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PREVALENCE OF SALMONELLAE IN SHEEP AND ITS ENVIRONMENT WITH SPECIAL REFERENCE TO DETECTION OF VIRULENCE ASSOCIATED GENE USING PCR.

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SUMMARY

The present investigation showed that the incidence of salmonellae in sheep and lambs was (4.49%) and (9.18%) respectively. The incidence in sheep differed according to age and health conditions as it was higher in lamb than in adult and in diseased than apparently healthy as it was (12.73%) and (4.65%) in diseased and apparently healthy lambs. Meanwhile, it was (6.67%) and (3.39%) in diseased and apparently healthy adults sheep respectively. The prevalence of salmonellae in slaughtered sheep was (4.44%) and it was higher in intestines (6.67%) than in livers and lymph nodes (3.33%) each. The prevalence of salmonellae in dead lambs was (11.76%) and this incidence was similar in the examined livers, intestines and lymph nodes (11.76 %) each. The incidences in feedstuffs, water, soil, waste samples and swabs from workers' hands in environment of living sheep, lambs and dead lambs were (5.71 %, 2.86%, 8.57%, 22.22% and 11.11%) respectively. While, the incidences from drain water, swabs from walls and workers' hands in environment of slaughtered sheep were (10%, 12.5% and 6.67%) respectively. The isolated salmonellae were belonged to 7 serovars and *S.* Typhimurium was most predominant isolate. Also *S.* Arizonae, *S.* Cerro, *S.* Enteritidis, *S.* Ferruch, *S.* Montevideo and *S.* Sandiego were isolated. *S.* Arizonae, *S.* Ferruch, *S.* Montevideo, *S.* Sandiego and *S.* Typhimurium harboured the virulence associated gene. While, *S.* Cerro, *S.* Enteritidis and S. Typhimurium (from slaughtered sheep) didn't harbour this gene. *S.* Arizonae, *S.* Cerro and *S.* Ferruch yielded (100%) mortality rate in each, followed by *S.* Sandiego (80%) then *S.* Typhimurium, *S.* Typhimurium (from slaughtered sheep) and *S.* En-

teritidis (60%) each, while *S.* Montevideo yielded lower mortality rate (40%). It was clear that (95.24%) of the isolates were susceptible to ciprofloxacin and enrofloxacin then, (90.48%) were susceptible to flumequine and (83.33%) were susceptible to amikacin. On the other hand the lower susceptibility incidences were observed to streptomycin (19.05%) and cefadroxil (23.81%). Enrofloxacin gave the highest inhibitory effect as its MIC was 0.195-3.12 µg/ml for sensitive isolates followed by ciprofloxacin and flumequine with MIC: 0.39-3.12µg/ml for sensitive isolates while chloramphenicol gave the lowest effect on the isolates as its MIC was 3.12- 12.5µg/ml for sensitive isolates.

INTRODUCTION

Sheep is the important animals in Egypt for wool production and considered a triple purpose animal which provided beside the wool, meat and milk. Salmonellae are among the most common bacteria that causes diseases to sheep and produced low production and great economic losses specially *S.* Abortusovis which leading to abortion. Also, salmonellae in sheep cause enteritis, metritis and septicemia; all are potentially lethal to both ewes and lambs (Moredun Foundation, 2007). Salmonellae constitute a hazard to public health as all serovares can produce diseases to human (WHO, 2006). It causes typhoid, paratyphoid, septicemia and enteritis to man. The environment plays a very important role in transmition of salmonellae to and among the animals. PCR technique is widely adopted during the last few years as it provides new strategy for rapid and sensitive detection of salmonellae as it allows the detection of single cell (Li et al., 1988) and measure the virulence of *Salmonella* by detecting the presence of virulence gene(s) without animal inoculation or plasmid profiling (Joseph et al.,1999) or amplification of characteristic genetic markers (Skwark et al., 2004) .Uncontrolled use of antibiotics in animal treatment or as prophylaxis leads to development of multi-resistant strains. The purpose of this work was to investigate the following items:

*Isolation and identification of salmonellae from apparently healthy and diseased sheep (adults and lambs) and their environment.

