Yemeni Journal of Agriculture & Veterinary Sciences ((2014) 1(3):70-82) ISSN:2311-7788

Faculty of Agriculture & Veterinary Medicine, Thamar University

Yemeni Journal of Agriculture & Veterinary Sciences

Available online at; WWW.YJAVS.COM

Prevalence of Salmonella in Human and Foods of Animal Origin with Antibiotic Resistance Patterns of Isolated Bacteria in Dhamar Governorate , Yemen

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Article history:

Received

15 September 2013

Accepted

ABSTRACT

Salmonellosis is a food borne zoonotic disease and one of the major public health problems. Assessment of the Salmonella contamination risk In Food of Animal Origin at Dhamar governorate is the main objective for this study. A total of 188 samples of animal food products, including, white meat, red meat, eggs and milk were collected from slaughterhouses, local markets and cows. In addition, a total of 35 stool and 45 blood samples from human were collected. All Samples were collected in aseptic manner, brought to laboratory and subjected to bacteriological, biochemical and serological tests accordingly. For assessment sensitivity of isolated *Salmonella* species to antibiotics, ten antibiotic drugs were used for this purpose. The results revealed that, out of 188 samples examined and tested for salmonella species, 37(20%) were positive. The positive rates according to sample-type were 0.11%, 0.14%, 0.05%, 0.11%, 0.05%, 0.16%, 0.19%, 0.14%, and 0.05% for native beef, imported beef, camel meat, minced meats, poultry meat, fish meat, table eggs, and raw milk respectively. In human, out of 35 stool and 45 blood samples examined, 8(22.8 %) and 22(48.9%) were positive for salmonellosis respectively. The serovars identified were; typhi (O) 49% typhi (H) 36% Paratyphi (B) 24%, but Paratyphi (A) hasn't found. All isolated salmonella were sensitive to chloramphenicol ciprofloxacin, nalidixic acid and gentamicin, and intermediate resistant for Erythromycin and Tetracycline, but complete resistant to Colistin, Doxycycline, Penicillin and Lincomycin antibiotics. This study confirmed that, food animal origin at Dhamar governorate are highly contaminated with some *Salmonella spp* and those species representing the main source of salmonella infections in patients in Dhamar governorate. Further studies on Salmonella

spp. Isolation, spread tracking through production cycle of farm animals, and control measures arerecommended.

Key words: Animals, Human, Dhamar, Prevalence, Sallmonella.

INTRODUCTION

Salmonellosis is a food borne zoonotic disease of primary concern in developed, as well as developing countries. The spread of this disease is favored by a variety of animal reservoirs and a wide commercial distribution of both animals and food products. This disease is among one of the major public health problems in terms of socio-economic impact **(Mushtaq et al., 2008).** About 95% of affected human with salmonella were infected by consumption the contaminated animal food origin such as meat, poultry, eggs, milk, seafood and fresh produce **(Callaway et al, 2000).** They cause a wide range of clinical illness: enteric fever, gastroenteritis, and bacteraemia, particularly in infants and in immunocompromised patients **(Fluit, 2005)**.Several studies from different countries have been reported prevalence and the relationship between Salmonella spp., foods of animal origin and public health problems **(Rasrinual et al., 1988; Boriraj et al., 1997; Boonmar et al., 1998);** Whereas, in Yemen, there are few studies about Salmonellosis that were mainly focusing on human infection **(Al-Haddad, 2004 and Saleh, 2010)** and local cooked foods **(AL-Ammari, 2012)**. Reports revealed that, in 2009, about 39,770 of Typhoid and Paratyphoid cases were recorded in Yemen **(CSO, 2009)**.

In Dhamar governorate, Salmonellasis is common and has been diagnosed as enteric fever in patients (16.4%) and food poising cases (15.2%) **(Taha et al., 2013)**. However, no data available in compiled form reporting the current status of salmonellosis and role of animal food origin as source for human infections. Therefore, this study was carried out to determine prevalenceof salemonella species and assess their susceptibility and resistance to some antimicrobial drugs.

MATERIAL AND METHODS:

The study had been conducted in Dhamar governorate through April until December 2013. A total of one hundred and eighty eight(188) samples of different animal product food were collected from different regions of Dhamar governorate includeds laughters, local markets and cows. also 35 clinical specimens (human stool samples) and 45 of blood samples had taken from patients with suspected enteric fever whom were attending General Dhamar hospital to determine the incidence of Salmonella infection**(Table 1)**.

