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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Isolation of *Salmonella* from slaughtered animals and sewage at Zakho abattoir, Kurdistan Region, Iraq

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Abstract

This study was conducted to determine the prevalence of *Salmonella* species in slaughtered animals and abattoir sewage from Zakho Abattoir, Kurdistan Region, Iraq. A total of 500 samples were collected including 200 from healthy sheep (100 rectal swabs and 100 gallbladder contents), 100 from healthy goats (50 rectal swabs and 50 gallbladder contents), 100 from cattle (50 rectal swabs and 50 gallbladder contents), and 100 sewage samples from the abattoir drains. From the total of 400 animals examined, 9 (2.25%) were positive for *Salmonella spp*., in which 5 (2.5%) were from sheep [2 (2%) from rectal swabs and 3 (3%) from gallbladder], 2 (2%) were from rectal swabs of goats, and 2 (2%) were from cattle [1 (2%) from rectal swabs and 1 (2%) from gallbladder]. Whereas, only 3 (3%) were positive for *Salmonella spp*., obtained from 100 sewage samples. Only three serotypes of *Salmonella* were detected in 12 (2.4%) samples from the total 500 samples. The isolated Salmonella serotypes were *Salmonella hato* 8 (66.66%), *Salmonella anatum* 3 (25%), and *Salmonella enteritidis* 1 (8.33%). The antimicrobial sensitivity test of all 12 isolates against 13 antibiotics was studied. Results revealed that all isolates were 100% sensitive to amoxicillin, amikacin, gentamicin, and ciprofloxacin, and 100% resistant to clindamycin, rifampin, vancomycin, cephalothin, lincomycin, and trimethoprim and sulfamethoxazole, chloramphinicol, doxycycline and tetracycline except *S. hato*.

Keywords: Zakho abattoir; *Salmonella*; Kurdistan Region; Iraq

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Introduction

Salmonella is considered one of the most causative agents of zoonotic disease in the world and their serotypes carried by animals act as sources of contamination (Teklu and Negussie, 2011). A number of researches were carried out in Iraq on *Salmonella*. In Duhok Abattoir, Kurdistan, Iraq, *S. newport* was isolated from faeces and gallbladder of slaughtered sheep (Taha, 2011) and *S. typhimurium* was isolated from faeces of cattle (Abdulrahman, 2010). Whereas, *S. hato, S. typhimurium, S. hadar*, and *S. enteritidis* were isolated from faeces and gallbladder of goats in four central provinces of Iraq (Yousif et al., 2010).

Furthermore, several studies have shown that this bacterium naturally exists in the intestinal tracts of birds and animals and they serve as the primary source of infection to human (Chadra et al., 2007; Willey et al., 2009). Contaminated meat by this pathogen may occur at abattoirs from the excretion of carrier animals, contaminated slaughterhouse equipment, floors and personnel in an abattoir. *Salmonella* could contaminate meat at any stage from abattoir to retail markets. Carcasses of slaughter animals and meat product contamination may occur during consequent of handling, processing, preparation and distribution (Hjartardottir et al., 2002; Woldemarium et al., 2005). In addition, consumption of fruits and vegetables with animal waste is another source of human infection (Woldemarium et al., 2005).

Moreover, this pathogen could survive and transmit by eating undercooked meat, or drinking water with

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contaminated faeces (Willey et al., 2009). Therefore, it could cause food poisoning in humans (Akoachere et al., 2009). According to the USA Food and Drug Administration (2007), contaminated food of animal origin, particularly meat products from cattle and pigs, is a major source of *Salmonella spp*. in human infections. This pathogen, as a result, causes morbidity and mortality in humans and animals (Akoachere et al., 2009). Callaway et al. (2008) reported that due to *Salmonella spp*. approximately 1.3 million people develop food-borne illness in the USA annually.

The wide spread of antimicrobial resistance has been resulted from the routine use in medical and agricultural farming and the antibiotic gene cassettes disseminate due to the development of genetic mechanisms efficient, particularly within and between two species of gram negative organisms (Ferber, 1998; Lindstedt et al., 2003). This leads to promote the development of the resistance organisms in animals that are eventually transmitted to human (Naeem, 2008).

Although, Popoff et al. (2004) indicated that 2541 *Salmonella* serovars have been identified worldwide which cause outbreak of food-borne illnesses, there were a limited number of *Salmonella* serovars that cause human infection. There is no available data about *Salmonella spp*. and their antimicrobial resistant profile in the slaughtered animals in Zakho Abattoir, therefore, this study was carried out to detect *Salmonella spp*. from clinically healthy sheep, goats and cattle, and sewage. To find the most prevalent *Salmonella* serotypes in the area and to study the antibiogram of isolated serotypes is also a part of this study.

Materials and Methods

Sampling

A total of 500 samples were collected from the abattoir from June, 2010 to February, 2011. Two hundred samples were taken from healthy slaughtered sheep (100 swabs from the rectum and 100 from the gallbladder). One hundred samples were taken from healthy slaughtered goats (50 swabs from the rectum and 50 from the gallbladder). One hundred samples were taken from healthy slaughtered cattle (50 swabs from the rectum and 50 from the gallbladder). One hundred swabs were obtained from the abattoir sewage.