*Serological identification of salmonellae.

*Detection of virulence associated gene in some isolated salmonellae using PCR and detection of their pathogenicity in mice.

*Determination of antibiotic sensitivity tests among the isolates by disc diffusion test and MIC.

MATERIALS AND METHODS

***Sampling:**

1-living sheep:

One hundred and eighty seven fecal samples were collected from grazing sheep in farms along Egypt or sporadically (89 adults and 98 lambs) either apparently healthy or diseased (showing symptoms of illnesses, diarrhea and septicemia) from rectum with sterile gloves.

2- Slaughtered sheep and dead lambs:
Liver, intestine and lymph node samples were collected 30 each from slaughtered sheep from various slaughter houses or outside it. Also the same samples were collected from dead lambs 34 each.

3-Environmental samples: including feedstuffs, water, soil, waste samples and swabs from workers' hands among living sheep and drain water, swabs from walls and workers' hands in slaughter houses.

Each sample was collected in sterile polyethylene plastic bag while water was collected in sterile glass bottle and send to laboratory as soon as possible.

*** Isolation of salmonellae by cultural method:** according to International Commission on Microbiological Specifications for Foods I.C.M. S. F. (1978) and American Public Health Association A.P.H.A. (1995):

A. Feedstuffs: 25grams of each sample were pre-enriched in 225 ml of buffered peptone water at 37°C for 24 hours. Then 10 ml of pre-enriched broth were added to 100 ml of Selenite F selective broth then incubated at 37°C for 18 hours.

B.Water: Water samples were centrifuged at 3000 r.p.m for 15 minutes and sediment were used for cultivation.

C. Swabs: each swab was put in 10 ml of buffered peptone water at 37°C for 24 hours. Then 1ml of pre-enriched broth were added to 10 ml of Selenite F selective broth then incubated at 37°C for 18 hours.

D. Fecal samples, organs and other samples: 10 grams of each sample were pre-enriched in 100 ml of buffered peptone water at 37°C for 24 hours. Then 1 ml of pre-enriched broth was transferred to 10 ml of Rappaport Vassiliadis selective broth then incubated at 42°C for 24 hours.

A loopfull from each selective enrichment broth of different samples was spread onto S.S agar, X. L.D. agar and Brilliant green agar. The plates were incubated at 37°C for 24 hours.

***Identification of salmonellae**: The suspected colonies were described for their morphological and characteristic appearance. Pure cultures of the isolates were identified biochemically according to Quinn et al. (2002).

***Serological identification**: of suspected *Salmonella* isolates were carried out using agglutination technique according to the Kauffmann - White scheme as described by Kauffmann (1973). The typing antisera were obtained from Denka Seiken Co. Ltd, Tokyo, Japan.

***Detection of virulence associated gene of salmonellae by PCR:** according to Rexach and Fach (1994) and Bakshi et al. (2003).

P.C.R. was performed in biotechnology center - faculty of Veterinary Medicine Cairo University.

Extraction of DNA: The isolated salmonellae were grown separately in 5ml of Luria Bertani broth and incubated at 37°C over night in shaking incubator. The bacterial culture were boiled for 10 minutes then immediately chilled in an ice and salt mixture and centrifuged at 9000 r.p.m. for 20 minutes to remove cell debris.

Amplification of virulence associated gene: was achieved on the thermocycler as followed: The primer sequence was done according to Bakshi et al. (2003). The forward primer corresponding to position 113-133 and the reverse primer to position 561- 583 in virulence plasmid gene. The sequence of the primer used for amplification were: forward primer 5' TTGTAGCTGCT-TATGGGGCGG -3' reverse primer, 5' TGGA-GAAACGACGCACTGTACTGC- 3'. Ten µl of supernatant were added to 5 µl of 10X PCR (500 m M Kcl, 100m M5 Tris Hcl PH 9.0 and 1.5 m M Mgcl), 1µl of Deoxy- Nucleotides Triphosphate solution (10 m M ultrapure water PH7.0), 1 µl of Primer1, 0.5µl of Primer 2, Taq DNA polymerase and completed to 50µl of Deionized water in Eppendorff tube then overlay with 40µl of paraffin oil. PCR consisted of 30 cycles of 1 minute at 94°C (denaturation), 30 seconds at 60°C (annealing) and 30 seconds at 72 °C. An additional cycle (primer extension) of 5 minutes at 72°C.

