

Fecal swabs from all examined animals were inoculated into MacConkey broth for 24hrs at 37°C aerobically. After that, swabs were streaked onto MacConkey agar and blood agar plates, as well as on Sorbitol MacConkey agar and incubated aerobically at 37°C for 24 hrs. Pure Lactose fermented colonies were subculture onto Eosin methylene blue (EMB) agar for 24hrs at 37°C for aerobically characteristic metallic sheen of *E. coli*. All isolates were refreshed onto nutrient ager and incubated at 37°C for 24 hrs. Suspected colonies of *E. coli* were exposed to biochemical testes as Oxidase, Catalase, In dole, Methyl red, Vogus proskouer. Citrate utilization, Hydrogen sulphide test and urease test according to **Kreig and Holt, (1984).**

3.3. Serotyping of *E. coli* isolates:-

The identified *E. coli* isolates were serotyped by commercially available kits using polyvalent and monovalent antisera O and K (Test Sera Enteroclon, Anti –Coli, SIFIN Berlin, Germany) Animal at Health Research Insititute, Serlogy Unit, Dokki, Giza. All the isolates were stored in Brain heart infusion broth with 30% glycerol at –70°C until required.

3.4. Anti-chemotheraeutic test:-

The susceptibility profiles of *E. coli* isolates from diarrhoeic calves were performed using disk diffusion technique according to the procedures of (**CLSI, 2011**). Pure colonies were picked up, cultivated on Muller Hinton broth and incubated at 37˚C for 24hrs then adjustment density with 0.5% of McFarland tube. Then the bacterial suspension was streaked on Mueller-Hinton agar plates using a dry sterile cotton swab using different chemotherapeutic sensitivity discs (Oxoid) namely tetracycline, gentamicin, erythromycin, penicillin, amoxicillin+clavulanic acid, ciprofloxacin, enrofloxa-

cin, sulphamethoxazole and norofloxacin and incubated at 37˚C for 24 hrs. The inhibition zone diameter of the cultured plates were recorded and measured.

4.1. Collection of blood samples:-
About 5 ml of blood was collected through jugular vein aseptically in the clean sterilized glass vials containing anticoagulant. In addition to this 10 ml of blood was collected without anticoagulant in clean and sterilized glass vials and allowed to clot at room temperature to separate serum. Samples were shifted immediately to the laboratory, to avoid any deleterious effect. The serum samples were separated and stored in deep freezer in vials till further use.

4.2. Hematological studies:-
Whole blood samples were analyzed for complete blood picture (Total white and red cells counts, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) These parameters were performed according to the routine hematological procedures adopted by **Feldman *et al*. (2000).**

5. Biochemical examination:-
Biochemical serum analysis, the concentration of copper, zinc, sodium, potassium, calcium, chloride, phosphorus, magnesium, glucose, total protein, Albumin, globulins, serum creatinine, urea, the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were all assayed by an automatic analyzer (Hitachi 912) in hospital Suez Canal university. Albumin and globulins were separated by cellulose acetate electrophoresis using Helena system (Helena France).

6. Molecular identification of *Escherichia coli* virulence genes *(Shiga toxins*):-
DNA extraction:-
DNA extraction from (10) isolates were performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. Samples were then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer:-
Primers used were supplied from **Metabion (Germany)** are listed in **Table (1).**

PCR amplification:-
Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix **(Takara, Japan)**, one µl of each primer of 20 p mol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied bio-system 2720 thermal cycler.

Analysis of the PCR Products:-
PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of each PCR product were loaded in each gel slot. Gene ruler 100 bp DNA ladder (Fermentas, Thermo) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software **Sambrook *et al.* (1989)**. For each PCR experiment, appropriate positive and negative controls were included.