The specimens were immediately collected after the animals were slaughtered. The gall bladder's contents were collected by sterile syringes. Then, the rectal swabs were obtained by inserting a sterile cotton swab into animal's rectum. Swabs from drains of abattoir were also collected from different area of Zakho Abattoir. All samples were immediately pre-enriched using tetrathionate broth 10 ml (Oxoid) and then, transferred to the Microbiology Laboratory of Zakho Institute and incubated at 37°C for 24-48 hours.

Isolation and Identification

Samples from enrichment broth were cultured on Deoxycholate citrate agar (DCA), and incubated at 37°C for 24-48 hours. All suspected colonies were submitted for the standard biochemical reactions according to ISO (2002) to confirm whether they belong to *Salmonella* spp. Then, they were serologically tested using *Salmonella* polyvalent H and O (Remel, USA) latex agglutination test. Finally, all isolated colonies were incubated in TSI agar and sent to laboratory of General Health Centre (unit of Enterobacter) in Baghdad for serotyping.

Antimicrobial Sensitivity Test

The *in vitro* sensitivity of all obtained isolates was tested using 13 different antibiotics by disc diffusion method (Bauer et al., 1966) on Mueller-Hinton agar (Oxoid). The antimicrobial agents include: amoxicillin (AMX 25 µg), amikacin (AK 30µcg), gentamycin (CN 10µg), chloramphinicol (Chl 30µg), doxycycline (DO 30µg), tetracycline (TET 30µg), ciprofloxacin (CIP 5µg), clindamycin (DA 2µg), cefotaxime CTX 30µcg, rifampin (RA 5 µg), vancomycin (VA 30µg), cephalothin (KL 30µg), lincomycin (L 20 µg), trimethoprim and sulfamethoxazole SXT (12.5 µg).

Results and Discussion

The typical colonies of *Salmonella* on Deoxycholate citrate agar (DCA) showed black colonies. All suspected colonies were submitted for the standard biochemical reactions according to ISO (2002). In addition, all isolates samples were positive (agglutination) for both *Salmonella* polyvalent 0 and H antisera. According to the cultural characteristics, biochemical reactions and serological identification, the isolates were specified as a genus *Salmonella*.

The results presented in Table 1 shows that the prevalence of *Salmonella* in apparently healthy sheep, and goats was 2.5%, and 2%, respectively. These findings are different from the report of Teklu and Negussie (2011) in an Export Abattoir, Modjo, Ethiopia which indicated 7.7% of the prevalence of *Salmonella* in sheep and 11.7% in goats. Furthermore, 2% *Salmonella* prevalence of apparently healthy cattle obtained in this study is lower than the findings reported by Akoachere et al. (2009) in Abattoir Drains in Buea, Cameroon which was 28.7%. In addition, the prevalence of *Salmonella* obtained from drains abattoir was 3%, and there is no available data to compare. This study is assuring that the 3% of spread *Salmonella* in sewage of the slaughterhouse came from contaminated domestic animals where they were brought to the place. The differences in these results with the previous studies might be associated with the procedure and plan of taking samples, types and tests of the bacteriological

Table 1: Results of *Salmonella* serovars isolated from different sources

Sample source and No.	No. & % of isolates *Salmonella* spp.	Swab sources and No.& % of isolates *Salmonella* spp.
Sheep 200	5 (2.5%)	100 rectal 2 (2%) 100 gallbladder 3 (3%)
Goat 100	2 (2%)	50 rectal 2 (4%) 50 gallbladder-
Cattle 100	2 (2%)	50 rectal 1(2%) 50 gallbladder 1(2%)
Sewage 100	3 (3%)	100 drain sewage 3 (3%)
Total 500	12 (2.4%)	12 (2.4%)

detection as well as the differences of occurrence and distribution of *Salmonella* (Nouichi and Hamdi, 2009; Teklu and Negussie, 2011).

In this study, 3% of isolated *Salmonella* from sheep's gallbladder was nearly similar to the 2% in Kars District, Turkey (Genc, 2002), while it was 0.8% in a modern abattoir in Duhok Governorate, Kurdistan-Iraq (Taha, 2011). However, Tadjbakhch and Haghigat (1992) did not isolate *Salmonella* from bile samples of sheep. Furthermore, the prevalence of *Salmonella* from rectal sheep was 2%. This result is nearly similar to the 2.1% reported by Woldemariam et al. (2005) in Debre, Zeit, Ethiopia. However, a study carried out to estimate the prevalence of faecal *Salmonella* in healthy sheep in Duhok abattoir yielded 0.8%, (Taha, 2011) and it was 0.1% in Great Britain (1999-2000) as reported by Davies et al. (2004) which are lower than the finding of the current study. In contrast, Karim et al. (2008) in Bangladesh and Edrington et al. (2009) in the USA, found higher prevalence of *Salmonella* in sheep fecal, (5.7% and 7%, respectively). Nabbut et al. (1982) and Venter et al. (1994) documented that *Salmonella* can survive and multiply in the rumen, when animals are starved as well as only a few *Salmonella* in healthy carriers intermittently excrete, unless the animals undergo some kind of stress such as transportation. As a result of the exposing animals to the different factors of stress prior to the slaughter such as starvation, transportation, overcrowding and longer staying in the confinement, the prevalence of *Salmonella* could become higher in faecal samples.