Agarose gel electrophoresis: Electrophoresis grad agarose was prepared in 1X electrophoresis buffer to reach 2%concentration and make a 4 mm thick layer. Then heated in boiling water bath to dissolve all granules. The agarose was allowed to cool at 70°C then 0.5µl of ethidium bromide was added and mixed well. The mixture was poured on gel casting apparatus after installation of comb. The gel was allowed to solidify. 1X electrophoresis buffer was added into a tank to alevel1-2mm above gel layer PCR product samples and molecular weight marker were loaded in the proper gel loading buffer the tank was covered and the power was attached. The parameters were run using 1-5 volts /cm of the tank length for min-igels, use 5-20 volts/cm. The bromophenol blue was allowed to run 2/3 of the gel length before termination of the run. Then observed under UV light (302nm). Positive bands of amplified region should be at 460bp.

***Pathogenicity test in mice:**

Forty five albino white mice with average weight of about 19- 20 g and aged 28-30 days old were used to investigate the pathogenicity of 8 salmonellae previously examined for presence of virulence associated gene. All mice were examined bacteriologically to ensure their freedom from pathogens. The mice were divided into groups each of 5 mice and inoculated I.P. with 0.1 ml of 5 X 10^8 C. F. U/ mouse of the tested strain and kept separately, last group was kept as control and injected only with saline. Mice were kept under observation for 7-10 days, the numbers of dead mice were recorded and re-isolation of inoculated strains were done,

***Antibiogram sensitivity test:**

Antibiogram was applied to determine susceptibility of isolated salmonellae to 12 chemotherapeutic & antibiotic agents (Oxoid), using disc diffusion technique according to Finegold and Martin (1982) and Quinn et al. (2002) with Muell-

Hinton agar, using the following discs, amikacin (30µg), amoxicillin (25µg), cefatroxil (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), colistin sulphate (50µg), enrofloxacin (5µg), flumequine (30µg), nalidixic acid (30µg), nitrofurantoin (300µg), streptomycin (10µg) and tetracycline (30µg). The results were interpreted according to Oxoid Manual.

Minimal inhibitory concentration MIC test: as described by Finegold and Baron (1986) and NCCLS (2002).Stock solution of the examined antibacterial agents (chloramphenicol produced by El-Nasr Company, Egypt and ciprofloxacin, enrofloxacin and flumequine produced by Amoun Industrial Company, Egypt) contained 200 µg /ml was prepared. Four colonies of examined strain were transferred to a tube contain 5ml of Mueller Hinton broth. The turbidity was adjusted to match a McFarland tube (0.5) corresponding to 1.5 X 10^8 C. F. U/ml. Then further diluted 1:200 in Mueller Hinton broth. The test was done in tubes each contain 1ml of Mueller Hinton broth. One ml of antibiotic stock solution was added into tube No.1 and 2. The content of second tube was mixed well then 1ml was transferred to the third one and soon, finally discarding 1ml from last tube then 1ml of diluted bacterial suspension were added in each tube and the concentrations of antibiotic were (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.0975, 0.0487µg/ml) the control –ve contained no antibiotic. The tubes were incubated at 37°C for 24 hours then visually examined for growth by turbidity .The lowest concentrations of antibiotic prevents the bacterial growth considered the MIC of the examined antibiotic. The steps were repeated with another strains and antibiotics.

RESULTS AND DISCUSSION

Salmonellae remain one of the most common causes of salmonellosis in animals and humans and among the most common causes of food born disease around the world. The present investigation showed that the incidence of salmonellae in sheep and lambs was (4.49%) and (9.18%) respectively. The incidence in sheep differ according to age and health conditions as it was higher in lamb than in adult and in diseased than apparently healthy as it was (12.73%) and (4.65%) in diseased and apparently healthy lambs. Meanwhile, it was (6.67%) and (3.39%) in diseased and apparently healthy adults sheep respectively as achieved in Table (1).