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences (5′ → 3′)	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondar denaturation	Annealing	Extension		
*stx*1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Dipineto *et al.* (2006)
*stx*2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	

7. Statistical analysis:-

All the observations recorded in this study were subjected to statistical analysis by **Snedecor and Cochran, (1994).**

Results:-

(1)- The clinical signs:-

The common clinical signs appeared on the control group were normal appetite, clear shiny eyes, bloomy coats, and the mean body temperature, pulse and respiratory rates were 38.5° C, 75 beats per minute and 25 breaths per minute, respectively. In diarrheic neonatal calves had thin and watery feces, signs of dehydration (sunken eyes, dry mucus membranes, and rough hair) **Photo (1, 2, 3 & 4)**, calf extremities were cold to the touch, loss of appetite, difficulty in getting up, tendency to lie down and unable to rise with loss of consciousness **Table (2)**.

Photo. (1)

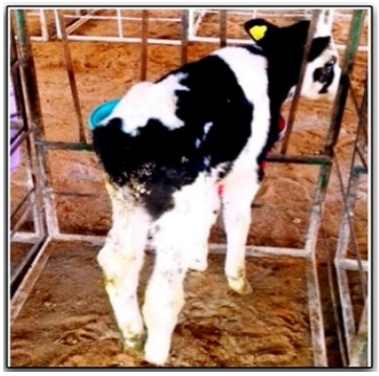

Photo. (2)

Photo. (3)

Photo. (4)

Photo. (1, 2, 3 & 4): Neonatal calves with severe diarrhea showed signs of dehydration; sunken eye and showed soiling of perineum and tail with yellow to green feces.

Table (2). Clinical examination profiles of apparently Healthy and diarrhoeic calves.

Parameters	Apparently Healthy	Diarrhoeic calves
Body temperature (C°)	38.20±0.19	40.02±0.09**
Respiration rate (/min)	25.00±1.12	42.61±2.82**
Heart rate (/min)	75.30±2.88	90.61±4.51**
Appetite	Normal	Loss of appetite
Eyes appearance	clear shiny eyes	Moderately deeply
General appearance	Alert and in standing position	Dull, depressed in standing position and some cases were recumbent
Mucous membrane	Rosy red	Dry and Pale
Faeces consistency	Semisolid	Soft to watery
Faeces color	Green to dark brown	Light green or brown tinged with mucous or blood

** Significant at $p < 0.01$

(2)- Prevalence of *E. coli* in the examined faecal swabs:-

Results illustrated in **Table (3)** revealed that *E. coli* was recovered from (3) in prevalence of (15%) for swabs from apparently healthy. On the other hand diseased calves showed and prevalence rate (**52.5%**) positive samples out of 80 examined..

Table (3). Prevalence of *E. coli* in the examined faecal swabs of apparently Healthy and diseased neonatal calves:-

Case of calves	No. of examined Samples	+ ve *E. coli*		-ve *E. coli*	
		No.	%	No.	%
Apparently Healthy	20	3	15%	17	85%
diarrhoeic calves	80	42	52.5%	38	47.5%
Total	100	45	45%	55	55%

(3)- Biochemical and serological identification of *E. coli*:-

All *E. coli* isolates were identified by cultural, morphological and biochemical charactering on MaCconkey agar and EMB. Agar character metallic sheen is seen in **Photo (5)** and biochemically for (**45**) as shown in **Photo (6) and Table (4)**. The *E. coli* isolated was sent to (serology units) in Animal Health Research Institute, Dokki- Giza to be serotyped.

Photo. (5): Cultural identification of *E. coli.* inoculated in EMB agar shows characteristic green metallic sheen colonies

(A) **(B)**

Photo. (6): Some biochemical tests for identification of *E. coli.*
(A) TSI (triple sugar iron) Agar and Simmon's Citrate Agar
(B) Kovac's indole reaction

Table (4). Biochemical identification of *E. coli* isolates (N= 45) from diarrheic calves.

Isolates	**Biochemical tests**					
	Test					
Isolates of diarrheic Calves	**MR**	**SC**	**VP**	**IT**	**UT**	**VP TSI**
	+ve	-ve	-ve	+ve	-ve	Acid butt slant with gas production

EMB): Eosin methylene blue; (+ve): positive; (-ve): negative; (acid): yellow color; (IT): indole production test; (MR): methyl red test; (VP): Voges-Proskauer test; (SC): Simmon's citrate test; (UT): urease test; (TSI): triple sugar iron.

