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Molecular study on *Salmonella* infection in lambs and the associated immunological and biochemical changes

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Abstract

A total of (100) samples were collected from diarrheic lambs aged (6-12 month), which were 50 fecal samples and 50 serum samples. These samples were collected from private farm in El-Qalubaya Governorate. Bacteriological examination of fecal samples revealed that the isolation of *Salmonellae* were 12%. Serological identification of *Salmonellae* revealed four strains of *Salmonella* Typhimurium and two strains of *Salmonella* Enteritidis. Antibiogram pattern were applied among the isolated *Salmonellae* showed that *Salmonellae* were sensitive to enrofloxacin and norfloxacin and resistant to ampicillin, amoxicillin, cefotaxime, flumoquine, gentamycin, nalidexic acid and neomycin. The MIC showed that enrofloxacin is sensitive for all tested strains of *Salmonella* while with flumoquine examined strains of *Salmonella* were resistant. Polymerase Chain Reaction (PCR) were applied to determined the virulence genes in isolated *Salmonellae* (invA, stn, avrA and bcfC) and antibiotic resistance genes (blaTEM, blaSHV, blaCTX and qnrS). The blood samples were collected to obtained serum using to determination the biochemical measures of liver enzymes function as Glutamic Pytuvate Transaminase (GPT), Glutamic Oxaloacetic Transaminase (GOT), Alkaline-phosphatase (ALP) parameters of immunity as Immunoglobin A (IgA), Immunoglobin G (IgG) and Immunoglobin M (IgM), and some antioxidant parameters as Total Antioxidant Capacity (TAC) and Nitric Oxide (NO).

Keywords: *Salmonella, Diarrhea, Lamb, PCR, Immunity and biochemical changes.*

Introduction

Sheep are the most important livestock that are noted for their ability to convert low opportunity cost feed into valuable good products as milk and meat **(Wilsmore, 2006).**

Salmonella is primary etiological agent of diarrhea and considered an important zoonotic pathogen worldwide. Salmonellosis is characterized by watery and mucoid diarrhea with the presence of fibrin and blood (**Fossler *et al.* 2005**).

Diarrhea is the major problem in livestock that may lead to economical losses in Egypt and the world (**Ghanem *et al.,* 2012**). In Salmonellosis, there is an excessive stimulation of action of chloride secretion with inhibition of sodium absorption lead to drawing of water tissue to gut causing diarrhea (**Radostits *et al.,* 2007**).

Antibacterial resistant of *Salmonella* are increase due to the wide use of antibacterial agents in animal feed at sub-therapeutic level or prophylactic dose (**Zewdu** and **Corneilus, 2009).**

PCR technique was used as a molecular tool to confirm the existed resistance pattern of Antibiogram test. The most common detected virulence gene (***invA, avrA,* and *bcfC***) using specific primer for each ***invA*** and ***bcfC*** gene were

expressed in all examined *Salmonella* serotypes followed by ***avrA*** (60%) from examined samples (**Mohammed, 2014**) and **PCR** was applied by testing the isolates existing resistance to antimicrobial agent for presence of the antimicrobial resistance genes as *Beta*-Lactamase antibiotic as (amoxicillin), cephalosporins as (cefotaxime) and fluroquinolones as (enrofloxacin).

This study was conducted to shed some light on the on the following points:-
1- Isolation and Identification of *Salmonella* from samples of diarrheic lambs,
2-Antibiogram pattern and MIC of isolated *Salmonellae*.
3- Detection of virulence genes and antibiotic resistance genes of *Salmonellae* using PCR.
4- Determination of biochemical and immunity changes.

Materials and Methods

Collection of Samples

A total number of 100 samples represented by fecal samples and blood samples (50 of each) of the same animals were directly collected from diarrheic lambs with age (6-12 month) suffering from diarrhea with high temperature in El-Qalubaya Governorate private Farms. Fecal samples collected in plastic bags and send to laboratory as soon as possible in ice box for bacteriological examination. The Blood samples were collected in sterile screw clean test tube without anticoagulant to separate serum for determination the biochemical changes of liver function enzymes, parameters of immunity and some parameters of antioxidant.