In this concern, Hjartardóttir et al. (2002) recorded that all fecal samples from sheep in the Southwest Iceland were negative, whereas samples

Table (1): Prevalence of *Salmonella* serovars among the examined sheep and lambs.

Sources of isolates	No of examined samples	No of positive samples	%*	Serovars & number
Living sheep:				
1-Apparently healthy	59	2	3.39	*S*.Cerro (1) & *S.* Typhimurium (1).
2-Diseased	30	2	6.67	*S.* Montevideo (1) &*S.* Sandiego (1).
Total	89	4	4.49	
Living Lambs:				
1-Apparently healthy	43	2	4.65	*S*.Cerro (1) &*S.* Typhimurium (1).
2-Diseased	55	7	12.73	*S.* Cerro (1), *S.* Montevideo (3), *S.* Sandiego (1) & *S.* Typhimurium(2).
Total	98	9	9.18	
Slaughtered sheep:				
1-Livers	30	1	3.33	*S.* Arizonae (1).
2-Intestines	30	2	6.67	*S.* Arizonae (1) & *S.* Typhimurium(1).
3-Lymph nodes	30	1	3.33	*S.* Typhimurium (1).
Total	90	4	4.44	
Dead lambs:.				
1-Livers	34	4	11.76	*S*.Cerro (1), *S.* Sandiego (1) & *S.* Typhimurium (2)
2-Intestines	34	4	11.76	*S*.Cerro (1), *S.* Sandiego (1)& *S.* Typhimurium (2)
3-Lymph nodes	34	4	11.76	*S.* Cerro (1), *S.* Sandiego (1) & *S.* Typhimurium (2)
Total	102	12	11.76	
Total	379	29	7.65	

*The percent was calculated according to the number of each examined samples.

from 3 farms obtained in the autumn in the Northwest were positive for *Salmonella*. Furthermore, Sandberg et al. (2002) examined rectal swabs from individual sheep of different age groups in fifty randomly selected sheep flocks from a region in central Norway for *S.* Diarizonae, seven flocks (14% - 95%) were positive. Helmy and Zaki (2003) mentioned that the incidence of salmonellae in diarrheic lambs in Kafr- El-Shiek Governorate was (11.1%). Meanwhile, Clark et al. (2004) stated that potential sources of infection with *S.* Brandenburg were carrier sheep in the South Island of New Zealand. In addition, Davies et al. (2004) found that samples of rectal feces in healthy sheep at slaughter contained (0.1%) *S.*Typhimurium.

Otherwise, Branham et al. (2005) mentioned that *Salmonella* spp. were found in (7.32%) of sheep grazing in the same rangeland with white-tailed deer in the United Sates. Also, Pao et al. (2005) detected *Salmonella* strains in 17 of 247 feces of tested small ruminants in Virginia. Kenilake and Perkins (2006) examined 405 sheep farms for S. Brandenburg in New Zealand. Of the 177 case farms, (97%) had diseased mixed-age ewes, (45%) had diseased two-mouth ewes.

