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Prevalence of Shiga toxin-producing *Escherichia coli* and *Salmonellae* and some associated hematologic and biochemical profile alterations in lambs

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Abstract

Lamb enteritis constitutes an economic burden on sheep production worldwide. We aimed to estimate the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonellae* among diarrheic lambs at Kafrelsheikh Governorate, Egypt and to detect the associated clinical, hematologic, biochemical, and antioxidant parameters. Fifty diarrheic and twenty apparently healthy control lambs were examined clinically, and hematologically. Diarrheic lambs had a significant elevated body temperature, respiratory and pulse rate, most of hemogram parameters, total proteins and albumin, oxidative stress markers malonaldiahyde and nitric oxide levels, liver enzymes, urea and creatinine than control group. On the other hand, these diarrheic lambs had significant reduction in total leukocyte count and lymphocytes, antioxidant biomarkers super oxide dismutase activities and reduced glutathione than control lambs. *E. coli* and *Salmonella* spp. were isolated from 32.00% and 16.00% of diseased lambs, respectively. Serotyping and biochemical tests of examined samples identified 16 *E. coli* isolates belonged to 10 different serotypes; O6, O8, O26:H11, O75, O84:H21, O103:H2, O114:H4, O121:H7, O128:H2 and O163:H2. All isolates are STEC as they harbor either Shiga-toxin 1 or Shiga-toxin 2 genes or both. One isolate carries intimin gene (*eae*A) and classified as EHEC; O26:H11. The obtained nine isolates of *Salmonella* carry enterotoxin (*Stn*) genes, eight of them carry hyper-invasive locus (*hilA*) gene, all isolates belonged to six serotypes; *S. Enteritidis*, *S. Heidelberg*, *S. Tsevie*, *S. Typhimurium*, *S. Essen*, and *S. Infantis*. Lamb diarrhea was prevalent in the studied area and might constitute a veterinary and public health threat. Alteration in hemato-biochemical parameters and oxidative–anti-oxidant balance could help adopt appropriate treatment regimens.

Introduction

Bacterial enteritis in lambs is a serious health disorder associated with diarrhea resulting from alterations in intestinal flora which is responsible for the disorder of the colonic environment and leads into economic losses resulted from low growth rates and death due to malnutrition and dehydration.[1,2]

Diarrhea associated with infectious agents in a herd is often difficult to be managed due to the large number of potential enteropathogens involved and the difficulty in confirming an etiologic diagnosis.[3] Several etiological agents are associated with infectious diarrhea in lambs like *E coli*, *Salmonella* spp., *Clostridium* spp., *Campylobacter* spp., *Cryptosporidium spp*., *Giardia* spp., *Adenoviridae*, *Coronaviridade*, and *Rotaviriadae*.[4]

Escherichia coli is considered as the most common and important pathogen of bacterial enteritis in lambs and kids and is associated with a serious health risk.[5] Pathogenic strains of *E. coli* are distinguished from normal flora by their possession of virulence factors. These pathogenic strains include Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) which considered the major cause of diarrhea in newborn farm animals.[6] STEC isolates carry genes encoding Shiga toxins and may possess other virulence genes for intimin and enterohemolysin. STEC strains, which also have *eaeA* and *ehly* genes, are called enterohemorrhagic *E. coli* (EHEC).[7]

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Enteric disease associated with *Salmonella* occurs only sporadically,[8] but outbreaks are typically associated with *Salmonella enterica* subspecies *enterica* infection, and serotype *typhimurium* is the most commonly linked to gastroenteritis in sheep.[9] Virulence of *Salmonellae* depends on the presence of genes which are responsible for invasion into the epithelial cells and colonization and secretory diarrhea; *sefA* and *stn*, respectively.[10]

The infected lambs with virulent strains of *E. coli* such as STEC and *salmonella* spp. and the presence of these microorganisms in lamb fecal excreta constitutes a veterinary and public health threat.[11] Therefore, serological and molecular techniques are essential for detecting and characterizing pathogenic bacteria and virulence markers, respectively which are responsible for bacterial enteritis.[12]

On the other hand, diarrhea in lambs is associated with hemato-biochemical and oxidative parameters alterations creating an imbalance in the electrolyte, fluid and acid-base balance in the animal body. These changes establish a high risk for animal mortality and, therefore the evaluation of such alterations is important for determining the proper medical intervention.[13]

The current work aimed to determine the prevalence of Shiga toxin-producing *Escherichia coli* and *Salmonellae* among lambs suffered from enteritis in Kafrelsheikh Governorate. Furthermore, we evaluated the clinical, hemato-biochemical, oxidative and antioxidant parameters consequences among these lambs.