Table 1:Samples from food of animal origin

Type of raw samples	**Red meat**				**White meat**			**eggs**	**milk**	**Human samples**		**Total**
species	**native beef**	**imported beef**	**Camel meat**	**Minced meats**	**Freeze chickens**	**fresh chickens**	**Fish meat**	**table eggs**	**Raw Milk**	**stool**	**serum**	
No. of collected samples	17	16	5	30	10	20	30	30	30	35	45	**268**

For meat samples, Approximately 25g of meat pieces were harvested from different parts of carcasses and each sample was aseptically placed into a sterile vacuum bag.Milk samples was collected by pouring approximately 50 ml into a sterile specimen cup. Blood samples were inoculated in sterile test tube for sera collection while stool samples were placed in selenite and tetrathionate broth.

Collected samples were taken, properly labeled and brought to, Faculty of Agriculture and Veterinary Medicine laboratories on the day of collection in Chilled ambient using thermos supplied with ice.

For bacteriological analysis, samples preparing, grinding and homogenizing of meats were carried out in sterile conditions,and then, 25g of meat or stool, 10 ml of milk and10 ml of egg yolk samples were added to 100 ml of selenite enrichment broth (**Himedia®**) and incubated at 37^{O}C for 24h under aerobic conditions.

The positive growth with selenite broth were transferred to selective media salmonella shigella agar (SSA) (**Himedia®**) and then streaked on Mac- Conkey's agar **(oxoid®)**. After 24 h incubation at 37^{O}C, two or more ofsuspect colonies from each agar plate (non-

lactose fermented coloniesin Mac- Conkey's agar), that revealed typical physiognomies of Salmonella were pickedout and submitted to biochemical analysis byusing sulfite-Indole-Motility agar (SIM) **(Himedia®)**,Oxidase, Catalase, Triple Sugar Iron Agar (TSI) **(Himedia®)**, Ureas (**Himedia®**) and simmon's citrate tests according to (**WHO, 2010**)and gram staining was carried outfor all apparent Salmonella colonies to morphological identification according to**(Benson, 2001).** Human serum samples were submitted to Slid (Widal) test (**Himedia®**) against s. typhi 'O', and 'H', and s. paratyphi A 'H' and s. paratyphi B 'H' antigens to determine the serovars present in human serum samples.

Sensitivity tests were conducted for confirmed *Salmonella* isolates using Muller-Hinton agar plates **(Himedia®**) by disk diffusion pattern using 10 types of antibiotic disks **(Himedia®**)as shown in table 2**.**

Table 2: antibiotic discs and their potency used in Sensitivity tests of isolated salmonella

Antibiotic	Lincomycin (L)	Penicillin (P)	Tetracycline (T)	Nalidixic Acid (NA)	Gentimycin (G)	Erythromycin (E)	Doxycycline (D)	Colistin (CL)	Ciprofloxacin (CIP)	Chloramphenicol (C)
Disk potency	2 µg	10 µg	30 µg	30 µg	10 µg	15 µg	30 µg	10 µg	5 µg	30 µg

RESULTS:

The results showed that, positive samples of food origin samples with *Salmonella* were 37 (20%) out of (188) different samples, individual percentage for each kind ranged from 0.05 % of raw milk to 0.19% of fish meat samples (**figure, 1**). Whereas, 8 of total 35 collected samples of human stool (22.8 %),were positive salmonella, and human blood samples had 48.9% positive serological results (**Table 3**).

About half of collected blood samples (22) showed positive reaction with Widal test against. typhi 'O', and 'H', and s. paratyphi A 'H' and s. paratyphi B 'H' antigens,and theserovars identified were; typhi (O) 49% typhi (H) 36% Paratyphi (B) 24%, but Paratyphi (A) hasn't found(**figure, 2**).

The microscopic future of gram stained isolates, exhibited a typical morphological characteristics of salmonella spp. in single, coupled, or clumped, gram positive road cells (**Figure 3).**

As showmen in **Table (4),** Isolated Salmonella were highly resistant tocolistin, doxycycline, penicillin, and lincomycin but moderate resistant was found against tetracycline and erythromycin, however, All isolated Salmonella were susceptible to ciprofloxacin, gentamycin, chloramphenicol and nalidixic acid.

Table 3:*Salmonella* positive of Human samples which collected at General Dhamar Hospital.