All 12 isolates were further characterized according to the Laboratory of General Health Centre (unit of Enterobacter) in Baghdad as shown in Table 2. The characterization revealed that there were three isolated serotypes of *Salmonella* from all isolates. Eight (66.66%) *S. hato*, four isolates were from sheep (2 from the rectum and 2 from gall bladder) and four isolates from cattle and sewage (one from cattle gallbladder and the other three were isolated from different areas of sewage). Three (25%) *S. anatum* were isolated from the rectum, two of them were from goats and the other was from cattle. Worth mentioning that only one (8.33%) *S. enteritidis* was isolated from sheep gallbladder.

The results were different from the results of Abdulrahman (2010) and Taha (2011) in which one serotype of *S. typhimurium* was isolated from three isolates of the cattle rectum and one serotype of *S. newport* from four isolates of sheep in Duhok Abattoir, respectively. Also, Yousif et al. (2010) isolated four serotypes (*S. hato, S. typhimurium, S. hadar, S. enteritidis*) from goats in the four provinces in central Iraq (Babylon, Karbala, Najaf, Baghdad). Furthermore, the percentage of isolated *S. anatum* from cattle was 8.3% of 50 samples compared with 2.6% in Tunisia between the years 1994-2004 (Hendriksen, 2010). On the other hand, there was not a statistical data reported that *S. hato* isolated from sheep, cattle and sewage in an abattoir, but it was isolated from gallbladder 3.06% and faeces 2% of goats (Yousif et al., 2010). In addition, according to Moussa et al. (2012), they isolated 13.8% S. *enteritidis* from 335 sheep faecal samples from Egypt in the year 2010 compared with 8.33% of it in our study. Brenner et al. (2000) stated that *S. enteritidis* has mainly been the cause of food borne diseases. Rodrigue et al. (1990) and Herikstad et al. (2002) reported that their number has been significantly increased worldwide during the last two decades.

The serotypes of non-typhoid *Salmonella* remains a possible threat to human health and the domestic animals are probable sources of these organisms in the environment (Abouzed et al., 2000). In spite of the non-typhoid Salmonellosis is commonly a self-limiting disease restricted to the intestinal tract of human, when infections spread beyond the intestine, it could have a serious effect requiring suitable antimicrobial treatment. Therefore, the examination of antimicrobial resistant strains is essential for effective treatment and the occurrence of resistant populations for current serotypes. Results from Table 3 revealed that the antibiogram of all isolates were 100% sensitive to amoxicillin, amikacin, gentamycin, and ciprofloxacin, whereas they were resistant to rifampin, vancomycin,

Table 2: The distribution of *Salmonella* serotype in different samples

Salmonella serotype	Sheep		Goat		Cattle		Swage	Total
	Rectal	Gall bladder	Rectal	Gall bladder	Rectal	Gall bladder		
Salmonella hato	2	2	-	-	-	1	3	8 (66.66%)
Salmonella anatum	-	-	2	-	1	-	-	3 (25%)
Salmonella enteritidis	-	1	-	-	-	-	-	1 (8.33%)
Total	5		2		2		3	12 (100%)

Table 3: Antibiotic Sensitivity test

Antibiotic disc	Code and Concentration	Zone of Inhibition (Diameter cm)		
		S. Hato 8	*S. Anatum 3*	*S. Enteritids 1*
Amoxicillin	AMX 25µg	S	S	S
Amikacin	AK 30µg	S	S	S
Ciprofloxacin	CIP 5µg	S	S	S
Gentamicin	CN 10µg	S	S	S
Chloramphinicol	C 30µg	S	R	R
Doxycycline	DO 30µg	S	R	R
Tetracycline	TE 30µg	S	R	R
Rifampin	RA 5 µg	R	R	R
Vancomycin	VA 30µg	R	R	R
Clindamycin	DA 2µg	R	R	R
Cephalothin	KL 30µg	R	R	R
Lincomycin	L 20 µg	R	R	R
Trimethoprim Sulfamethoxazole	SXT 12.5 µg	R	R	R

S-sensitive; R- resistant

clindamycin, cephalothin, lincomycin, trimethoprim and sulfamethoxazole. These results corroborated the findings of Dahal (2007) who reported that *S. enteritidis* was 100% sensitive to gentamycin. The other active agents observed were amoxicillin 86.37%, ciprofloxacin 97.73%, nearly 98% chloram phenicol and amikacin (90.6%). In this study, all *S. hato* isolates were sensitive to chloramphenicol, doxycycline, and tetracycline compared with a marked resistance to *S. anutum and S. enteritids*. However, these results differ with the results reported by Akoachere et al. (2009) who found that there was a marked resistance of non-typhoid *Salmonella* to doxycycline (68%) and amoxicillin (90.7%).

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