Materials and Methods

Animals and samples. A total of 70 (apparently healthy; n = 20 and diarrheic lambs; n = 50) lambs of both sexes and aged from 1 to 60 days from Kafrelsheikh Governorate were examined and sampled. The animals were from a large sheep farm out of the six governmental farms in the governorate.

Clinical examination. All animals were subjected to clinical examination including general health condition, body temperature, pulse, respiration, character of mucous membranes, auscultation of chest and abdomen and characters of the diarrhea according to Radostits *et al*.[9]

Blood collection for hematological examination and antioxidant biomarkers. Two blood samples were collected from jugular vein of each lamb. The first sample was collected in Vacutainer™ tubes (BD, Franklin Lakes, USA) containing EDTA for hematological studies according to standard techniques described by Feldman *et al*.[14] using Vet analyzer (Medonic CA620/530; Boule Medical AB, Stockholm, Sweden) and for anti-oxidant biomarkers super oxide dismutase (SOD) according to Abelson *et al*.[15] and reduced glutathione (GSH) according to Pertile *et al*.[16] The second sample was collected without anticoagulant and allowed to clot at room temperature, then centrifuged at 3,000 rpm for 10 min for serum separation. Serum samples were stored at – 20.00 ˚C for further biochemical studies.

Serum biochemical parameters and oxidative stress markers. The following biochemical parameters were determined in serum: serum total protein, serum albumin according to Henry *et al*.,[17] serum globulin was calculated as the difference between total protein and albumin together with albumin to globulin ratio (A/G) according to Kaneko *et al*.[18] Serum alanine amino transferase (ALT), aspartate amino transferase (AST) according to Reitman and Frankel,[19] and alkaline transferase (ALP) according to Rec.[20] Glucose according to Nagy *et al*.[21] urea nitrogen according to Patton and Crouch,[22] creatinine according to Young,[23] L-malondialdehyde (L-MDA) according to Esterbauer *et al*.[24] Nitric oxide (NO) according to Aebi,[25] Spectrophotometrically (Optizen 3220 UV; Mecasys Co. Ltd, Daejeon, South Korea) using diagnostic test kits (Spinreact, Girona, Spain for serum proteins and Spectrum Diagnostics, Cairo, Egypt for other parameters).

Samples for bacteriological examination. Rectal swabs were taken from diarrheic (lambs) by means of sterile cotton swabs and transported to laboratory as soon as possible in sterile MacConkey broth (Oxoid Ltd., Basingstoke, UK) and incubated at 37.00 ˚C for 24 hr for increasing chances of isolation. The samples (rectal swabs) were cultivated aerobically then bacterial isolates were subjected for characterization by studying their cultural, and biochemical characteristics according to Quinn *et al*.[26]

Isolation and identification of causative agents. For isolation of *Salmonella* strains, a loopful from the MacConkey broth was inoculated into selenite F broth with overnight incubation at 37.00 ˚C. Then, a loopful was streaked out onto MacConkey's agar, xylose lysine deoxycholate (Oxoid Ltd.) and *Salmonella–Shigella* agar media (Oxoid Ltd.) and incubated at 37.00 ˚C for 24 hr. Suspected colonies were subjected to biochemical testing according to Collee *et al*.[27] For isolation of *E. coli* strains, a loopful from the MacConkey broth was inoculated into MacConkey's agar and incubated at 37.00 ˚C for 24 hr. Lactose fermenter (pink) colonies were streaked onto and Eosin Methylene Blue agar and confirmed as *E. coli* using the standard biochemical tests according to Collee *et al*.[27]

Biochemical identification of *E. coli.* Standard biochemical tests for detection of *E. coli* were performed for 16 positive isolates according to Kreig and Holt,[28] including indole production test, methyl red test, nitrite reduction, ONPG, Sugar fermentation as lactose and arabinose.

