Original article

Sources and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002

Bayleyegn Molla, Daniel Ajemayehu , Woubit Salah

Abstract

Background: Foods of animal origin are considered to be the major sources of foodborne salmonellosis. A periodic surveillance of the sources, distribution and prevalent Salmonella serotypes in slaughtered food animals, retail meat products and environment is necessary to control the spread of the pathogen and infection of man through contaminated animal products..

Objectives: The purpose of this study was to find out the sources and distribution of Salmonella serotypes isolated from apparently healthy slaughtered cattle and camels, retail meat products (minced beef and chicken) and slaughterhouse personnel over a 5-year period (1997-2002).

Methods: Three thousand eight hundred ninety-eight samples from apparently healthy slaughtered cattle and camels, slaughterhouse personnel, minced beef and chicken meat and giblets were examined for the presence of Salmonella. Salmonellae were isolated and identified according to the techniques recommended by the International Organisation for Standardisation (ISO 6579, 1998).

Results: A total of 412 Salmonella isolates consisting of 25 different serotypes were identified from slaughtered cattle (4.2%), camels (16.2%), slaughterhouse personnel (6.0%), minced beef (12.1%), chicken meat and giblets (23.6%). The predominant serovars were *S. braenderup*, *S. dublin* and *S. saintpaul* followed by *S. typhimurium* (including var. Copenhagen) and *S. anatum* Salmonella *enteritidis* was detected from chicken, cattle and camel meat. *Salmonella typhimurium*, *S. anatum* and *S. dublin* were isolated in man as well as in food animals and meat products.

Conclusion: Isolation of Salmonella from a wide range of sources suggests that Salmonella is widespread in food animals and meat products and underlines the necessity for a joint and coordinated surveillance and monitoring programs for salmonellosis and other major food borne zoonotic diseases in Ethiopia. [*Ethip.J.Health Dev.* 2003;17(1):63-70]

Introduction

Salmonellosis is considered as one of the most widespread foodborne zoonoses in industrialized as well as developing countries even though the incidence seems to vary between countries. It is usually difficult to evaluate the situation of salmonellosis in developing countries because of the very limited scope of studies and lack of co-ordinated epidemiological surveillance systems (1,2). In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis in some developing countries including Ethiopia. The increased global population coupled with mass production of animal and human food and the rapid international trade in agriculture, aquaculture and food products could worsen the problem (3).

Department of Clinical Studies, Faculty of Veterinary Medicine, Addis Ababa University, P.O.Box 34, Debre Zeit, Ethiopia E-Mail: vetmed.rgs@telecom.net.et, Tel: 338917/ 338062; Fax: 33993

Food animals harbour a wide range of Salmonella serotypes and so act as source of contamination, which is of paramount epidemiological importance in non-typhoid human salmonellosis (2,4). The process of removing the gastrointestinal tract during slaughtering of food animals is regarded as one of the most important sources of carcass and organ contamination with Salmonella at abattoirs (4,5). Food items such as poultry, meat and meat products are the common sources of foodborne salmonellosis (1,3,6). Contamination of meat by Salmonella may occur at abattoirs from the excretion of symptomless animals, contaminated abattoir equipment, floors and personnel and the pathogen can gain access to meat at any stage during butchering (5). Cross-contamination of carcasses and meat products could continue during subsequent handling, processing, preparation and distribution.

A periodic surveillance of the level of Salmonella contamination in the different food animals, food products and environment is necessary to control the spread of the pathogen and infection of man. As the movement of people, food animals and food stuffs across national boundaries expands, the risk of salmonellosis and other foodborne zoonoses from importation/exportation also increases. Therefore, the knowledge on the prevalent Salmonella serotypes in a country is important to understand the distribution and means of introduction into a country (3,7,8). The purpose of this study was, therefore, to find out the sources and distribution of Salmonella serotypes isolated from food animals, minced beef, chicken meat and giblets and slaughterhouse personnel at different abattoirs and supermarkets in Ethiopia over a 5-year period (1997-2002).

Methods

Sources of samples

One thousand eight hundred fifty-six samples (faeces, mesenteric lymph nodes and muscle) from apparently healthy slaughtered cattle at Addis Ababa and Deber Zeit and 714 samples from camels (faeces, mesenteric lymph nodes, liver, spleen and muscle) at Dire Dawa and Jijiga slaughterhouses were obtained during slaughtering operations (Table 1).