(4)- Serotypes of isolates:-

The serotypes found were. (N=6) O157:H7, (N=6) O26:H11, (N=7) O119:H4, (N=5) O63:H5 and (N=11) O125: H6, while the untypable isolates was (N=10) **Table (5).**

Table (5). prevalence of detected serotypes based on total number of *E. coli* isolates (n=45)

Types of *E. coli* isolates	**Serotypes**	**No. of isolates**	**%**
E. coli	**O26:H11**	6	13.33 %
	O157:H7	6	13.33 %
	O63:H5	5	11.20 %
	O119:H4	7	15.55 %
	O125:H6	11	24.44 %
Untypable	**untypable**	10	22.20 %
Total		45	100 %

The results of anti-chemotherapeutic sensitivity test were recorded in **Table (6)**

Table (6). Anti-chemotherapeutic sensitivity test of *E. coli* isolated from diarrheic calves:-

Bacterial isolates / Anti-chemotherapeutic	*E. coli* N= 45			
	S	**%**	**R**	**%**
Gentamicin (120 µg)	18	40	27	60
Erythromycin (15 µg)	9	20	36	80
Tetracycline (10 µg)	7	15.5	38	84.5
Enrofloxacin (5 µg)	17	37.77	28	62.22
Sulphamethoxazole (25µg)	-	-	45	100
Penicillin (10 µg)	-	-	45	100
Norfloxacin (10 µg)	13	28.9	32	71.11
Amoxicillin+clavulanic acid (10 µg)	-	-	45	100
Ciprofloxacin (5 µg)	-	-	45	100

S: sensitive strain R: resistant strain

(5)- Molecular identification:-

A total of 10 positive serotyped *E. coli* were analyzed by PCR for detection of *shiga toxin* genes, (*stx*1 and *stx*2). Molecular characterization of serologically identified *E. coli* isolates revealed 3 isolates from 3 diarrheic calves were positive for *stx*2 at 779 base pair (bp) belonged to O26:H11, O157:H7, and O63:H5. One isolates from diseased animals were positive for *stx*1 at 614 bp, belonged to O157:H7. These data recorded in **Table (7)** and **Photo (7 and 8).**

Table (7). Detection of *E. coli* virulence genes *(Shiga toxins*) in the isolated strains from fecal samples of diarrheic calves:-

Serotype	*E. coli* virulence genes	Positive samples	%
O157:H7	***stx*1**	1	(10 %)
O26:H11, O157:H7, O63:H5	***stx*2**	3	(30 %)

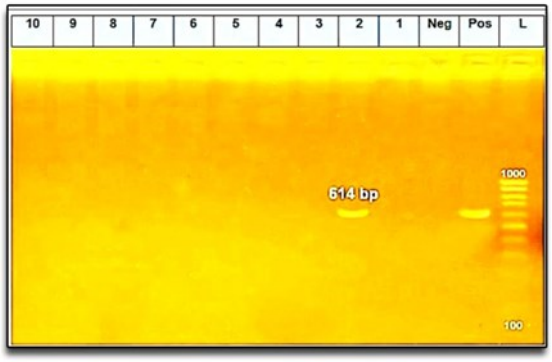

Photo. (7)

Photo. (8)

Photo. (7): Shows agarose gel electrophoresis of PCR amplified products of stx1 (614 bp) and Photo. (8) Shows agarose gel electrophoresis of PCR amplified products of stx2 (779 bp) genes for characterization of *Enteropathogenic E. coli.* Lane L: DNA molecular size marker (100 bp), lanes 1-10: The examined isolates lane (+ve): positive control and lane (-ve): negative control. The size in base pairs (bp) of each PCR product is indicated above the bands.

