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ORIGINAL ARTICLE

Occurrence and antimicrobial resistance of *Salmonella* serovars in apparently healthy slaughtered sheep and goats of central Ethiopia

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Abstract The present study was undertaken to determine the occurrence, distribution and antimicrobial resistance pattern of *Salmonella* serovars in apparently healthy slaughtered sheep and goats in central Ethiopia. A total 1224 samples consisting of faeces, mesenteric lymph nodes, liver, spleen, and abdominal and diaphragmatic muscle samples were collected from 104 sheep and 100 goats. *Salmonella* was isolated from 12 of 104 (11.5%) sheep and 3 of 100 (3%) goats. Of the total 624 and 600 samples examined from sheep and goats, 18 (2.9%) and 4 (0.7%), respectively, were *Salmonella* positive. The 22 *Salmonella* isolates belonged to 9 different serovars. The common serovars isolated were *S. typhimurium*, followed by *S. heidelberg*, *S. reading*, *S. give*, and *S. poona*. Seven of the 22 isolates (31.8%) were multidrug-resistant to various antimicrobials.

Keywords Slaughter sheep and goats · *Salmonella* · Antimicrobial resistance · Ethiopia

Abbreviations

ABM	abdominal muscle
Amc	amoxicillin/clavulanic acid
Amp	ampicillin
Cef	cephalothin
Chl	chloramphenicol
DIM	diaphragmatic muscle
ICEPT	International Centre for Enteric Phage Typing
ISO	International Organization for Standardization
MLN	mesenteric lymph node
NCCLS	National Committee for Clinical Laboratory Standards
NLEP	National Laboratory for Enteric Pathogens
Spt	spectinomycin
Str	streptomycin
Sul	sulfisoxazole
Sxt	sulfamethoxazole/trimethoprim
Tet	tetracycline
Tmp	trimethoprim

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Introduction

Previous reports have indicated that sheep and goats harbour a wide range of *Salmonella* serovars and act as one of the sources of infection of human salmonellosis (Nabbut and Al-Nakhli, 1982; Abdel-Ghani *et al*., 1987). Contamination of red meat by *Salmonella* may occur at abattoirs from carrier animals excreting the organism and by contaminated abattoir equipment and floors (Smeltzer *et al*., 1980). It is obvious that salmonellae in the mesenteric lymph nodes, which could be incised during the inspection process, and in faeces, constitute sources for contamination of red meat and other edible parts of the carcasses (Smeltzer *et al*., 1980; Nabbut and Al-Nakhli, 1982).

Antimicrobial resistance of *Salmonella* is a global public health and veterinary concern. The widespread administration of medically important antimicrobials to food animals at subtherapeutic or prophylactic doses may promote on-farm selection of antimicrobial-resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products (D'Aoust *et al*., 1992). Furthermore, in most sub-Saharan African countries like Ethiopia, antimicrobial agents are indiscriminately used in both veterinary and human health practices and a high level of antimicrobial resistance in *Salmonella* has been reported (Leegard et *al*., 1996; Mache *et al*., 1997; Alemayehu *et al*., 2003).

Studies conducted in Ethiopia indicated the presence of *Salmonella* in various food animals (Alemayehu *et al*., 2003; Molla *et al*., 2004) and in humans (Mache *et al*., 1997). However, information on salmonellosis in sheep and goat is very limited (Woldemariam *et al*., 2005). This study was therefore undertaken to determine the occurrence, distribution and antimicrobial resistance pattern of *Salmonella* serovars isolated from apparently healthy slaughtered sheep and goats at the Addis Ababa and Modjo abattoirs, central Ethiopia.

Materials and methods

Collection of samples

A cross-sectional study on *Salmonella* was undertaken from September 2003 to February 2004 at the Addis Ababa abattoir (Addis Ababa) and Modjo abattoir (Modjo), Ethiopia. Modjo is a small town located 73 km south-east of Addis Ababa. The sheep and goats slaughtered originated from markets and were brought to the abattoirs either on foot or by truck. In Addis Ababa abattoir, an average of 250 sheep, 75 goats and 700 head of cattle are slaughtered daily. In Modjo abattoir on average 50 sheep and 500 goats are slaughtered daily. On each sampling day, six types of samples were collected from 12 randomly selected sheep and goats ($n = 72$). Faecal, mesenteric lymph nodes, liver, spleen, diaphragm and abdominal muscle samples were collected separately in sterile containers. A total of 624 and 600 samples were collected from 104 sheep and 100 goats respectively. The samples were transported to the laboratory for analysis in an icebox packed with ice.