An average of 600 heads of cattle were slaughtered daily at Addis Ababa abattoir of which 20 to 25 animals were randomly selected and sampled weekly. At Debre Zeit abattoir 15 to 20 animals were slaughtered two times a week and samples were obtained from all slaughtered cattle. About 2 to 4 camels (only male and adult) were slaughtered per day and samples were taken from all slaughtered camels. Three hundred human stool samples were collected from voluntary and accessible apparently healthy personnel at Addis Ababa abattoir in collaboration with medical personnel at the clinic. A total of 380 minced beef and 648 chicken samples (liver, gizzard, heart and meat) were randomly collected from 24 out of 42 different supermarkets in Addis Ababa. Each sample was obtained aseptically and transported in iceboxes packed with ice to the microbiology laboratory of the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit for analysis. The samples were examined upon arrival or were stored at freezer temperature for no longer than 24 hours.

Isolation and identification of Salmonella

Salmonellae were isolated and identified according to the techniques recommended by the International Organisation for Standardisation (ISO) 6579 (9). Briefly, 25 g of each sample of meat and meat products was weighed and cut into smaller fine pieces with sterile scalpel blades. Each sample was put in a sterile stomacher bag and 225 ml of buffered peptone water (BPW; Merck, Darmstadt, Germany) was added, homogenized using a stomacher (Colworth Stomacher 400, London). Faecal samples and samples smaller than 25 g were pre-enriched in BPW in a ratio of 1g of the sample to 9 ml of BPW. The pre-enriched

Samples were incubated for 16 to 20 hours at 37^0 C. One ml and 0.1 ml of the pre-enrichment broth was transferred aseptically into 10 ml of selenite cysteine (SC; Difco, Detroit, USA) and 10 ml of Rappaport Vassilliadis (RV; Merck, Darmstadt, Germany) and incubated for 18 to 24 hours at 37^0 C and 42^0 C respectively. This was followed by streaking from RV and SC broths onto brilliant green-phenol red-lactose-sucrose agar (BPLS; Merck Darmstadt, Germany) and MacConkey agar (Difco, Detroit, USA) and incubated at 37^0 C for 24 to 48 hours. The plates (BPLS and MacConkey agar) were examined for the presence of Salmonella colonies. Suspect Salmonella colonies were biochemically characterized according to ISO 6579 (9) and putative Salmonella isolates were examined for agglutination using polyvalent I and II Salmonella anti-sera (Sifin, Berlin, Germany).

Salmonella serotyping and phage typing were done at the Health Canada, Office International des Epizooties (OIE) Reference Laboratory for Salmonellosis in Guelph, Ontario, Canada (n = 221) and at the National Reference Laboratory for Salmonella of the Federal Institute for Health Protection of Consumers and Veterinary Medicine (Bg VV), Berlin, Germany, (n=191).

Results

Of the total 1856 samples examined from apparently healthy slaughtered cattle for human consumption, 79 (4.2%) were Salmonella positive (Table 1). Sixty-three of the 116 (5.6%) meat samples (diaphragmatic and abdominal muscle) were contaminated with Salmonella. One hundred and sixteen (16.2%) Salmonella positive samples were detected from a total of 714 organ and faecal samples from 119 apparently healthy slaughtered camels

Table 1: **Salmonella isolates from food animals, slaughterhouse personnel and minced beef samples (1997-2002).**

	Number of samples		
Source of Samples	**Examined**	**Positive**	**%**
Slaughter cattle:			
Faeces	370	7	1.9
Mesenteric lymph nodes	370	9	2.4
Muscle (Abdominal and diaphragmatic)	1116	63	5.6
Total	1856	79	4.2
Slaughter camels:			
Faeces	119	18	15.1
Mesenteric lymph nodes	119	19	15.9
Liver	119	14	11.8
Spleen	119	17	14.3
Muscle (Abdominal and diaphramatic)	238	48	20.1
Total	714	116	16.2
Slaughterhouse personnel:			
Human stool	300	18	6.0
Supermarket:			
Minced beef	380	46	12.1
Chicken meat and giblets:			
Meat	452	54	8.3
Liver	111	33	29.7
Gizzard	116	48	41.4
Heart	85	18	21.2
Total	648	153	23.6

in Eastern Ethiopia. Of the 380 minced beef samples from supermarkets and 300 human stool samples examined from slaughterhouse personnel, 46 (12.1%) and 18 (6.0%) of them contained Salmonella, respectively (Table 1). Out of the 648 chicken samples (meat, liver, gizzard and heart) examined, 153 (23.6%) were contaminated with Salmonella. A high level of Salmonella contamination was detected in edible chicken gizzard and liver followed by heart and meat.