(6) Haematological studies:-

The results of haematological studies are presented in Table (8). It is evident that there was significant (P ≤0.05) increase in Hb (14.51 ± 0.74),TEC (8.51 ± 0.51) and highly significant (P ≤0.01) increase in PCV (45.98 ± 4.24) values in diarrhoeic calves as compared to healthy calves. There was significant decrease of MCH (13.51 ± 1.94) , MCHC (27.14 ± 2.10) and non-significant increase in MCV(60.90±5.95). Highly significant (P ≤0.01) increase in TLC (**11.10 ± 0.72**), neutrophils (48.89 ± 5.2), while highly significant decrease in lymphocyte (42.47 ± 4.42) was observed in diarrhoeic calves whereas significant increase in value of monocytes (4.72 ± 0.21) and eosinophils (3.92 ± 0.1) was observed in diarrhoeic calves, respectively.

Table (8). Mean value (±SE) of hematological parameters in diarrhoeic calves compared to apparently healthy group.

Parameters	Apparently Healthy calves (n=20)	Diarrhoeic calves (n=45)
RBCs ($\times 10^6$/µl)	6.68±0.92	8.51 ±0.51*
Hb (g/dl)	11.39±1.25	14.51±0.74*
PCV (%)	36.10±3.18	45.98±4.24**
MCV (fl)	54.48±4.02	60.90±5.95
MCH (pg)	16.89±1.02	13.51±1.94*
MCHC (g/dl)	32.01±2.12	27.14 ±2.10*
WBCs ($\times 10^3$/µl)	8.25±.1.24	11.10 ±0.72**
Neutrophils %	36.34±3.28	48.89±5.2**
Lymphocytes %	55.27±5.92	24.47±4.42**
Monocytes %	3.51 ±0.3	4.72 ±0.21*
Eosinophils %	2.91±0.1	3.92±0.1**

(*): means significant changes from control at p≤0.05, (**) means high significant changes from control at p≤0.01

7- Biochemical studies:-

The results of serum biochemical studies of apparently healthy and diarrhoeic calves are depicted in **Table (9&10)**. It is evident from serum biochemical studies that A highly significant (P ≤0.01) increase in activities of AST (**135.6±8.14**), ALT (**78.7 ±3.06**), alkaline phosphatas (**134.6±7.1**) and also hypoglycemia (**48.69±2.49**) was noticed in comparison to with value of apparently healthy calves. There was significant (P ≤0.05) increase in values of Creatinine (1.73±0.04) and Urea (27.53±1.95) . There was significant (P ≤0.05) decrease in values of serum total protein (**6.55±0.10**), albumin (**3.60±0.42**), globulin (**2.95±0.11**) and albumin: globulin ratio (**1.22±0.02**) in diarrhoeic calves.

Table (9). Mean value (±SE) in serum liver, Kidney enzymes and Glucose in diarrhoeic calves compared to Apparently healthy group.

Parameters	Apparently Healthy calves (n=20)	Diarrhoeic calves (n=45)
AST (U/L)	71.42 ±3.6	135.6±8.14**
ALT (U/L)	39.5 ±2.43	78.7 ±3.06**
ALP (U/L)	96.17±2.99	134.6±7.1**
Creatinine (mg/dl)	1.01±0.01	1.73±0.04*
|Urea (mg/dl)	21.82±1.75	27.53±1.95*
Glucose (mg/dL)	71.83±4.2	48.69±2.49**

(*): means significant changes from control at p≤0.05, (**) means high significant changes from control at p≤0.01

Table (10). Mean value (±SE) serum total protein and protein electrophoresis in diarrhoeic calves compared to apparently healthy group.

Parameters	Apparently Healthy calves (n=20)	Diarrhoeic calves (n=45)
Total protein (g/dl)	7.95±0.20	6.55±0.10*
Albumin (g/dl)	4.74±0.28	3.60±0.42*
Globulin (g/dl)	3.21±0.13	2.95±0.11*
A/G ratio	1.47±0.04	1.22±0.02*
Alpha globulins (g/dl)	1.01±0.03	1.10±0.01
Beta globulins (g/dl)	0.98±0.014	0.84±0.034
Gamma globulins (g/dl)	1.22±0.087	1.01±0.062*

(*): means significant changes from control at p≤0.05

Serum electrolytes showed a significant decrease on sodium, calcium, chlorides, zinc, copper, phosphorous, iron and magnesium while in potassium, showed a significant increase on in all diarrhoeic calves group **Table (11).**

Table (11). Changes in serum minerals and electrolyte in diarrhoeic calves compared to apparently healthy group.