Biochemical identification of *Salmonella.* Standard biochemical tests for detection of *Salmonella* were performed for nine positive isolates according to Kreig and Holt,[28] including motility positive, methyl red test, citrate utilization, H_2S, ODC, LDC and arginine dihydrolase while with Sugar fermentation only xylose.

Serological identification of *E. coli.* The isolates of *E. coli* were serologically identified according to Kok *et al*.[29]

using rapid diagnostic *E. coli* antisera sets (Denka Seiken, Tokyo, Japan) for diagnosis of the enteropathogenic types.

Serological identification of *Salmonella*. The isolates of *Salmonella* were serologically carried out according to Kauffman,[30] for determination of somatic (O) antigen by Slide agglutination test and flagellar (H) antigen using tube agglutination test.

Multiplex polymerase chain reaction for detection of virulence genes. The PCR was applied on *E. coli* isolates as well as *Salmonella* isolates.

Genomic DNA extraction. It was carried out following Sambrook *et al*.[31] Genomic DNA from individual pure cultures of *E. coli* isolates and *salmonella* isolates was extracted by GeneJET Genomic DNA Purification Kit (Thermo-Fisher, Waltham, USA) according to manufacturer's guidelines.

Primer sequences of *E. coli* used for PCR identification. Application of PCR for identification of Shiga toxins (*stx1* and *stx2*) and intimin (*eaeA*) genes of *E. coli* was performed essentially using primers (Amersham Pharmacia Biotech, Orsay, France), (Table 1).

Primer sequences of *Salmonella* species used for PCR identification. Application of PCR for identification of virulence factors including enterotoxin (*stn*), and hyper-invasive locus (*hilA*) genes of *Salmonella* species were synthesized (Table 1).

Statistical analysis. All data were presented by the means ± standard error. All pair-wise comparison of infected lambs to control was analyzed by one way analysis of variance using SPSS Software for data analysis (version 23.0, IBM Corp., Armonk, USA). Unless otherwise indicated, all differences were considered statistically significant at $p < 0.05$.

Results

Clinical Findings. The control lambs had a normal appetite with normal defecation in form of small hard pellets. On the other hand, lambs suffered from enteritis manifested clinical signs of diarrhea i.e., profuse and watery in some cases, and pasty white or yellowish and rancid in the others. The fecal materials were accumulated on the tail and hind limbs. These lambs suffered from fever associated with dullness, anorexia with congested mucous membrane. Body temperature, respiratory rate and pulse rate among diseased lambs were significantly higher than among the control lambs at $p \leq 0.05$; 41.10 ± 0.08 ˚C, 37.33 ± 0.88 and 121.60 ± 2.60 among diseased lambs and 39.20 ± 0.17 ˚C, 23.66 ± 1.20 and 81.00 ± 1.52 among control lambs, respectively.

Hematological findings. Lambs suffered from diarrhea had an elevated RBCs count, Hb concentration, PCV neutrophils and monocytes than control lambs. On the other hand, diarrheic lambs had a significant decrease in total leukocyte count and lymphocytes compared to the control lambs (Table 2).

Serum biochemical analysis. There was a significant increase in serum protein profile and serum enzyme activities among diarrheic than the control lambs. However, diarrheic lambs had a significant decrease in serum glucose concentration than the control ones (Table 2).

Oxidative stress and antioxidant biomarkers. The MDA and NO levels were significantly increased in diarrheic lambs compared to apparently healthy lambs ($p < 0.05$), while SOD activities and GSH levels were significantly reduced among diseases lambs ($p < 0.05$), (Table 2).

Microbiological findings. The *E. coli* and *Salmonella* were the main cause of bacterial enteritis in examined lambs. The percentage of isolated bacteria was calculated relative to the total diseased lambs. The bacterial isolates from collected fecal samples of diarrheic lambs found that *E. coli* was present in 16 samples (32.00%), *Salmonella* in nine samples (18.00%), *Enterobacter* spp. in five samples (10.00%), *proteus* in four samples (8.00%), *Citrobacter* spp. in four samples (8.00%), *Klebsiella* spp. in four samples (8.00%), *Providencia* spp. in three samples (6.00%), *Serrattia* spp. in two samples (4.00%) and about three samples (6.00%) were mixed infections. A total of 10 different *E. coli* serotypes were identified biochemically as O6, O75, O8, O114, O128, O26, O84, O103, O121 and O163 (Table 3) and a total number of six different *salmonella* serotypes were identified biochemically as *S. Enteritidis*, *S. Heidelberg*, *S. Tsevie*, *S. Typhimurium*, *S. Essen*, and *S. Infantis* (Table 3).