The prevalence of salmonellae in slaughtered sheep was (4.44%). There were variations among the incidences according to examined internal organs as the incidence was higher in intestines (6.67%) than in livers and lymph nodes (3.33%) each. As shown in Table (1).These may be attributed to fact that intestine is a predilection site for salmonellae. Also, isolation of *Salmonella* from slaughtered sheep may be due to contamination during slaughtering or illness before slaughtering. Many investigators concerned with salmonellae isolated from slaughtered sheep as Vanderlinde et al. (1999) isolated salmonellae from (5.74%) of Australian sheep carcass. Meanwhile, Alvseike and Skjerve (2002) found that the regional prevalences of *S. Diarizonae* in 133 Norwegian sheep herds from (10 different) abattoirs varied from (0 to 45%) and were more prevalent in adults than in lambs. Furthermore, Hjartardóttir et al. (2002) recorded that the incidence of *Salmonella* was (2%) in caecum and (8%) in tonsils in lambs at slaughter. In addition, Davies et al. (2004) found that samples of rectal faeces in healthy sheep at slaughter in Great Britain contained (0.1%) *S. Typhimurium*. Also, Zweifel et al. (2004) found that the prevalence of *Salmonella* spp. was (11.0%) in caecum samples collected from 653 slaughtered sheep from two Swiss abattoirs. While, Molla et al. (2006) examined 104 apparently healthy slaughtered sheep in central Ethiopia and found that the incidence of *Salmonella* was (11.5%). Otherwise, Small et al. (2006) mentioned that *Salmonella* was found on (9.6%) of 240 lamb carcasses in five commercial abattoirs in Southwest England.

Concerning the results in Table (1) the prevalence of salmonellae in dead lambs was (11.76%) and this incidence was similar in examined livers, intestines and lymph nodes (11.76 %) each. This indicated that the deaths usually occurred due to septicemia from *Salmonella* infection which appeared severe in young lambs. These data coincided with Helmy and Zaki (2003) who found that the incidence of *Salmonella* in intestine and liver of dead lambs in Kafr- El-Shiek Governorate was (13%).The obtained data showed that the isolated *Salmonella* serovars among the examined sheep and lambs belonged to 5 serovars and *S.Typhimurium* was most predominant isolate. Although there was no clear pattern could be established relative to isolation of specific serovars of salmonellae in relation to specific type and

condition of examined sheep or lambs there was difference in isolated serovars as *S*.Cerro, *S*. Montevideo, *S*.Sandiego, and *S*. Typhimurium were isolated from diseased lambs. While, *S*. Arizonae, and *S*. Typhimurium were isolated from slaughtered sheep. Also, *S*. Cerro, *S*. Sandiego and *S*. Typhimurium were isolated from dead lambs This result nearly coincided with those reported by Hjartardöttir et al. (2002) isolated *S*. Thompson and *S*. Montevideo from sheep in Iceland. Also, Helmy and Zaki (2003) isolated *S*. Typhimurium, *S*. Dublin, *S*. Anatum and *S*. Paratyphi B from diarrheic lambs. While, *S*.Typhimurium, *S*. Dublin and S. Enteritidis from dead lambs in Kafr- El-Shiek Governorate. Otherwise, Molla et al. (2006) recorded that 22 salmonellae isolated from apparently healthy slaughtered sheep and goats of central Ethiopia belonged to 9 different serovars. The common serovars isolated were *S*. Typhimurium.

The environment of sheep and lambs plays a very important role in spread of *Salmonella* infection between animals and to human. The incidence of salmonellae in environment of sheep and lambs varied according to nature of environmental samples and site from which they were taken. The incidences of salmonellae in feedstuffs, water, soil, waste samples and swabs from workers' hands in environment of living sheep, lambs and dead lambs were (5.71 %, 2.86%, 8.57%, 22.22% and 11.11%) respectively. While from drain water, swabs from walls and workers' hands in environment of slaughtered sheep were(10%, 12.5% and 6.66%) respectively and *S*. Arizonae, *S*. Enteritidis, *S*. Ferruch, *S*. Montevideo and *S*. Typhimurium were isolated from environment of sheep and lambs as illustrated in Table (2) One point of interest in this work was the isolation of *S*. Ferruch from environment of sheep and lambs for first time this may be due to presence of rodents and ferrets.

Table (2): Prevalence of salmonellae among sheep and lambs environment..