Parameters	Apparently Healthy calves (n=20)	Diarrhoeic calves (n=45)
K (mmol/L)	3.9 ±0.27	5.51 ±0.30*
Na (mmol/L)	121 ±3.24	106.1 ±3.17*
Cl (mmol/L)	81.36 ±1.75	63.28 ±1.94*
Ca (mg/dL)	10.26 ±0.21	8.19 ±0.28*
P (mg/dL)	6.53 ±0.42	3.92 ±0.18*
Mg (mg/dL)	1.50 ±0.03	1.01 ±0.01*
Cu (mg/dl)	128.2±3.68	108.29±4.72
Zn (mg/dl)	180.37±11.61	135.58±10.36*
Fe (mg/dl)	193±8.37	138.2±5.24*

(*): means significant changes from control at p≤0.05

Discussion:-

The rate of prevalence of *E. coli* **Tables (3)** isolated from fecal swabs of diarroeic calves indicated that, the *E. coli* was isolated from 42 samples out from total 80 rectal swabs collected from diarrhoeic cavles with a percentage of (52.5%) and 3 from 20 healthy calves with percentage of (15%) which total percentage become (45%) (N=45) from 100 examined calves. Our results agree with **Paul *et al.* (2010)**, who showed that *E. coli* was isolated with an incidence of 50%. Meanwhile, other researchers isolated *E. coli* from calves with lower incidence as described by **Anwarullah *et al.* (2014),** who isolated *E. coli* with an incidence of 14.6%. On the other hand, higher incidence of *E. coli* was recorded by **Majueeb *et al.* (2014)** who isolated *E. coli* with an incidence of 72.8%.

The recorded results in **Table (5)** revealed that the incidence of *E. coli* infection in diarrheic calves was 54.21% where 45 isolates of *E. coli* were serologically identified. The detection of *E. coli* in calves was extremely lower than those obtained by **Osma *et al.* (2013)** (63.6%), **Hassan, (2014)** (50%), **El-Seedy *et al.* (2016)** (75.6%) and **Shaaban *et al.* (2018)** (20.8%) while it was higher than those of **Azzam *et al.* (2006)** (5.4%). The differences of the detection rates of *E. coli* may be attributed to the geographical locations and poor management practices as well as standards of hygienic measures. Further, *E. coli* was a commensal organism and was responsible for diarrhea in calves, particularly calves receiving less or no maternal antibodies through colostrum where milk is mainly used for commercial purposes **Malik *et al.* (2012**). Also, the presented data in Table (5) showed the serological identification of the obtained isolates and it revealed the detection of serotypes O26 (6), O119 (7), O125 (11), O157:H7 (6) and O63 (5).

Shiga toxigenic *E. coli* (STEC) have been incriminated in foodborne infections in humans. It's defined based on the pathogroup-associated virulence genes: *stx* encoding *Shiga* toxin for STEC. The work examined the toxin genes of serologically typed pathogenic *E. coli*. Along with the common pathogroups of *E. coli* and STEC strains were detected. STEC and other diarrheogenic *E. coli* are able to acquire virulence genes via horizontal gene transfer from other pathogroups leading to the development of divergent pathogroups **Johura *et al.* (2016**). Existing of STEC associated virulence genes in *E. coli* strains of human, animal, and environmental origins has been reported in Germany, United States and Slovakia **Fratamico *et al.* (2008**), some of which have been associated with human disease **Prager *et al.* (2011**). Previous studies have identified STEC from patients and animals in Finland **Martikainen *et al.* (2012**) and from animal derived food in Burkina Faso **Nyholm *et al.* (2015a**). Comparative genomics and characterization study of such strains has been conducted by **Nyholm *et al.* (2015b)** to determine their phylogenetic position among *E. coli* and to define the virulence genes they harbor.