Human specimens	Number of samples	Positive samples					Positive%
stool	35	8					22.8
blood	45	22	Paratyphi (A)	Paratyphi (B)	typhi (O)	typhi (H)	48.9%
			-	11	22	16	
total	80	30					37.5

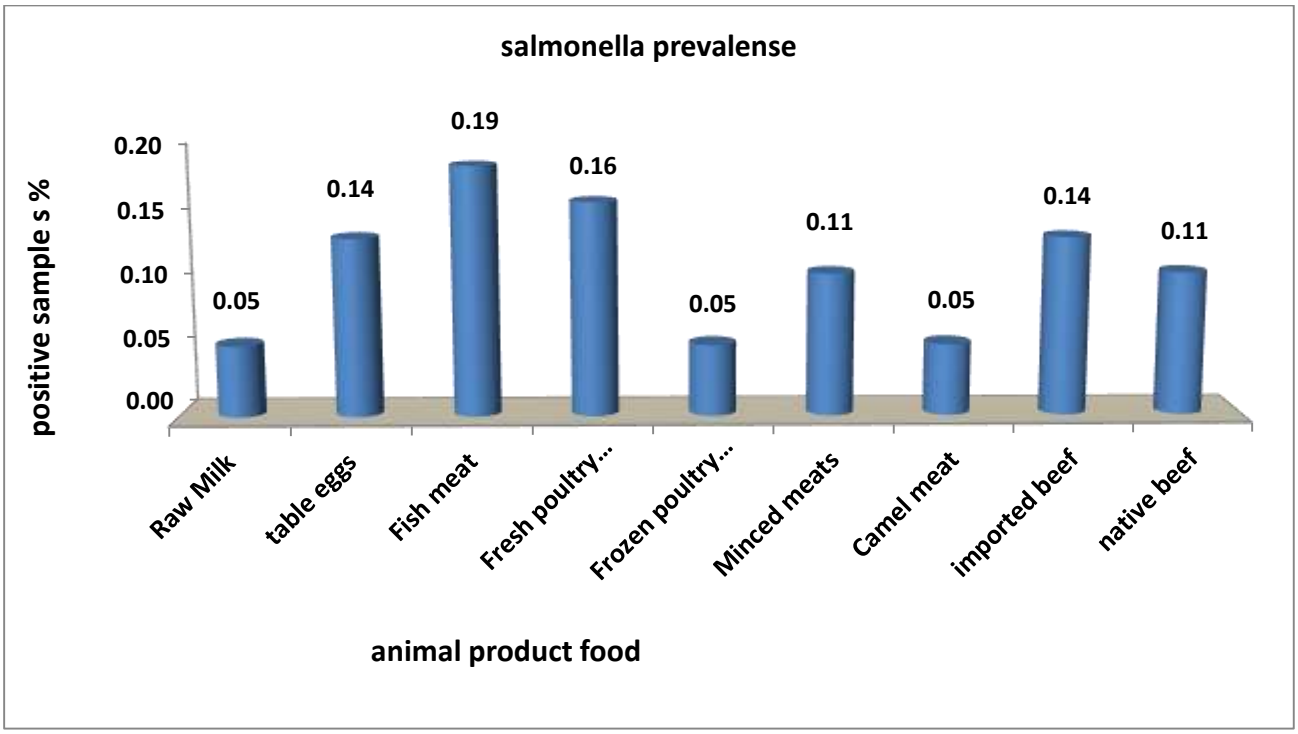

Figure 1: The distribution rates of positive *Salmonella* samples.