Isolation and identification of *Salmonella*

Salmonellae were isolated and identified according to the techniques recommended by the International Organization for Standardization (ISO 6579, 1998) and Quinn and colleagues (2004) as described previously (Alemayehu *et al*., 2003; Molla *et al*., 2004). Presumptive *Salmonella* isolates were shipped to the Public Health Agency, Office International des Épizooties (OIÉ) Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada for serotyping, phage typing and antimicrobial resistance testing.

Serotyping

For serotyping, the somatic (O) antigens of the *Salmonella* isolates were determined with a slide agglutination test as described by Ewing (1986), whereas the flagellar (H) antigens were identified by using a microtechnique that employs microtitre plates (Shipp and Rowe, 1980). The antigenic formulae of *Salmonella* serovars as listed by Popoff and Le Minor (1997) were used to name the serovars.

Phage typing

The standard phage typing technique described by Anderson and Williams (1956) was followed. *S. enteritidis* strains were phage typed with typing phages obtained from the International Centre for Enteric Phage Typing (ICEPT), Central Public Health Laboratory, Colindale, United Kingdom (Ward *et al*., 1987) via the National Laboratory for Enteric Pathogens (NLEP),

Table 1 *Salmonella* in apparently healthy slaughtered sheep and goats at Addis Ababa and Modjo abattoirs

	Occurrence of *Salmonella* by species and abattoir					
	Addis Ababa abattoir		Modjo abattoir		Total	
Sample type	Sheep	Goats	Sheep	Goats	Examined	Positive (%)
MLN[a]	8.7 (4/46)	0 (0/45)	6.9 (4/58)	3.6 (2/55)	204	10 (4.9)
Faeces	6.5 (3/46)	2.2 (1/45)	3.5 (2/58)	1.8(1/55)	204	7 (3.4)
Liver	2.2 (1/46)	0 (0/45)	1.7 (1/58)	0 (0/55)	204	2 (0.9)
Spleen	2.2 (1/46)	0 (0/45)	0 (0/58)	0 (0/55)	204	1 (0.5)
ABM[a]	2.2 (1/46)	0 (0/45)	1.7 (1/58)	0 (0/55)	204	2 (0.9)
DIM[a]	0 (0/46)	0 (0/45)	0 (0/58)	0 (0/55)	204	–
Total	3.6 (10/276)	0.4 (1/270)	2.3 (8/348)	0.9 (3/330)	1224	22 (1.8)

[a]MLN, mesenteric lymph node; ABM, abdominal muscle; DIM, diaphragmatic muscle

Health Canada, Winnipeg, Manitoba. The phage typing scheme and phages for *S. typhimurium* developed by Callow (1959) and further extended by Anderson (1964) and Anderson and colleagues (1977) were obtained from ICEPT via NLEP. The *S. heidelberg* phage typing scheme and phages were supplied by NLEP (Demczuk *et al*., 2003). Strains that did not conform to any recognized phage type were considered atypical (AT).

Antimicrobial resistance testing

The *Salmonella* isolates were tested for resistance to 24 selected antimicrobial drugs by the agar dilution method (Larkin *et al*., 2004). The antimicrobials and breakpoints were amikacin (Amk) 16 μg/ml; ampicillin (Amp) 32 μg/ml; amoxicillin/clavulanic acid (Amc) 64/16 μg/ml; apramycin (Apr) 32 μg/ml; carbadox (Crb) 30 μg/ml; cephalothin (Cef) 32 μg/ml; ceftriaxone (Cro) 8 μg/ml; ceftiofur (Ctf) 8 μg/ml; cefoxitin (Fox) 32 μg/ml; chloramphenicol (Chl) 32 μg/ml; ciprofloxacin (Cip) 0.125 μg/ml; Florfenicol (flo) 16 μg/ml; gentamicin (Gen) 16 μg/ml; kanamycin (Kan) 64 μg/ml; nalidixic acid (Nal) 32 μg/ml; neomycin (Neo) 16 μg/ml; nitrofurantoin (Nit) 64 μg/ml; spectinomycin (Spt) 64 μg/ml; streptomycin (Str) 32 μg/ml; sulfisoxazole (Sul) 521 μg/ml; sulfamethoxazole/trimethoprim (Sxt) 76/4 μg/ml; tetracycline (Tet) 16 μg/ml; tobramycin (Tob) 8μg/ml and trimethoprim (Tmp) 16 μg/ml. The reference strains described by the National Committee for Clinical Laboratory Standards (NCCLS, 1999) for antimicrobial susceptibility tests of bacteria isolated from animals were used as controls. An isolate was defined as resistant if it was resistant to one or more antimicrobials, whereas isolates that were resistant to two or more antimicrobials were considered as multidrug-resistant strains.