Isolation of Salmonella

The fecal samples were prepare as 25 gm of samples mixed with 225 ml of sterile buffered peptone water were incubated at 37^{o}C for 24 hours (pre-enrichment media) incubated at 37^{o}C for 24 hours. After incubation 1 ml of pre -enrichment broth was transferred into 10 ml of Muller-Kauffmann Tetrathionate/Novobiocin broth, and 0.1 ml of pre-enrichment broth to 10 ml of Rappaport-Vassiliadis with Soya and incubate at 41.5^{o}C for 24 hours, each pre incubated culture was streaked onto XLD and S.S., then incubated at 37°C for 24 hours. Suspected typical colonies were examined for morphological and characteristic appearance, and pure culture of the isolates were identified biochemically according to (**Quinn *et al.,* 2002**), final results were confirmed by using the API 20 E (Biochemical identification kit).

Serological Identification according to (**ISO-6579/2014**)
The suspected *Salmonella* was carried out by using (Slide Agglutination Technique, according to Kauffmann White Scheme). A diagnostic poly and monovalent *Salmonellae* O and H (phaseI and phase II) antisera were obtained from DENKA SEIKN, Co., LTD, Tokyo, Japan).

Dermination of biochemical and immunity parameters

Blood samples without anticoagulant to separate serum for measure liver enzymes as (GPT), (GOT (ALP) and some antioxidant parameters as (TAC) and (NO) and other parameters of immunity as (IgA), (IgG) and (IgM), Interleukin-6 (IL-6). The animals divided into four groups, the number of each group (10) animals, Apparent healthy group were kept as (control negative), diseased group, the lambs had clinical symptoms (control positive) and two treated groups, third group treated with cefotaxime (dose 1 gram of cefotaxime per 50 kg. I/M daily for successive 3 days) and fourth group treated with cefotaxime + Vit E antioxiden (Tocosel-forte injection 5ml dose 5ml./50 kg I/M). Drug treatment have been compare for the rates of clinical signs and bacteriological signs, were clinical signs cure are based on resolution of diarrhea, fever and other signs while bacteriological cures become free fecal samples after therapy.

Antibiogram Pattern

Antibiogram pattern was applied to determine susceptibility of isolated *S.* Typhimuirum and *S.* Enteritidis to antibiotic agents discs (Oxoid). This apply by using Disc Diffusion Technique with Mueller Hinton agar according to (**Quinn, *et al.,* 2002).** The results were interpreted according to **(CLSI, 2014)**.

Minimum Inhibitory Concentration (MIC):

MIC defined as the lowest concentration of

antibiotics that prevents the in vitro growth of bacteria (break point represents the MIC). The lowest concentration of antibiotic agent in the series that prevents in vitro the growth of the organisms was taken expressed as micrograms or (unit) ug/mL. MIC as described by **(NCCLS, 2014).**

PCR Technique

This technique according to (**Bakshi *et al.*, 2003**). Molecular Identification of DNA extraction, detection of virulence genes ***(invA,.stn, avrA* and befc**) in *Salmonella* serovars where antibiotic resistance genes.

DNA extraction

DNA extraction from samples was 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. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

Oligonucleotide Primer

Primers used were supplied from (**Metabion-Germany)** are listed in Table (1): detected the primers sequences, target genes, amplicon size and cycling condition of virulence genes of *Salmonella* isolates.

PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 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

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Appli chem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. A 100 bpDNA Ladder (Qiagen, Germany, GmbH) and generuler 100 bp ladder (Fermentas, Germany) were 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.

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions of virulence genes of *Salmonella* isolates

Target gene	Primers sequences (5/- 3/)	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
bcfC	ACCAGAGACATT-GCCTTCC CTTCTGCTCGCCGC-TATTCG	467	94˚C 5 min.	94˚C 45 sec.	53˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Huehn *et al.* (2010)**
avrA	CCTGTATTGTT-GAGCGTCTGG AGAAGAGCTTCGTT-GAATCC	422	94˚C 5 min.	94˚C 45 sec.	58˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	
stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	94˚C 5 min.	94˚C 45 sec.	59˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Murugkar *et al*., (2003)**
invA	GTGAAATTATCGCCAC-GTTCGGGCAA TCATCGCAC-CGTCAAAGGAACC	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Oliveira *et al*., (2003)**

Table (2). Primers sequences, target genes, amplicon sizes and cycling conditions of antibiotic resistance genes of *Salmonella* isolates