Table 1. Primer sequences of *E. coli* and *Salmonella* spp. virulence genes used for PCR identification.

Microorganism	Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
E. coli	*stx1* (F)	5′ ATAAATCGCCATTCGTTGACTAC ′3	180	Paton and Paton[48]
	stx 1 (R)	5′ AGAACGCCCACTGAGATCATC ′3		
	stx 2 (F)	5′ GGCACTGTCTGAAACTGCTCC ′3	255	
	stx 2 (R)	5′ TCGCCAGTTATCTGACATTCTG ′3		
	eaeA (F)	5′ GACCCGGCACAAGCATAAGC ′3	384	
	eaeA (R)	5′ CCACCTGCAGCAACAAGAGG ′3		
Salmonella spp.	*Ssn* (F)	5′ CTTTGGTCGTAAAATAAGGCG ′3	260	Makino *et al*.[49]
	stn (R)	5′ TGCCCAAAGCAGAGAGATTC ′3		
Salmonella spp.	*hilA* (F)	5′ CTGCCGCAGTGTTAAGGATA ′3	497	Guo *et al*.[50]
	hilA (R)	5′ CTGTCGCCTTAATCGCATGT ′3		

Table 2. Hemato-biochemical and antioxidants parameters among diarrheic and control lambs.

Parameters	Control	Diseased
Whole blood sample		
RBCs (10^6 mm^{-3})	7.90 ± 0.25	9.25 ± 0.24*
Hb (g dL^{-1})	10.50 ± 0.45	12.14 ± 0.14**
PCV (%)	29.12 ± 0.56	34.15 ± 1.15**
WBCs (10^3 mm^{-3})	9.92 ± 0.68	8.80 ± 0.56*
Lymphocytes (%)	55.50 ± 2.08	37.80 ± 2.6**
Neutrophil (%)	39.50 ± 2.00	54.50 ± 3.2**
Monocytes (%)	2.52 ± 0.04	5.01 ± 0.14**
Eosinophils (%)	2.20 ± 0.21	2.10 ± 0.45
Basophils (%)	1.24 ± 0.12	1.00 ± 0.12
SOD (U mL^{-1})	205.12 ± 10.45	152.94 ± 9.14**
GSH-R (mmol L^{-1})	6.24 ± 0.10	5.12 ± 0.14*
Serum sample		
Total protein (g dL^{-1})	6.29 ± 0.21	8.37 ± 0.22*
Albumin (g dL^{-1})	3.33 ± 0.45	5.09 ± 0.09*
Globulin (g dL^{-1})	2.96 ± 0.50	3.28 ± 0.15
A/G ratio	1.13 ± 0.12	1.55 ± 0.12
ALT (U L^{-1})	50.12 ± 2.56	68.45 ± 4.22**
AST (U L^{-1})	29.16 ± 2.56	38.55 ± 1.18**
ALP (U L^{-1})	16.20 ± 2.21	33.30 ± 1.45**
Urea (mg dL^{-1})	37.16 ± 0.45	51.22 ± 2.71**
Creatinine (mg dL^{-1})	1.01 ± 0.04	1.12 ± 0.24*
Glucose (mg dL^{-1})	55.12 ± 1.12	48.12 ± 2.12**
MDA (mmol mL^{-1})	1.69 ± 0.21	4.25 ± 0.14**
NO (µmol L^{-1})	6.96 ± 1.50	9.31 ± 0.15*

RBCs: Red blood cells, Hb: Hemoglobin, PCV: Packed cell volume, WBCs: White blood cells, SOD: Superoxide dismutase, GSH-R: Reduced glutathione, A/G: Albumin to globulin ratio, ALT: Serum alanine amino transferase, AST: Aspartate amino transferase. ALP: Alkaline transferase, MDA: Malonaldiahyde, NO: Nitric oxide. *,** indicate significant differences compared to the control values at $p \leq 0.05$ and $p \leq 0.001$, respectively.