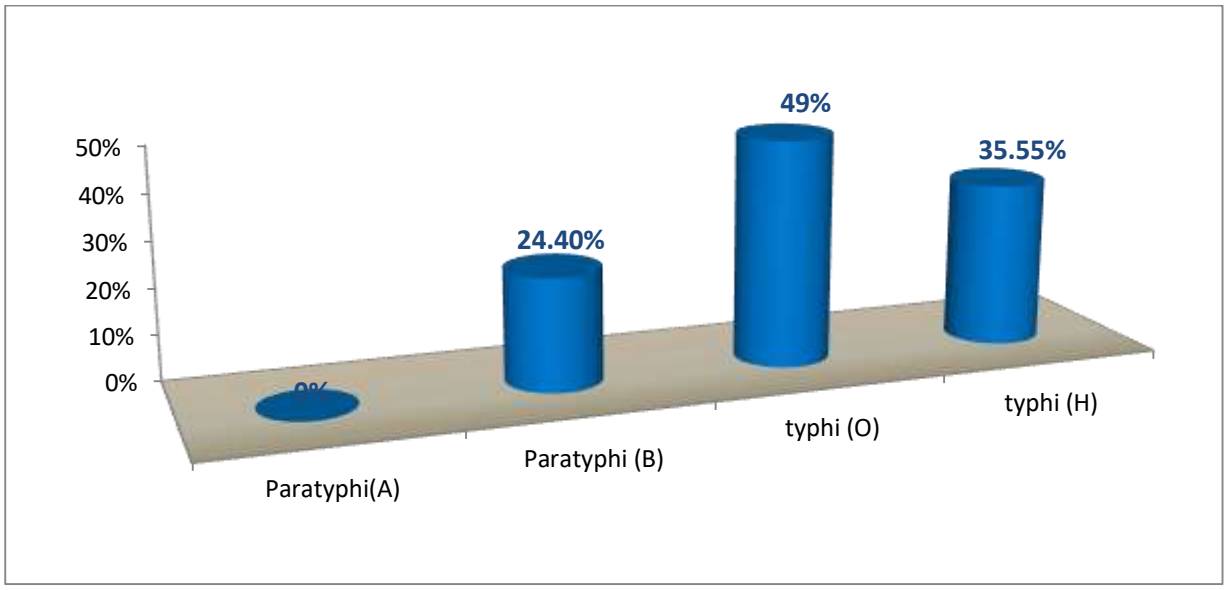

Figure 2: The positive *widal test* percentage of each kind of samples.

Figure 3: *Salmonella* gram stain (Gram negative, short rod).

Table 4: Zones of clearing for different antibiotics against isolated salmonella

Antibiotic	Disk potency	Sensitivity	
		Inhibition zone diameter / mm	Result
Chloramphenicol (C)	30 µg	27 – 30	Susceptible
Ciprofloxacin (CIP)	5 µg	25	Susceptible
Colistin (CL)	10 µg	-	Resistant
Doxycycline (D)	30 µg	-	Resistant
Erythromycin (E)	15 µg	14 – 11	Intermediate
Gentimycin (G)	10 µg	17 – 20	Susceptible
Nalidixic Acid (NA)	30 µg	18 – 20	Susceptible
Tetracycline (T)	30 µg	16	Intermediate
Penicillin (P)	10 µg	-	Resistant
Lincomycin (L)	2µg	-	Resistant

DISCUSSION:

In Yemen, high contamination of cocked and ready-to eat food with salmonella,also, the incidence of Salmonella infection in peoples suffering from enteric fever and food poisoning had detected **(AL-Ammari, 2012 and Taha et al., 2013),** but no satisfied investigation or data about the mainsources or reservoirs of these infections. This study designed for solution of this riddle. The present results showed that the highest contamination rates were in fish meats(19%), followed by fresh poultry meats (16%),which agree with many former studies (**Dhaher, et al, 2011)**. The cause of this phenomenon may due to high contamination of poultry feed **(Jones and Richardson, 2004, and EFSA, 2008a.)**in addition to contamination through transportation and processing in unplanned and muddled local slaughters that poorly equipped don't followed the minimal hygiene precautions.whereas, the mentioned two types of foods are public and the cheapest source of protein in Yemen, hence, in many developed countries,the chicken meats are the main source of human infections, they make a potential threaton nation public health .**(Al-Matar et al, 2005 and Xia et al., 2009).**The table eggs were at the third rank of positive samples which were 0.14% this rate is constant with **Herdberg, (1993) ICMSF.**

(1996)., and Poppe,et al.,(1998), whose found positive samples ranged between (%0.4) and(0.17 %). The lowest rates of positive samples were in raw milk **(0.05%)**, which agreed with **AL-Ammari, (2012)** who found similar results in animal product cocked food. .Also camel meats and frozen chicken samples had low rates (0.05) of salmonella positive, this ratified with **Lake et al., (2002),**who found low contamination of child chicken but for camel meats, in present study, this may due to the paucity of this type of meat in Dhamar that lead to low number of collected samples.

The high percentages of positive stool samples exposed a high incidence of salmonellosis (22.8) in peoples whom were attending General Dhamar hospital. These findings were similar to those reported by **Al-Haddad, (2004) and saleh, (2010).** These results may be due to the most of studied patients had a history of direct contacts with domestic animals or its products that may be the main source of their infections**(Al-Matar et al., 2005 and Xia et al.,2009).**

The positive serological results of human blood,revealed that typhi (O) was the predominant serovars(49%) followed bytyphi (H) (36%) whereas,Paratyphi (B) was the lowest(24%), and no positive results had found with Paratyphi (A), these results had Approved With **Taha et al., (2013)**. The high prevalence of Salmonella Typhi may be due to the fact that Salmonella Typhi is spread predominantly within the household, whereas Salmonella Paratyphi is mainly transmitted outside the home**(Rice et al.,2003and Massi et al., 2005).**

Isolated Salmonella were highly resistant tocolistin, doxycycline, penicillin, and lincomycin. This outcome is consistent with **Taha et al., (2013) and Gautam (2002)** this resistance may be due to miss-used of those antibiotics without medical superintendence in Yemeni's animal and poultry farms as treatment or growth promoters.All isolated Salmonella were susceptible to ciprofloxacin, gentamycin, chloramphenicol and nalidixic acid.