Target gene	Primers sequences (5/- 3/)	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
BlaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516	94˚C 5 min.	94˚C 45 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Colom *et al*., (2003)**
blaCTX	ATGTGCAGYACCAG-TAARGTKATGGC TGGGTRAARTARGTSAC-CAGAAYCAGCGG	593	94˚C 5 min.	94˚C 45 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Archambault *et al*., (2006)**
qnrS	ACGACATTCGTCAACTG-CAA TAAATTGGCACCCTG-TAGGC	417	94˚C 5 min.	94˚C 45 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Robicsek *et al*., (2006)**
blaSHV	AG-GATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Colom *et al*., (2003)**

Results

Table (3). Clinical symptoms of diarrheic lambs:-

Examined Samples	No. of Examined Samples	Types of Diarrhea	Positive Samples	%
Fecal Samples	50	Semi solid Diarrhea Watery Diarrhea Bloody Diarrhea	35 10 5	70% 20% 10%

Table (4). Incidence of isolated enteric bacteria from examined fecal samples:-

No. of examined samples	Enteric bacteria isolates	Positive samples	% of positive samples
50	*Escherichia coli* *Salmonella* spp. *Proteus* spp. *K.* oxytoca *K.* pneumoniae	10 6 4 4 1	20% 12% 8% 8% 2%
Total		25	50%

*Percentage calculated according to number of samples

Table (5). Isolation, percentage and antigenic structure of *S.* serovars isolates

No. of fecal *S*amples	*S.* serovars	No.	%	*S*omatic antigen (O antigen)	Flagular antigen (H antigen) (Phase I)	(Phase II)
50	*S.* Typhimurium *S*, Enteritidis	4 2	8% 4%	1, 4, [5], 12 1, 9, 12	i g,m	1, 2 --

from fecal samples :-

* The percent was calculated according to number of examined samples (50)

Table (6). Antibiogram pattern of isolated *Salmonella* serovars:-

Salmonella serovars / Antibiotic discs	Symbol	Conc.	*S.* Typhimurium No.=4 strains		*S.* Enteritidis No=2 strains	
		(ug)	S	R	S	R
Ampicillin	AM	10ug	0/0	4/4	0/0	2/2
Amoxicillin	AMK	25ug	0/0	4/4	0/0	2/2
Cefotaxime	CTX	30ug	4/4	0/0	2/2	0/0
Enerofloxacin	ENR	10ug	4/4	0/0	2/2	0/0
Flumoquine	UB	30ug	0/0	4/4	0/0	2/2
Gentamycin	GN	10ug	0/0	4/4	0/0	2/2
Nalidexic acid	NA	30ug	0/0	4/4	0/0	2/2
Norfloxicin	NOR	10ug	4/4	0/0	2/2	0/0
Neomycin	N	10ug	0/0	4/4	0/0	2/2

Table (7). MIC of antibacterial agents of isolated *Salmonella* serotypes:-

Antibiotic agents *S.* serovars	Flumoquine (UB-30ug.)	Cefotaxime (CTX-30ug)	Enrofloxacin (ENR-30ug.)
***S.* Tyhimurium** ***S.* Enteritidis**	12.5 ug/ml 6.25ug/ml	6.5ug 3.125ug	0.39ug/ml 0.78ug/ml

The MIC correlates of: Fluomquine and Enrofloxacin were R>_1 and S<_ 4 while Cefotaximee was R >_64 and S <_16

Table (8). Virulence genes of isolated *Salmonella :-*

Samples	Sample ID	Results			
		invA	***stn***	***avrA***	***bcfC***
1	***S.* Typhimurium.**	+	+	+	+
2	***S.* Enteritidis**	+	+	+	+

The products of PCR 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 the products was loaded in each gel slot. A 100 bp DNA Ladder (Qiagen, Germany, GmbH) and generuler 100 bp ladder (Fermentas, Germany) were 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.