	Type of examined samples	No. of examined samples	No. of positive samples	%*	Serovars
A-environment of living sheep, , lambs and dead lambs	Feedstuffs	35	2	5.71	*S.* Arizonae &*S.* Enteritidis
	Water	35	1	2.86	*S.* Montevideo
	Soil	35	3	8.57	*S.* Arizonae, *S.* Montevideo & *S.* Typhimurium
	Waste samples	9	2	22.22	*S.* Ferruch & *S.* Typhimurium
	Swabs from workers' hands	18	2	11.11	*S.* Montevideo & *S.* Typhimurium
B-environment of slaughtered sheep	Water (drain)	10	1	10	*S.* Typhimurium
	Swabs from walls	8	1	12.5	*S.* Arizonae
	Swabs from workers' hands	15	1	6.66	*S.* Typhimurium

*The percentage was calculated according to the number of each examined samples.

These were recorded by many authors as Clark et al. (2004) considered contaminated water sources and contaminated sheep yard dust as potential sources of infection with *S.* Brandenburg in the South Island of New Zealand. In addition, Branham et al. (2005) stated that *Salmonella* spp. were found in (5%) of sampled water troughs of sheep grazing in the same rangeland with white-tailed deer in the United States. Otherwise, Hutchison et al. (2005) isolated *Salmonella* spp. from lambs' waste samples. Meanwhile, Purvis et al. (2005) found that *S.*Typhimurium was isolated from (59%) of soil and lamb feces were taken from four holding paddocks used to retain sheep before slaughter in the North of England.

PCR was used to detected nucleic acid (DNA) in especial sequence depend on sequence of used primer so it makes the results more reliable. In this work PCR was used to detect and amplify virulence associated gene in 8 salmonellae which is corresponding to *mfka* gene and present in 55 kb plasmid in *S.* Typhimurium Bakshi et al. (2003). On examination of selected 8 salmonellae isolated from different source of sheep (adult, lamb &

slaughter) and their environment showed that, *S.* Arizonae (from slaughtered sheep), *S.* Ferruch, (from waste samples) *S.* Montevideo, *S.* Sandiego and *S.* Typhimurium (from diseased lambs) harboured the virulence associated gene. In all positive cases a single amplified fragment of 460 bp was present. While, *S.* Cerro (from dead lambs), *S.* Enteritidis (from feedstuffs) and *S.* Typhimurium (from slaughtered sheep) didn't harbour this gene so there were no amplified fragment of 460 bp. photo (1).