Table 3. Virulence genes distribution of *E. coli* and *Salmonella* spp. isolated from diarrheic lambs.

Serovars	Extracted isolates (n)	Genes (n)		
Salmonella		***stn***		***hilA***
S. Enteritidis	3	3		3
S. Essen	1	0		1
S. Heidelberg	1	1		1
S. Infantis	2	0		2
S. Tsevie	1	1		0
S. Typhimurium	1	1		1
E. coli		***stx1***	***stx2***	***eaeA***
O6	1	1	0	0
O8	2	2	2	0
O26 : H11	1	1	1	1
O75	3	2	0	0
O84 : H21	2	0	2	0
O103 : H2	1	1	1	0
O114 : H4	1	1	0	0
O121 : H7	1	0	1	0
O128 : H2	3	3	0	0
O163 : H2	1	0	1	0

Multiplex PCR of the virulence of *E. coli* serogroups and *Salmonella* serotypes. All of *E. coli* isolated was identified by PCR as Shiga toxin producing isolates (prevalence 100%). The production of *stx 1, stx2 and eaeA* genes was varied among the isolated serogroups. The *stx 1* was shown in serogroups: (mainly O6), (O75), (O8), (O103), (O114) and (O128). Moreover, *stx 2* was shown in serogroups: mainly (O8), (O26), (O84), (O103), (O121) and (O163). On the contrary, the production of *eaeA* was shown among the recovered serogroups: mainly (O26), (O8) and (O78), (Fig. 1A). The production of *Stn and hilA* genes was varied among the isolated serogroups. *Stn* was shown in *S. Enteritidis*, *S. Heidelberg*, *S. Tsevie* and *S. Typhimurium*. Moreover, the production of *hilA* was shown among *S. Enteritidis*, *S. Essen*, *S. Heidelberg*, *S. Tsevie*, *S. Infanti* and *S. Typhimurium* (Table 3, Fig. 1B).

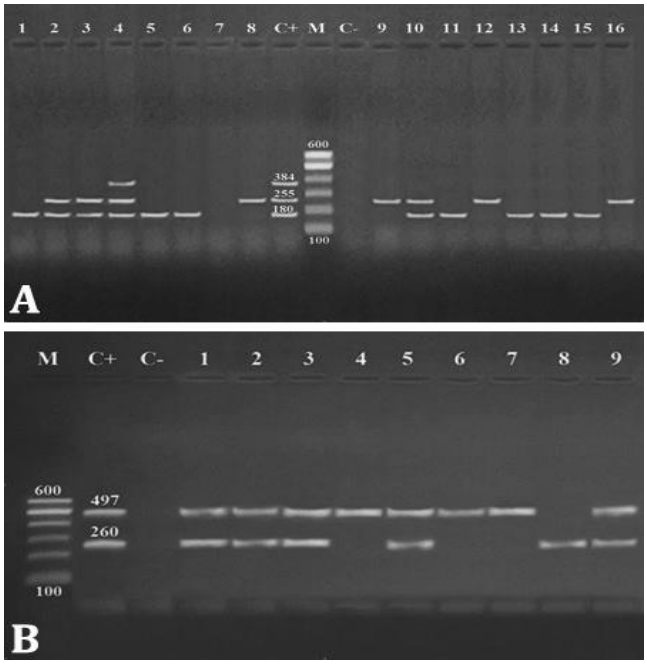

Fig. 1. Agarose gel electrophoresis of multiplex PCR. **A)** *stx1* (180bp), *stx2* (255 bp) and *eaeA* (384 bp) virulence genes for characterization of *Enteropathogenic E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes. Lane C-: Control negative. Lanes 1 (O6), 5, 6 (O75), 11 (O114), 13, 14 and 15 (O128): Positive *E. coli* for *stx1*gene. Lanes 8, 9 (O84), 12 (O121) and 16 (O163): Positive *E. coli* for *stx2* gene.Lanes 2, 3 (O8) and 10 (O103): Positive *E. coli* for *stx1* and *stx2* genes. Lane 4 (O26): Positive *E. coli* for *stx1*, *stx2* and *eaeA* genes. Lane 7 (O75): Negative *E. coli* for *stx1*, *stx2* and *eaeA* genes. **B)** stn (260 bp) and hilA (497 bp) virulence genes for characterization of *Salmonella* species. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive strain for *stn* and *hilA* genes. Lane C-: Control negative. Lanes 1, 2, 3 (*S. Enteritidis*), 5 (*S. Heidelberg*) and 9 (*S. Typhimurium*): Positive *Salmonellae* for *stn* and *hilA* genes. Lanes 4 (*S. Essen*), 6 and 7 (*S. Infantis*): Positive *Salmonellae* for *hilA* gene. Lane 8 (*S. Tsevie*): Positive *Salmonella* strain for *stn* gene.