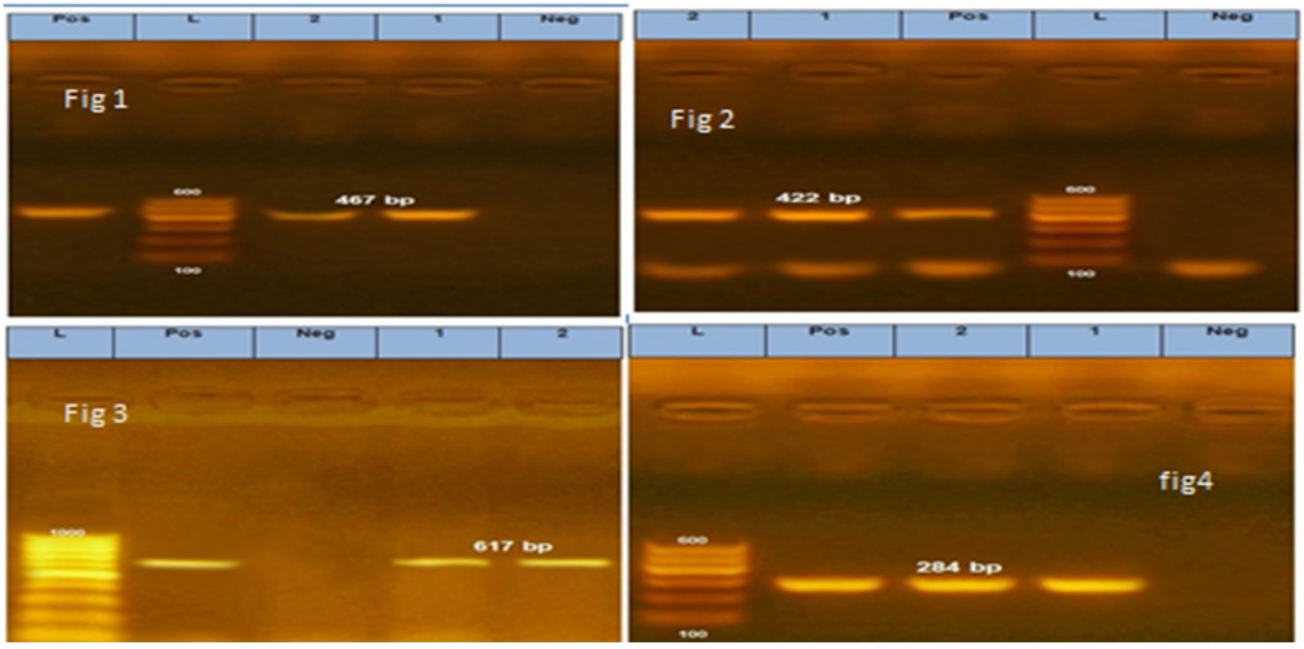

Fig. (1): The agarose gel electrophoresis of **PCR** for detection of **bcfC** virulence gene positive sample with band of amplication size at **467**bp.
Fig. (2): The agarose gel electrophoresis of **PCR** for detection of **avrA** virulence gene positive sample with band of amplication size at **422**bp.
Fig. (3): The agarose gel electrophoresis of **PCR** for detection of **stn** virulence gene positive sample with band of amplication size at **617**bp.
Fig. (4): The agarose gel electrophoresis of **PCR** for detection of **ivaA** virulence gene positive sample with band of amplication size at **284**bp

Table (9). Antibiotic resistance genes of isolated *Salmonella*

Samples	Results			
	blaTEM	***blaSHV***	***blaCTX***	***qnrS***
***S.* Typhyimurium**	+	+	+	+
***S.* Enteritidis**	+	+	+	+