The results of **Table (7)** and **Photo (7 and 8)** showed that the *shiga* toxin virulence genes were the most prevalent in all *E. coli* isolates. In the present study, out of the 10 *E. coli* isolates one was positive for *stx*1 (10%) belonging to serotypes O157:H7 and 3 were positive for *stx*2 (30%) belonging to serotypes O63:H5, O26:H11 and O157:H7. *stx*1 virulent gene was the predominant virulence gene (10%). These results agree with **Hashish *et al.* (2016)** who recorded that, most of the positive isolates of *E. coli* contained genes for *shiga* toxin (*stx*1) which would suggest the emergence of a new phenotype causing diarrhea in calves in Egypt. Moreover high frequency of *E. coli* isolates carrying the *stx*1 gene was observed **Wani *et al.* (2007)**. However, high prevalence of *stx*2 gene had been reported by **Irino *et al.* (2005)**. The current work demonstrated the presence of STEC among *E. coli* isolates and strongly suggested that this strain might contribute in the diarrhea in neonatal calves and affect public health threat in Egypt.

According to anti-chemotherapeutic sensitivity test the results recoded in **Table (6)**, revealed that isolated *E. coli* were highly sensitive to Gentamycin 120 µg at rate 18/45 (40%) and Enrofloxacin 5 µg at rate 17/45 (37.77%) These results nearly agreed with **Nazir, (2004) and Paul *et al.* (2010**) reported that the isolated *E. coli* was highly sensitive to Enrofloxacin. While revealed that the high resistance rates of *E. coli* isolates against amoxicillin-clavulanic acid, penicillin, ciprofloxacin and sulphamethoxazole (100% each). These come agree with **Ammar *et al.* (2015**) recorded that, multiple antibiotic-resistant were alarmingly observed in all *E. coli* isolates, with the majority of isolates displaying resistance to amoxicillin-clavulanic acid, sulfamethoxazole and ciprofloxacin (100% each) respectively. In Egypt documented higher resistance rates among this proves that the increased prevalence of resistance of *E. coli* isolates to these antibiotics is due to their regular usage for control of pathogenic *E. coli* in many districts in Egypt because of their low cost and availability.

Hematological alterations can help in identification of inflammatory response associated with enteritis and helps in the early detection of enteritis and prediction of disease severity.

Significant increase in RBCs count, PCV value and Hb concentration these may be due to Loss of extracellular fluid in diarrhoea leads to haemoconcentration and hypovolemia and inadequate intake of milk and fluids during diarrhea, these results were similar to other studies **Malik *et al.* (2013).** There was significant increase in MCV in diarrhea, and this may be attributed to production of immature RBCs of large size **Ahmed and Hassan, (2007**). There was significant decrease in MCH of mild and server than control, and this may be attributed to loss of blood and low level of hemoglobin in erythrocytes.

The leukocytosis in diarrhoea might have occurred due to normal reaction of body defence

mechanism against infections. Present findings are in accordance with those as obtained by **Brar *et al.* (2015)** and also attributed mainly to neutrophilia **Eddy and Pinsent, (2004**). The marked neutrophilia with lymphopenia is characteristic of bacterial enteritis and indicated the presence of intestinal infection **Malik *et al.* (2013**). The leukocytosis, neutrophilia, monocytosis and lymphopenia may be attributed to inflammatory mechanism of the causative agents these parallel with results reported by **Shekhar *et al.* (2017).**

Serum liver function tests of diarrheic calves showed significant ($P<0.05$) increase in ALT and AST. This result might be attributed to pathological affection of the liver and digestive tract and inflammation of gastrointestinal tract of diarrheic calves which occur due to bacterial infection and its toxins, these results agree with **Ghanem *et al.* (2012) and Fatma *et al.* (2014**). Significant increase in activities of serum AST, ALT and ALP in diarrhoeic calves are indicating that there might be some cellular level damage in tissues of major organs like liver, heart, kidney and musculature of calves.