Discussion

Bacterial enteritis in lambs is a serious problem facing the international intensified livestock production. The disease morbidity and mortalities result mainly from the severe alterations in the hemato-biochemical parameters and oxidative-antioxidative balance in affected lambs.[11] In the current study, the clinical and hemato-bicoemical parameters disturbances and the oxidative-antioxidative imbalance among lambs with bacterial enteritis were studied at Kafrelsheikh governorate, Egypt. Furthermore, the prevalence of STEC and *Salmonella* spp. enteritis among these lambs were determined. Our results are in agreement with what had been reported by Radostits *et al*.[9] who stated that the clinical signs of bacterial enteritis among lambs characterized by feces of clay to yellowish gray or grayish to greenish color containing mucous and sometimes blood. Many cases showed a rise in body temperature with congested mucous membrane.

Similarly, the recorded anorexia, depression, dullness and muscular weakness among lambs might be due to escape of intracellular potassium, hyperkalemia and hypoglycemia as confirmed in hemato-biochemical alterations obtained in the current study. The most isolated bacterial species in the current study were *E. coli* and *Salmonella* spp. and these bacteria are responsible for the clinical signs. *E. coli* bacteria adhere to the apical portion of microvilli which fuse with one another and become atrophic resulting in indigestion and malabsorption.[32] In salmonellosis, there is an excessive stimulation of active chloride secretion with inhibition of sodium absorption resulting in drawing of water tissue to gut leading to diarrhea.[9]

The current study demonstrates a highly significant increase in PCV, RBC count and Hb value than those in healthy ones. The increase in hematological parameters may be attributed to hemo-concentration, excessive loss of body fluid and dehydration which lead to decrease plasma volume.[9] Leukogram in diarrheic lambs found to be significantly depressed for total WBCs than the corresponding values in healthy ones. Differential leucocyte count revealed that there was marked lympho-penia, neutrophilia and monocytosis. The decrease of total leucocytic count in diarrheic lambs may be attributed to the stress of malnutrition. This suggestion was supported by the result obtained by Mgongo *et al.*[33] Lymphopenia might have been due to stressful condition produced by multiple etiological agents.

Serum analysis of diarrheic lambs showed significant decrease in serum glucose levels with compared to the control. The occurrence of hypoglycemia in diarrheic lambs may be attributed to weak or absence of normal suckling affinity and altered intestinal epithelial transport and developing endotoxic-septic shock.[34] On the other hand, increase in the concentrations of serum total proteins and albumin in diarrheic lambs are in line with Guzelbektes *et al*.[35] who showed that diarrhea also influenced the plasma protein profile increasing values for total serum protein and serum albumin concentration.

Ghanem *et al*. stated that inflammation of gastro-intestinal tract of diarrheic sheep and cellular destruction of the liver and intestinal mucosa lead to significant increase in serum enzyme activities of ALT, AST and ALP in diarrheic animals compared to the control healthy ones[36] which are in agreement with our findings.

Kidney function tests of diarrheic lamb in this study are in agreement with Singh *et al*. who stated that**,** uremia is a constant finding especially in the late stage of neonatal calf diarrhea with marked increase in serum urea and exerts its due effect in the pathogenesis of diarrhea.[13] This may be due to decrease in renal function and reduction in glomerular filtration rate caused by hypovolemia, systemic arterial hypotension and vasopressin release.