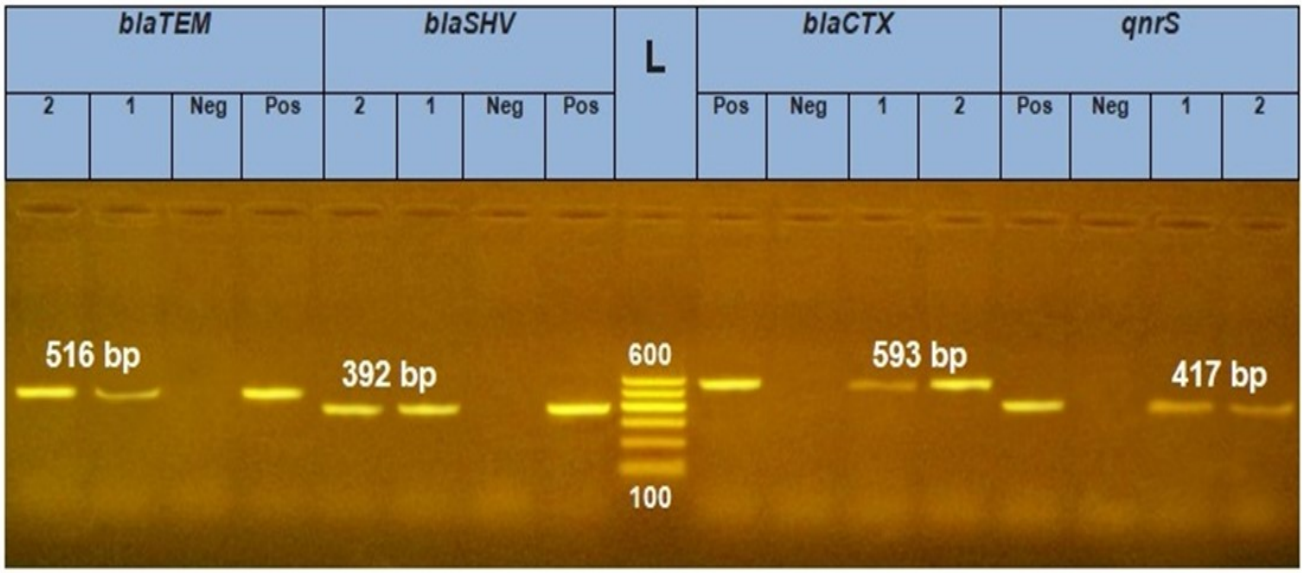

Fig (5): Antibiotic resistance genes of *Salmonella* isolates:-
The agarose gel electrophoresis of **PCR** for detection of **antibiotic resistance genes:**
blaTEM-positive samples with band of amplicon size at **516bp**
blaSHV-positive samples with band of amplicon size at **392bp**
blaCTX-positive samples with band of amplicon size at **593bp**
qnrS-positive samples with band of amplicon size at **417bp**

Table (10). Immunity changes of *Salmonella* infection and methods of therapy on lambs :-

Groups / Parameters	Apparent healthy group (No.=10)	Diseased group (No.=10)	Third group Treated with Cefotaxime (No.=10)	Fourth group Treated with Cefotaxime+vitE (No.=10)
IgA mg/ml	0,5463±0,04335[a]	0,1323±0,009597[b]	0,1910±0,03512[c]	0,2907±0,03518[ac]
IgG mg/ml	13,30±0,5434[a]	7,527±0,5434[b]	9.480±0,3205[c]	9,170±0,2843[ac]
IgM mg/ml	2,450±0,2802[a]	0,9833±0,1065[b]	1,710±0,3079[c]	1,260±0,1127[ac]

Table (11). Biochemical changes of *Salmonella* infection and methods of therapy on lambs :-

Groups / Parameters	Apparent healthy group No.=10	Diseased group No.=10	Third group Treated with cefo-taxime No.=10	Fourth group Treated with cefo-taxime+vit E No.=10
NO nmol/ml	8,030±1,518[a]	28,47±5,4011[a]	18,98±1,796[c]	11,20±0,5438[ac]
TAC nmol/ml	5,223±0,5315[a]	1,333±0,2318[b]	1,657±0,1963[c]	2,430±0,3350[ac]
IL6 pg/ml	7,863±0,5985[a]	35,42±5,475[b]	20,56±8,118[c]	12,96±1,500[ac]
GPT u/l	57.65±4.666[a]	159.5±10.36[b]	88.95±4.748[c]	67.6±6.295[ac]
GOT u/l	59,21±9,836[a]	369,6±64,91[b]	168,8±12,15[c]	56,33±8,697[ac]
ALP u/l	78,63±0,5985[a]	354,2±5,475[b]	205,6±8,118[c]	129,6±1,500[ac]

Statistical analysis
The results were analyzed by a one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparisons using computer software, Duncan's Multiple Range Test

Discussion

Salmonella infection in sheep characterized by major syndroms were enteritis and septicemia in addition to abortion in pregnant females. The most common form in adult animals was acute enteric, while septicemia is the most common form in young lanbs **(CFSPH, 2005)**
In this study, **Table (3)** stated that the diarrhea classified into semi solid diarrhea, watery diarrhea and bloody diarrhea with percentage 70%, 20%, 10% respectively, The most diseased lambs are normal temperature ranged from (38°C - 39.3°C) while the raise in temperature in early stages of infection then disappeared. These finding are nearly similar to the previous reported by (**Mahamoud, 2014**) who found that the pasty (semi solid), watery and bloody diarrhea were 63.8%, 24.3%, and 2.6% respectively.