Significant increase in urea and creatinine levels in this work was attributable to dehydration as a result of diarrhea and hypovolemia occur which lead to increase the concentration of the plasma solutes with proportionate increase in both parameters, impaired excretion of urea and creatinine may occur, secondary due to reduced renal blood flow and GFR (glomerular filtration rate). These come agree with **Singh *et al.* (2014).** Also may be due to excessive protein catabolism and present azotemia **(Coles, 1986).**

There was significant hypoglycemia in calf diarrhea. Hypoglycaemia may occur as a result of reduced rate of conversion of lactic acid to glucose, anorexia, alteration in tissue metabolism caused by decreased blood flow and oxygenation associated with hypovolemic shock which has been invariably present in hypoglycaemic diarrhoeic calves **Naylor, (2002**) and might be attributed to lack of glucose absorption from damaged intestine **Ahmed, *et al.* (2009**).

The mean values of total serum proteins and albumin were significantly lower in diarrhoeic calves than control group and this could be attributed to anorexia in diarrheic calves. The significant decrease in serum globulin in diarrheic calves in this study was in coincidence with **Ghanem *et al.* (2012).** The decreased in mean values of gamma (γ) globulin in diarrheic calves may be attributed to failure of calves to receive adequate quantity of colostrum after birth or due to action of the pathogenic agents on the immune system leading to suppressing of the response to any pathogenic agent **Ghane *et al.* (2012**). In the present work a decrease in the serum albumin level consequently a decrease in the A/G ratio these results were agree with **Hashish *et al.* (2016)**.

Hyponatremia in diarrheic calves in our work, may be due to excessive secretion of the Na^+ ions by intestinal villus cells which are lost through the intestinal tract particularly in *enterotoxigenic E. coli* induced diarrhoea **Radostits *et al.* (2007**). While hyperkalaemia in diarrheic calves may be due to increased potassium retention by kidney, also due to cellular damage and also indicated by increasing in the values of serum urea and creatinine lead to shift in potassium ions from intracellular fluid to extracellular fluid (K^+ - H^+ exchange) takes place in response to acidosis and hyperkalemia will developed **Singh *et al.* (2014**). Our results were in coincidence with **Shekhar *et al.* (2017)** who reported hyponatremia and hyperkalaemia in diarrheic calves as compared with healthy calves.

In diarrhoeic calves the significant decrease in Ca, P, Mg, Cl, Zn, Cu, and Fe than control may be due to, in Ca level might be attributed to persistent diarrhea and dehydration with loss of Ca in feces **Chernecky and Berger, (2008)**, the decrease in P was attributed to greater electrolyte loss than water loss **El-Dessouky and Nabila, (2005)**, The low serum Mg might be attributed to decreased absorption or diarrhea Also the low serum Cl was attributed to loss of

large amounts of Cl related to increased intestinal secretion and diarrhea **Blood and Radostits, (1989)** and finally the significant decrease in Cu, Zn, and Fe levels might be attributed to decrease in absorption of food nutrient through the intestine and losses in faeces **Khan, *et al.* (2009)**. These result come in harmony with **Ghanem, *et al.* (2012)**.

The work indicated that diarrheagenic *E. coli* have a role in causing diarrhea in young calves and produce several toxins and some of it may be involved in human diseases STEC (*stx*1 10%; *stx*2 30%). The obtained results revealed that the isolated *E. coli* strains susceptible to antimicrobial drugs as well as it more resistance to other drugs. And also significant increase in values of Hb, PCV and TEC along with leucocytosis due to absolute neutrophilia and lymphocytopenia in diarrhoeic calves. Decrease in values of serum total protein, albumin and globulin in diarrhoeic calves. Significant increase in activities of serum AST, ALT and ALP in diarrhoeic buffalo calves. So alterations of haemato-biochemical parameter in diarrhoeic calves can be used by veterinarian as indicator for applying adequate preventive and therapeutic measures to prevent further losses.

Therefore, to prevent and control of *E. coli* in calves, the following suggestions should be applied:-

Isolation of any calves having diarrhea in their case history. Using of good hygiene roles to prevent spreading of pathogens. Improvement of calves' immunity to reduce infection. Keep farms and all containers clean and disinfected to prevent infection. Separate the neonatal calves from the older one. Minimizing the non-responsible use of antibiotics for treatment of diarrhea in calves' farms. Reduction of pathogen load in the calf environment will reduce the burden of illness on the calves, as well as reduce the potential for pathogens to reach the human population.

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