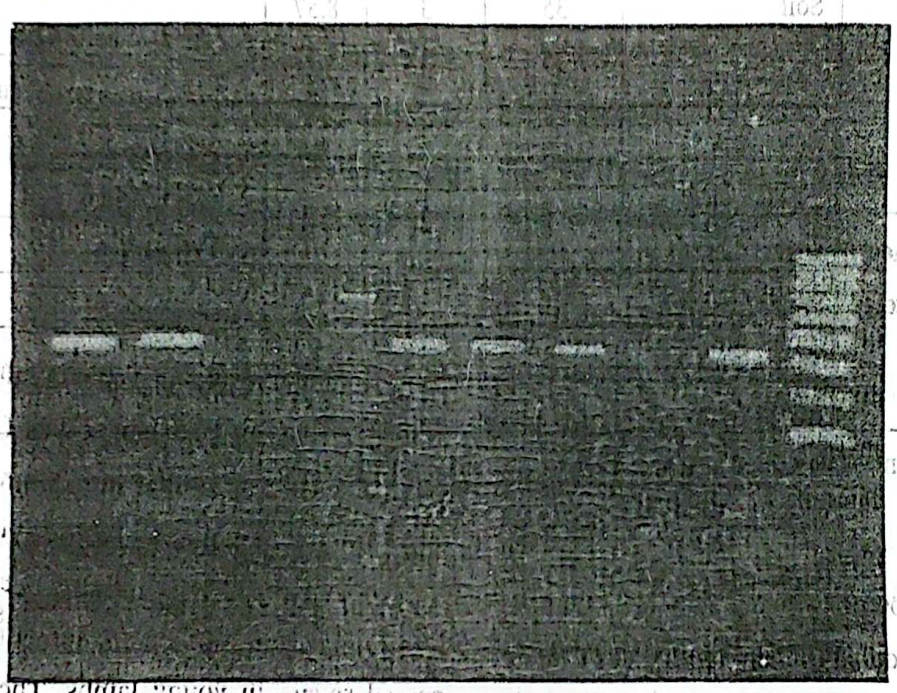

Fig. (1): PCR amplification mixture were run on polyacrylamide gel stained with ethidium bromide for detection of the virulence associated gene: Lanes 1, molecular weight marker (100-1000 bp). Lanes 2, positive control where the amplified fragment 460bp presented. Lanes 3, *S.* Cerro (from dead lambs). Lanes 4, *S.* Montevideo. Lanes 5, *S.* Sandiego Lanes 6, *S.* Typhimurium. each (from diseased lambs) Lanes 7, *S.* Typhimurium (from slaughtered sheep) Lanes 8, *S.* Enteritidis (from feedstuffs). Lanes 9 *S.* Arizonae (from slaughtered sheep), Lanes 10, *S.* Ferruch, (from waste samples).

It was clear that *Salmonella* isolates which had a virulence gene showed mortality rate varied between (40-100%) Table (3) *S.* Arizonae,, *S.* Cerro and *S.* Ferruch yielded (100%) mortality rate in each, followed by *S.* Sandiego (80%) then *S.* Typhimurium, *S.* Typhimurium ((from slaughtered sheep) and *S.* Enteritidis (60%) in each, while *S.* Montevideo yielded lower mortality rate (40%).On studying the relation between virulence associated gene and pathogenicity in mice, the results indicated that presence of this gene not considered as indicator for pathogenicity as *S.* Cerro that isolated from dead lambs and yielded high mortality rate in mice (100%) didn't harbour the virulence associated gene. On the other hand, *S.* Montevideo (from diseased lambs) and yielded lower mortality rate (40%) harboured this gene.

Table (3): Pathogencity test in mice and occurrence of virulence associated gene in some salmonellae isolated from sheep and its environment.

Source of isolation	diseased lambs			Slaughtered sheep		Dead lambs	Feedstuffs	From waste samples
Serovars	*S.* Montevideo	*S.* Sandiego	*S.* Typhimurium	*S.* Arizonae,	*S.* Typhimurium	*S.* Cerro	*S.* Enteritidis	*S.* Ferruch
Mortality rate &percent	2(40)	4(80)	3 (60)	5(100)	3 (60)	5(100)	3 (60)	5(100)
Presence of virulence associated gene.	+	+	+	+	-	-	-	+

The percent was calculated according to total number of mice (5) in each group.

Examination period for 7 -10 days.

The data presented in Table (4) showed that (95.24%) of the isolates were susceptible to ciprofloxacin and enrofloxacin, (90.48%) were susceptible to flumequine and (83.33%) were susceptible to amikacin. On the other hand lower susceptibility incidences were observed to streptomycin

(19.05%) and cefadroxil (23.81%). This may due to the extensive use of antibiotics, not only in human and veterinary medicine, but also in livestock production for disease prevention or as growth-promoting feed additives, has led to a serious increase in, and spread of, multiple antibiotic-resistant bacteria. Helmy and Zaki (2003) found that *S.* Typhimurium isolated from diarrheic lambs and *S.* Enteritidis from dead lambs in Kafr-El-Shiek Governorate were highly sensitive to ciprocin, nitrofurantoin and oflocin (100%) and resistant to colistin sulphate and tetracycline. Furthermore, Molla et al. (2006) mentioned that (31.8%) isolates of salmonellae from apparently healthy slaughtered sheep and goats in central Ethiopia were multidrug-resistant to various antimicrobials.

The results achieved in Table (5) showed the minimum inhibitory concentration MIC of salmonellae isolates from sheep and its environment to chloramphenicol, ciprofloxacin, enrofloxacin and flumequine. There were variations in inhibitory effect of different antimicrobials on different isolates. Enrofloxacin gave the highest inhibitory effect as its MIC was 0.195-3.12 µg/ml for sensitive isolates, followed by ciprofloxacin and flumequine with MIC: 0.39-3.12µg/ml for sensitive isolates while chloramphenicol gave the lowest effect on the isolates as its MICwas 3.12- 12.5µg/ml for sensitive isolates. these agree to some extant with Ibrahim et al. (2001) who stated that MIC of enrofloxacin and flumequine among 10 isolates of *S.* Typhimurium ranged from 0.78 to 1.56 µg/ml and 0.39 to 6.25µg/ml respectively . Also, Andrewe et al. (2002) recorded that MIC of chloramphenicol and ciprofloxacin on *S.* Typhimurium were 8 and 0.125 µg/ml respectively.