Our result reported that infection with *Salmonella* of clear clinical signs as fever, anorexia, diarrhea of offensive odor, fleece and hind quarter soiled with feces, because *Salmonella* enter epithelium mucosa by endocytosis and secrete enterotoxins lead to acute inflammation, inhibition protein synthesis and increase secretion of fluid in large and small intestinal bowel is increased with decreased absorption the result diarrhea **(Coburn *et al.,* 2007**).

From **Table (4)** showed that the bacterial agents involving in diarrheic lambs including *E.coli* which represent 20%, *Salmonella* spp. With 12% then *Proteus* spp. and *Klebsiella* spp. are 8%, this results are nearly similar with that obtained by (**Zain-Eldin *et al.,* 2013**) who isolated *E.coli* (30%), *Salmonella* spp. (15%), then *Klebsiella* spp. (8.33%) and *Proteus* spp. (5%).

Bacterial agents involving in diarrhea represents (50%) of enteric bacteria from the examined samples of diarrheic lambs. Several investigation isolated the same organisms with various percentage, *E.coli* adhere to the apical portion of microvilli, this fuse with one another and become atrophic resulting indigestion and mal-absorption (**Schoenian, 2009**). But in *Salmonella* there in an excessive stimulation of active chloride secreation with inhibition of Sodium and absorbtion resulting in drawing of water tissue to gut which leading to diarrhea **(Radostis *et al.,* 2007**). While *Klebsiella* spp. and *Proteus* spp. were appear to play a minor role as causative agents of diarrhea in sheep such observation was previously recorded by **(Joshua *et al.*, 2011)**
The present study in **Table (5**) showed that, the incidence of *Salmonella* were isolated 6 strains of *Salmonella* from 50 examined samples with percentage **(12%**). This results agree with to recorded by (**Amany and Hala, 2013**) who determined *Salmonella* from lambs was 11.6%

and (**Ibrahium and EL-Gohary, 2007**) who recorded the incidence of *Salmonella* was 12.73%. but our data higher than (**Nasr *et al.,* 2014**) who found the prevalence of *Salmonella* associated with diarrhea in lambs was 5.26% and by (**Manar, 2017**) who reported the rate of isolated of *Salmonella* from diarrheic lambs are (1.52%).

In the current study, also from Table (5) showed that, the serological typing of *Salmonella*, and antigenic structure of *S.* serovars that could be isolated four strains of *S.* Typhimurium (8%) and two strains of *S.* Enteritidis with percentage (4%) from (6) stains of *Salmonella*e with percentage of 12% of all fecal samples of diarrheic lambs. The results of the current study for serotype of *Salmonella* are nearly similar to that recorded by (**Amany and Hala, 2013**) who recorded that the isolated of *S.* Typhimurium with 5% of isolated from lambs

The isolated serotypes of *Salmonella* were plays an important role in inducing diarrhea in lambs and sheep, and *Salmonella* is importance in both human and animal's infections (**CFSPH, 2005**).

Table (6) showed the results of Sensitivity test of *Salmonella* serovars showed all isolates of *Salmonella* serovars (*S.* Typhimurium and *S.* Enteritidis) were sensitive to enrofloxacin, norfloxacin, followed by cefotaxime, while the same isolates of *Salmonella* serovars were resistant to amoxicillin, ampicillin, flumoquine, nalidaxic acid and neomycin. This results are nearly similar to those recorded by **(Tanios *et al.,* 2014**), (**Abd EL-Tawab *et al.,* 2016**) and (**Manar, 2017**) who obtained that cefotaxime, norfloxacin are sensitive, while resistance were recorded against ampicillin, aomxicillin, levofloxacin and ciprofloxacin were 100%, 62.5%, 62.5% and **50%** repectivelly. The results of our sensitivity in this study agree with that recorded by **(Atfeehy, 2007)** who stated that *Salmonella* isolated (*S.* Typhimurium) were resistant to amoxicillin, flumoquine and intermediate to ciprofloxacin and sensitive to Aflaxacin also (**Gebreyes *et al.,* 200**9) who recorded that *S.* Typhimurium isolated from lambs and sheep are highly sensitive to enrofloxacin, then amikacin but resistant against streptomycin.