Concerning oxidative stress and antioxidant status of diarrheic lambs, our results are in agreement with previously report by Ahmed and Soad, as they mentioned that, the reduced SOD activities in diarrheic sheep lead to accumulation of oxidant substances and free radicals that caused cellular damage to the intestinal lining mucosa.[37] Higher MDA levels in serum of diarrheic lambs suggested increased production of lipid peroxidation in the liver, and indirectly pointed to enhanced free radical generation, lipid peroxidation and oxidative stress.

Bacterial isolates from collected fecal samples of diarrheic lambs revealed that many bacterial species are incriminated as causative agents in such problem, especially when the respective organisms have been isolated in pure culture as declared by Wani *et al*.[38] who isolated similar bacteria from fecal samples of diarrheic lambs and Nasr *et al*.[39] who isolated *E. coli* (34.20 %), *Salmonella* (5.26%), *Proteus* (13.10%), *Klebsiella* (7.89%) and mixed infection (21.00%).

In the current study *E. coli* and *Salmonella* were the main cause of bacterial diarrhea in lamb. Several investigations isolated the same organisms with various percentages.[40] The prevalence of *Salmonella* in the present study was higher than that reported by Younis *et al*.[41] (4.09%). Much more prevalence of *Salmonella* was reported by Moussa *et al*.[42] (43.53%). Differences of the prevalence rates of *Salmonella* in diarrheic lambs in comparison to the previous studies could be explained in the light of species and geographical locations and hygienic measures. These factors significantly influence the prevalence of salmonellosis.[41] The prevalence of *E. coli* in the current study was nearly coincided with the findings of Bendali *et al*.[43] in France (20.30%), but higher than those of Azzam *et al*.[44] (5.40%), and lower than that recorded by Osman *et al*.[45] (63.60%). The differences of the prevalence rates of *E. coli* in diarrheic lambs may be attributed also to the geographical locations and management practice as

well as hygienic measures where ETEC infection occurs mainly through ingestion of contaminated food or water.[6] *Proteus sp. and Klebsiella sp*. appear to play a minor role as causative agents of diarrhea in sheep.[40]

Up to our knowledge, the current study is one of the first researches on the characterization of STEC and *salmonella* spp. responsible for diarrhea among neonatal lambs in Egypt. The virulence of *E. coli* serogroups mainly controlled via the production of virulence encoding genes, in particular *stx1, stx2* and *eaeA.* Our results detected that all of serotypes are STEC and one serotype belonged to EHEC and these serotypes are considered major causes of enteritis among animals and hemorrhagic enteritis among humans.[12] O8 and O75 serogroups are known to be ETEC which commonly isolated from diarrheic lambs.[12] In the current study, these 2 serotypes carry the Shiga toxin producing genes and this may be attributed to the nature of horizontal gene transfer (HGT) among different *E. coli* serotypes,[46] which is responsible for evolution of new pathogenic serotypes of *E. coli*. The limitation of our study was that we did not identify *sta* genes for these 2 serotypes to confirm the existence of new hybrid serotype STEC-ETEC and further work is required to confirm that finding. However, we believe that this finding is not far from the reality because we depend on gold standard serological tests for identification of *E. coli* serotypes.

The data demonstrated that a wide variation of STEC and ETEC serogroups were incremented in the incidence of diarrhea in small ruminates in Egypt as similar to the results obtained by Aref *et al.*[46] The coexisting between STEC and ETEC associated virulence genes in *E. coli* strains of human, animal, and environmental origins has been reported in Germany, United States and Slovakia and some of which have been associated with human disease.[47]

The virulence of *salmonella* serogroups mainly controlled via the production of *Stn and hilA* genes which were varied among the isolated serogroups. *S. Enteritidis, S. Heidelberg and S. Typhimurium* were all commonly isolates from diarrheic lambs.[38]

In conclusions*, E. coli* and *Salmonella* spp. are the most important cause of bacterial enteritis and diarrhea among lambs at Kafrelsheikh governorate, Egypt. Among the isolated bacteria, STEC especially EHEC and *salmonella* spp. are the most prevalent serotypes and this represents a veterinary and public health threat. The alteration in hemato-biochemical parameters and the disturbance in the oxidant-antioxidant balance among affected lambs could be used to adopt new strategy towards more suitable treatments and preventive measures against such problem.

Conflict of interest

The authors declare no financial or conflict of interest regarding this study that could inappropriately influence the work.

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