Conclusions and recommendations:

Salmonella was present in allsample kinds that were examined in Dhamar governorate .This study has confirmed that, animal food origin at Dhamar province, were highly contaminated with Salmonella and believed that, those products (particularly, chicken and fish meat, which are public and the cheapest source of protein in Yemen) are the main source of salmonella infection in Dhamar's people.

From our results, it recommended that, more studies on spread tracking of Salmonella spp. and control measures in the production

cycle of farm animals are required. The good hygiene, good cocking for animal product foods and medical superintendence on animal farms and slaughters should be directed to controlling the spread of Salmonella, and reduce the number of Salmonella infections.

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انتشار السالمونيلا في الإنسان والأغذية ذات المنشأ الحيواني ودراسة حساسيتها للمضادات الحيوية في محافظة ذمار / اليمن

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الخلاصة:

تعتبر السالمونيللا من الامراض الغذائية المشتركة وتشكل أحد المشاكل الرئيسية للصحة العامة. أجريت هذه الدراسة بهدف تقييم خطر انتشار السالمونيلا في الأطعمة ذات المنشأ الحيواني في محافظة ذمار. جمعت عدد مائة وثمان وثمانون عينة (١٨٨) من أغذية المنتجات الحيوانية المختلفة حيث جمعت من المسالخ والمجازر المحلية والبقالات وجمع الحليب من الأبقار. كذلك تم جمع خمس وثلاثون (٣٥) عينة من البراز البشري. جرى تجهيز هذه العينات وتحضيرها وخضعت للفحص البكتيري وتم تأكيدها بالاختبارات البيوكيميائية لتحري وجود السالمونيللا. سحبت خمس وأربعون عينة دم من الحالات البشرية المشتبهه لتحديد ما اذا كانت مصابة بالسالمونيلا باستخدام الاختبار السريع على الشريحة (اختبار وايدل). خضعت عشرة أنواع من المضادات الحيويلأختبار حساسية عزلات السالمونيلا المدروسة لها. أضهرت النتائج وجود السالمونيللا في حوالي ٢٠ % من مجموع العينات المدروسة، توزعت النتائج الموجبة على الأغذية المختلفة بحيث كانت: ٠,١١ %، ٠,١٤%، ٠,٠٥%، ٠,١١%، ٠,٠٥%، ٠,١٦%، ٠,١٩%، ٠,١٤%، و٠,٠٥ % للحمِ البقرالمحليٍ، لحم البقرالمستورد، لحم جَملِ، اللحم المفروم، لحوم الدجاج، لحوم السمكِ، بيض المائدةِ، والحليب الخام على التوالي. ٢٢,٨ % مِنْ مجموع عينات البراز البشري، كانتْ إيجابية تحتوي على السالمونيلا ، بينماأظهرت عينات الدمّ البشري نتائج ايجابية في٤٨,٩ % منها بالاختبارات المصلية، شكّل النمط المصلي (O) للسالمونيلا تايفي ٤٨% والنمط المصلي (H) ٣٦% بينما وجد النمط المصلي للسالمونيلا نظيرة التايفويد ٢٤% ولم يوجد النمط المصلي (A) للسالمونيلا نظير التايفويد. أضهرت السالمونيلات المعزولة حساسية للكلورامفينيكول والسيبروفلوكساسين والناليديكسيك أسيد والجنتامايسين، وحساسية متوسطة للإريثرومايسين والتتراسيكلين، ولكنها اظهرت مقاومة كاملة للكولستين والدوكسيسيكلين والبنسلين واللينكومايسين. لقد أكدت هذه الدراسة أن الأطعمة ذات المنشأ الحيواني في محافظة ذمار تحتوي على تلوث عالي بالسالمونيلا ويعتقد أن هذه الأطعمة هي المصدر الرئيسي لعدوى السالمونيلا لدى سكان ذمار. ويوصى بمزيد من الدراسات لتتبع انتشار السالمونيلا وطرق السيطرة عليها في في حلقات الانتاج للحيوانات الزراعية.