A total of 412 Salmonella isolated consisting of 25 different serotypes were identified (Table 2). The predominant serotypes were *S. braenderup*, *S. dublin* and *S. saintpaul* followed by *S. typhimurium* (including var. *Copenhagen*) and S. anatum. Some phage types of *S. typhimurium*, *S. enteritidis* and *S. heidelberg* were also identified. Table 3 shows the distribution and sources of Salmonella isolates from slaughter cattle, camel, slaughterhouse personnel, minced beef and chicken samples. Salmonella *typhimurium* and Salmonella *anatum* were isolated from all sources including man whereas *S. melagridis* was detected only in man. *S. dublin* was isolated from minced beef, slaughter cattle and slaughterhouse personnel. Salmonella *enteritidis* was detected from camel meat, chicken and slaughtered cattle. Some serovars such *S. heidelberg*, *S. derby*, *S. butantan* and *S. havana* were isolated only from camels whereas *S. uganda* and *S. virchow* were detected only from chickens.

Table 2: **Salmonella serotype isolated from different sources**

Serogroup	Serotype	Number isolated	%	Phage types (PT) identified
B (O:4)	*S. saintpaul*	62	15.0	-
	S. typhimurium var. Copenhagen	28	6.8	PT 104, PT 120
	S. typhimurium	28	6.8	PT1, PT2, PT 79
	S. heidelberg	2	0.5	PT 19
	S. derby	1	0.2	-
	S. haifa	2	0.5	-
	Salmonella II 4:12	2	0.5	-
C1 (O:7)	*S. braenderup*	78	18.9	-
	S. infantis	5	1.2	-
	S. virchow	1	0.2	-
	Salmonella I 6:7:eh:-	1	0.2	-
C 2-3 (O:8)	*S. muenchen*	12	2.9	-
	S. kottbus	12	2.9	-
	S. hadar	5	1.2	-
	S. bovismorbificans	2	0.5	-
D 1 (O:9)	*S. dublin*	71	17.2	-
	S. enteritidis	8	1.9	-
E1 (O:3,10)	*S. anatum*	53	12.9	PT 8
	S. Uganda	6	1.5	-
	S. meleagridis	5	1.2	-
	S. butantan	2	0.5	-
G(O:13)	*S. mishmarhaemek*	12	2.9	-
	S. Havana	6	1.5	-
M (O:28)	*S. Guildford*	3	0.7	-
Others	*S. rough form*	5	1.2	-
	Total	412		

Table 3: **Distribution of Salmonella serotypes by source**

Serotype	Source and number of serotypes					
	Cattle	Minced beef	Abattoir personnel	Chicken meat and giblets	Camel	Total
S. saintpaul	9	-	-	8	45	62
S. typhhimurium var. Copenhagen	-	-	-	24	4	28
S. Heidelberg	-	-	-	-	2	2
S. derby	-	-	-	-	1	1
S. Dublin	47	20	4	-	-	71
S. Uganda	-	-	-	6	-	6
S. typhimurium	5	2	2	18	1	28
S. braenderup	-	-	-	52	26	78
S. infants	-	-	-	2	3	5
S. muenchen	1	1	-	-	10	12
S. kottbus	-	-	-	5	7	12
S. meleagridis	-	-	5	-	-	5
S. mishmarhaemek	12	-	-	-	-	12
S. hadar	-	-	-	2	3	5
S. bovismorbificans	-	-	-	1	1	2
S. enteritidis	3	-	-	4	1	8
S. Guildford	3	-	-	-	-	3
S. anatum	8	13	7	22	3	53
S. butantan	-	-	-	-	2	2
S. Haifa	-	-	-	2	2	2
S. Havana	-	-	-	-	6	6
S. virchow	-	-	-	1	-	1
Salmonella II 4: 12B	-	-	-	2	-	2
S.rough form	-	1	2	2	-	5
Salmonella I 6:7:eh:-	-	-	1	-	-	1
Total	79	46	18	153	116	412

Discussion

During the period 1997-2002 a wide range of Salmonella serotypes were isolated from different sources. Analysis of the results indicated that of the 412 Salmonella serotypes, 153 (37.1%) originated from chicken meat and giblets, 116 (28.1%) and 79 (19.2%) from apparently healthy slaughtered camels and cattle respectively. Forty-six (11.2%) of the Salmonella serotypes were from minced beef samples obtained from supermarkets and 18 (4.4%) from slaughterhouse personnel. Eventhough there have been studies on Salmonella infection in apparently healthy slaughtered cattle and camels, there has been no uniformity with respect to the materials examined, the sampling and culture techniques and distribution of salmonellae in a lot examined, consequently the results may not be comparable (4). About 4.2% and 16.2% of samples from slaughtered cattle and camels respectively were positive for Salmonella. This is particularly important in Ethiopia where raw and undercooked meat is consumed. A relatively high prevalence rate of Salmonella in both cattle and camels had also been reported by various authors elsewhere (5,10,11). The relatively low prevalence of Salmonella contamination in cattle might be due to a low Salmonella carrier rate among the cattle as

compared to camels in the study populations. The existing poor hygienic practices and facilities in both cattle and camel slaughterhouses could also exacerbate the contamination of carcasses and edible organs.