Sensitivity test is one of the steps in treatment of *Salmonellosis* in animals by use of the chemotherapeutic agents and variable from place to place and from case to another. The result due to wide use of antibiotics may be produce new resistant bacteria for this reason the sensitivity test is the steps of controlling of *Salmonellosis*.

The extensive use to antibiotics not only in human and Veterinary medicine but excessive used in livestock production for disease prevention or as growth promoting feed additives which lead to serious problem and spread of (MDR) bacteria with increase produced new strains of bacteria.
From table (7) showed MIC of *Salmonella* isolates. From fecal samples of diarrheic lambs to antimicrobial agents (flumoquine, cefotaxime and enrofloxacin), there were variations in inhibitory effect of different antimicrobials on different isolates of *Salmonella.* enerofloxacin gave highest inhibitory effect as it is MIC was **(**0.39ug/ml) for *S.* Typhimurium and **(**0.78ug/ml) for *S.* Enteritidis, whil**e** flumoquine gave lowest effect on MIC (12.5ug/ml and 6. 25ug/ml) for *S.* Typhimurium and *S.* Enteritidis respectively .This results are agree with that obtained by **(Ibrahium *et al.,* 2001)** who record that MIC of enerofloxacin of *S.* Typhimurium ranged from (0.68ug/ml. to 1.56 ug/ml**.**) while, the results disagree with **(Andrewe *et al.,* 2002)** who recorded that MIC of Ciprofloxacin on *S.* Typhimurium was 0.125 ug/ml.

In this study from table (8) detection of virulence genes of *Salmonella* by PCR (invA, stn, *avrA* and *bcfC*) considered important cause of diarrhea and isolated from all isolates of *Salmonella* which considered important cause of diarrhea. The invA gene is detected from all isolates which invasion of the intestinal epithelium cells to allow *Salmonella* organism to enter the host this gene detected in all *S.* serovars this nearly similar to (**Shekhar and Singh 2014**). The stn gene is responsible for production of enterotoxin of *Salmonella* infection which causing diarrhea this gene isolated from all isolates of *Salmonella* serovars this agree with that recorded by (**Murugkar *et al.,* 2003).**

AvrA gene it is responsible of *Salmonella* spp. virulence by limitation of the host responces especially macrophages. In this study this gene present in all isolates this agree with (**Borges *et al.,* 2013**) who found that avrA in 100% of the isolates. The bcfc gene is responsible for intestinal colonization which present in all isolates this results similar to the finding of **(Mohamed, 2014**) who detected bcfc in all isolate.

The virulence genes of *Salmonella* depends on virulence factors encoded by specific genes, The virulence genes of *Salmonella* can be considered as important virulence genes play an important role in development of diarrhea originated from *Salmonella* infection.

From table (9) showed the antimicrobial resistant genes (blaTEM, blaSHV, blaCTX and qnrS) were detected in all isolated of *Salmonella*. Our results are compatible with the results of **(Tanios *et al.,* 2014**) who detected the presence of blaTEM 100% in all isolates but this results not compatible with (**Amira, 2016**) who applied PCR on all isolates which exist and not exist resistance to antibiotic and found (blaTEM 90%) and (qnrS 70%).

Antimicrobial resistant genes were detected by using PCR technique which is as molecular tool to confirm the existed resistance pattern of Antibiogram test. This was applied by testing the *Salmonell*a isolates existing resistance to antimicrobial agents for presence of antimicrobial resistance genes were 4 antimicrobial resistance genes (blaTEM, blaSHV, blaCTX) as *Beta*-Lactamases antibiotics genes and **(**qnrS) as Quinolones were responsible for the following antimicrobial resistant genes such as Amoxicillin , Ampicillin (*β*-Lactamases).