Table (4): Sensitivity of salmonellae isolated from sheep and its environment for chemotherapeutic and antibiotic agents.

Salmonella serovars / Chemotherapeutic & antibiotic discs	*S.* Arizonae, (5)		*S.*Cerro (6)		*S.* Enteritidis (1)		*S.* Ferruch (1)		*S.* Montevideo (7)		*S.* Sandiego(5)		*S.* Typhimurium (17)		Total (42)	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	NO. Of total sensitive	%**
Amikacin (30)*	4	80	5	83.3	1	100	1	100	6	85.7	5	100	13	76.5	35	83.33
Amoxicillin (25)	3	60	4	66.7	1	100	1	100	0	0	4	80	10	58.8	23	54.76
Cefadroxil (30)	3	60	1	16.7	0	0	0	0	1	14.2	0	0	5	29.4	10	23.81
Chloramphenicol (30)	2	40	6	100	1	100	1	100	4	57.1	3	60	15	88.2	32	76.19
Ciprofloxacin (5)	4	80	6	100	1	100	1	100	6	85.7	5	100	17	100	40	95.24
Colistin sulphate (50)	2	40	4	66.7	0	0	0	0	4	57.1	3	60	8	47.1	21	50
Enrofloxacin (5)	4	80	6	100	1	100	1	100	6	85.7	5	100	17	100	40	95.24
Flumequine (30µg)	4	80	6	100	1	100	1	100	6	85.7	4	80	16	94.1	38	90.48
Nalidixic acid (30)	2	40	1	16.7	1	100	0	0	6	85.7	5	100	9	52.9	24	57.14
Nitrofurantoin (300)	2	40	5	83.3	1	100	0	0	5	71.4	4	80	12	70.6	29	69.05
Streptomycin (10)	0	0	1	16.7	0	0	0	0	1	14.2	0	0	6	35.3	8	19.05
Tetracycline (30)	3	60	6	100	1	100	1	100	3	42.9	5	100	10	58.8	29	69.05

* Concentration in micrograms. NO. = positive number & the percent was calculated according to the number of each examined isolated salmonellae.

**The percent was calculated according to the total number of examined isolated salmonellae(42).

Table (5): Minimum inhibitory concentration of some antibacterial agents on salmonellae isolates from sheep and its environment.

Antibacterial agents / Serovars & No.	Chloramphenicol	Ciprofloxacin	Enrofloxacin	Flumequine
S. Arizonae (5)	6.25-50	0.78-6.25	0.39-6.25	1.56-6.25
*S.*Cerro (6)	0.78-6.25	0.78-3.125	0.195-0.39	1.56-3.125
S. Enteritidis (1)	6.25	1.56	0.78	3.125
S. Ferruch (1)	3.125	1.56	0.39	0.78
S. Montevideo(7)	12.5-50	3.125-6.25	1.56-6.25	3.125-6.25
S. Sandiego (5)	0.78-6.25	1.56-	0.195-0.39	1.56-6.25
S. Typhimurium (17)	6.25-50	0.39-1.56	0.39-3.125	0.39-6.25

The approximate MIC correlates of chloramphenicol were R > = 32 and S < = 8 µg/ml. While of ciprofloxacin, enrofloxacin and flumequine were R>=4 and S<=1 µg/ml. according to NCCLS (2002).

Conclusion: continuous examinations of sheep and lambs and their environments to detect the carriers and any ill animal to prevent spread of salmonellae to human. The maintenance of slaughter hygiene is consequently of crucial importance and regular microbiological monitoring of carcasses. Finally correct use of antibiotics.

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