Of the meat samples analysed 6.5% were positive for Salmonella and was in agreement with D'Aoust (8) who reported that the contamination rate of beef carcasses with Salmonella varies from 0.2 to 21.5%. The detection of 60% of the serovars in meat as well as in faeces and mesenteric lymph node samples suggests that the process of evisceration could be the main source of carcass contamination in additon to the carrier state. Cross-contamination can also occur during the skinning process as a result of poor hygienic conditions. The other probable source of contamination is infected abattoir personnel. Eighteen of the 300 stool samples (6%) from apparently healthy abattoir personnel in Addis Ababa were positive for Salmonella and some of the serovars therein were also isolated from slaughtered cattle including *S. typhimurium*, *S. anatum* and *S. dublin*. This may be interpreted as evidence of an association between contamination of cattle carcasses and people. Salmonellae have been isolated previously in man in Ethiopia (12,13,14). Forty-five Salmonella strains were isolated from adult diarrhoeal out patients in Addis Ababa of which 84.4% belong to non-typhoid serogroups (14) whose source of infection was most probably contaminated food and food products.

The high level of Salmonella contamination of chicken samples (23.6%) confirms the findings of previous studies on salmonellosis in chicken and chicken products (15,16,17). Out of the total 648 samples, 312 (48.1%) were giblets, which had higher contamination level of Salmonella (15.3%) than carcass samples (8.3%). Comparison could also be made with the other reports in which a high level of Salmonella contamination was detected in retail chicken meat and giblets (16,17). Cross-contamination of Salmonella from giblets to carcass could occur during handling, processing, packing and distributions. In addition to this, scalding water can become contaminated with Salmonella from faeces, plucking equipment, cages or floors. Workers could also spread the contamination during retailing.

It is known that the serovars involved in salmonellosis vary geographically. While some serovars maintain their dominant role over many years, others emerge, re-emerge or decrease over time. A rapid international trade in agriculture, aquaculture and manufactured food products has facilitated the introduction of Salmonella serovars within the geographic boundaries of importing countries (3,15). Of the 25 different Salmonella serotypes identified, *S. braenderup*, *S. dublin*, *S. saintpaul*, *S. anatum* and *S. typhimurium* were the prevalent ones. Previously other workers have reported some of these and other non-host adapted Salmonella serotypes in food animals, meat products and man (7,10,16,17,18). Analysis of the results of serotyping of the Salmonella isolates from different sources suggests that an association exists between the occurrence of certain Salmonella serotypes in food animals, meat products and in man, which could be acquired by man through ingestion of contaminated food and food products. Other contaminated food products can only justify the origin of *S. meleagridis* as it was detected only from the stool of abattoir workers. In addition to the serotyping, the identification of phage types of pathogenic and invasive Salmonella strains could be important in the future to be used as markers in epidemiological studies of salmonellosis (8) in the different species of food animals, meat and meat products and man in Ethiopia.

It should be noted that the detection of invasive and pathogenic Salmonella serotypes such as *S. typhimurium*, *S. enteritidis*, *S. uganda* *S.*

heidelberg, *S. dublin* and others is of public health significance since contaminated meat and meat products may pose health hazards. The high-risk part of human population, that is infants, elderly, immunocompromised and malnourished persons are highly susceptible and the presence of Salmonella even in low numbers constitutes a major public health concern (1,6,7,19). The risk may further be accentuated if meat is consumed raw or undercooked or if cross-contamination of the kitchen with Salmonella during meal preparation occurs. The control of Salmonella contamination and other foodborne pathogens in the food chain includes the introduction of good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) concepts together with stringent control of all aspects of meat production, preparation, storage and distribution.

Isolation of various serotypes of Salmonella from a wide range of sources indicated the presence and widespread distribution of Salmonella of food animal and human origin, which is of significance in the veterinary and public health sectors in Ethiopia. It also underlines the necessity for a joint and coordinated surveillance and monitoring programs for salmonellosis and other major foodborne zoonotic diseases in the country.

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