Table (10) Showing that infection with *Salmonella* associated with alteration in immunity system (IgA, IgG, IgM), significant changes when compare groups of apparent healthy and diseased group and groups of treatment, also found significant changes between this treatment (third and groups), because *Salmonella* invade immune system of infected animals that agree with (**Tsolis, 1999)** who reported that the virulence of *Salmonella* in invading the intestinal mucosal epithelia and surviving within macrophages, multiplying in lymphoid tissues, and evade host defense immunity systems, leading to systemic disease (enteropathyogenicity). reated groups in this study when compare with control groups after 15 days from treatment founded that group that received Cefotaxime and Vit. E was the best immunity response because the use of Cefotaxime is a newer cephalosporin antibiotic with good in vitro activity against most tested strains of *Salmonella* to give good therapeutic effect and addition of Vit. E has antioxidant effect which modulate oxidative effect of infection and improve response of immune system , these results agree with (**Araceli, 2003**) who found that the use of Cefotaxime in dose (1 gm./daily for 7 days I/M) or (1gm /3 daiys for only 2 to 3 days I/M), and Vit.E (Tocoselforte injection 5ml per50kg, I/M which produced by Pharma Swede-Egypt Vit. E and Selenium as anticxident) and reported Vit. E has an effect on the humoral immune and ,cell mediat system in the absence of an adequate supply of tocopherols lipid hydroperoxides lead to direct cellular damage because Vit.E reacts as a chain-breaking antioxidant, there by neutralising free radicals and preventing the oxidation of lipids.

From table (11) showing Increased (NO) level in the diseased group (28,47±5,4011^{b} nmol/ml) than healthy animals (8,030±1,518^{a} nmol/ml) indicates that the *Salmonella* has oxidative effect on diseased animals and decrease antioxidant which stimulate macrophages to produce Free radicals (NO) that produced primarily as effectors molecules of the host defense response. On the other hand, (NO) frequently is an important mediator in intracellular inhibition of bacteria multiplication, which results in lower bacteria activation and more efficient host clearance of the infection, hence recovery these results were agree with (**Çenesiz *et al.,* 2007**) who detected that (NO) is a plays important role in many physiological and pathological processes.

Our results from this table showed **(TAC)** were decreased in diseased sheep (**1,333±0,2318^{b}**) than the results in apparent healthy sheep (**5.223±0.53154 ^{a}).** So we must give antioxidant as Vit.E which act as scavenging to free

radial decreasing oxidative stress of *Salmonella* infection and produce balance between radial oxidant and antioxidant balance (Redox balance) by preventing lipid peroxidation by donation to free radial.

This result reported that infection with *Salmonella* has effects of liver enzymes functions disorder on diseased animals lead to increase GOT to five times normal concentration than apparent healthy group also GPT increase due to infection and ALP. Liver disorders appear among control and treated groups and significant change between two treated groups since fourth group that receive Vit. E achieved better results due to antioxidants effect of Vit. E on cells this study came agree with (**Francesco *et al.,* 1993**). Serum liver function of diarrheic lambs showed significant changes this results attributed to inflammation of gastrointestinal tract of diarrheic lamb and cellular destruction of the liver and intestinal mucosa (**Ghanem *et al.,* 2012)**.

Our results showed inflammatory response with *Salmonella* infection lead to increase in cytokine IL-6 in diseased group than other groups, these results agree with (**Henry *et al*., 2002**), who reported that infection of *Salmonella* produce inflammation lead to increase of IL-6, also these increase of came agree with (**JasmineKaur, 2012**) who said the activation of macrophages by lipopolysaccharide (LPS) from *Salmonella* species, also results in the release of inflammatory cytokines in infected animal such as IL-6.

Conclusions

1- S*almonella* is still an important problem and most common bacteria causing disease in livestock and human, the importance of this organism as a source of infection to human and other animals which indicates the close relationship between animals and human (Zoonotic diseases).

2- *Salmonella* infection, were the clinical symptoms as diarrhea and fever are improvement within 5 days after start of therapy, while the bacteriological cure free from (day 4 up day 15 after start of therapy (Fecal samples are negative).

3- We must be raise awareness about antibiotic resistance in diarrheic sheep and the veterinarian should exercise caution when prescribing antibiotics and *Salmonella* in sheep needs more investigation of the disease to control and eradication of the disease which has a great economic and public health importance.

4-Effect of antibiotics on the levels of TAC, NO, IL-6, Liver enzymes as GOT, GPT and ALP, Immunoglulins (IgA, IgM and IgG) were measured in the serum of blood samples (control and treated groups of lambs).

5- Therapy of infected lambs with *Salmonella* occur on the day 4 and 15 after start of therapy with either (Cefotaxime(1g/day I/M for 3 days) or Cefotaxime (1g/day I/M for 3 days+Vi.t E) which Vit. E has an effect of the humoral immune and cell mediate system.

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