Results

Out of the 104 sheep and 100 goats examined for the presence of *Salmonella* in the body tissues and faecal samples, *Salmonella* was isolated from 15 (7.4%) of the animals of which 12 (11.5%) and 3 (3%) were sheep and goats, respectively (Table 1). There was a statistically significant difference ($p < 0.05$) in the isolation of *Salmonella* between sheep and goats. From a total of 1224 tissue and faecal samples of sheep ($n = 624$) and goats ($n = 600$) from the two abattoirs, *Salmonella* was detected in 22 (1.8%) samples. Of the 624 samples examined from sheep, 18 (2.9%) were *Salmonella* positive. *Salmonella* was isolated from 4.8% and 7.7% of each 104 faecal and mesenteric lymph node samples. *Salmonella* was not isolated from any of the 104 diaphragmatic muscle samples examined. The difference in the frequency of *Salmonella* isolation among faecal, mesenteric lymph nodes, liver, spleen, abdominal muscle and diaphragmatic muscle samples from sheep was statistically significant ($p < 0.05$). Only 4 (0.7%) *Salmonella*-positive samples were detected from a total of 600 samples from goats (Table 1).

Twenty-two *Salmonella* isolates belonging to 9 different serovars were identified from sheep and goats (Table 2). The common serovars were *S. typhimurium*, followed by *S. heidelberg*, *S. reading*, *S. give* and *S. poona*. The maximum number of isolates in one

Table 2 Distribution of *Salmonella* serovars by animal species and abattoir

	Source and number of serovars						
	Addis Ababa abattoir		Modjo abattoir		Total		
Serovar	Sheep	Goats	Sheep	Goats	Sheep	Goats	Total
S. typhimurium	6	1	1	–	7	1	8
S. typhimurium var. Copenhagen	1	–	–	–	1	–	1
S. heidelberg	–	–	4	–	4	–	4
S. reading	2	–	–	–	2	–	2
S. give	–	–	2	–	2	–	2
S. poona	–	–	–	2	–	2	2
S. enteritidis	1	–	–	–	1	–	1
S. niederoderwitz	–	–	1	–	1	–	1
S. I: 6,7,14: -: enz15	–	–	–	1	–	1	1
Total	10	1	8	3	18	4	22

animal was four (in faeces, mesenteric lymph nodes, liver and spleen) consisting of two serovars (*S. typhimurium* and *S. typhimurium* var. Copenhagen). Two serovars (*S. heidelberg* and *S. typhimurium*) were also detected from one sheep.

All the eight *S. typhimurium* isolates belonged to 5 different phage types (Table 3). All isolated phage types were detected in sheep. In goats only *S. typhimurium* phage type 193 was detected. *S. typhimurium* phage types 46, 193, 2, 79, and phage type U285 were detected. *S. typhimurium* var. Copenhagen phage type 104 and *S. enteritidis* phage type 5a were also identified in mesenteric lymph nodes and faeces, respectively. All the identified phage types of *S. heidelberg* were atypical.

Seven (31.8%) of the 22 *Salmonella* isolates were multidrug-resistant (resistance to two or more drugs) (Table 3). Only strains of *S. typhimurium* and *S. reading* were resistant. Of the *S. typhimurium* strains, phage types 46 and 193 were resistant. All resistant

Table 3 Distribution of antimicrobial resistance of *Salmonella* isolates by source

				Number of strains	
Species	Source[a]	Serovar	Phage type	Tested	Resistant (pattern)[b]
Sheep	Faeces	*S. enteritidis*	5a	1	–
		S. typhimurium	193	1	1 (StrSulSxtTetTmp)
	Faeces, MLN	*S. reading*	–	2	2 (StrSulTet)
	Faeces, MLN, ABM	*S. heidelberg*	Atypical	4	–
	MLN, liver	*S. give*	–	2	–
	MLN	*S. niederoderwitz*	–	1	–
		S. typhimurium var. Copenhagen	104	1	–
		S. typhimurium	79	1	–
		S. typhimurium	2	1	–
		S. typhimurium	U285	1	–
	Liver, spleen, ABM	*S. typhimurium*	46	3	3 (AmpCef)
Goats	Faeces	*S. typhimurium*	193	1	1 (AmpAmcCefChl-SptStrSulSxtTmp)
	Faeces, MLN	*S. poona*	–	2	–
	MLN	*S.* I: 6,7,14:-:enz15	–	1	–
Total				22	7 (31.8)

[a]MLN, mesenteric lymph node; ABM, abdominal muscle; DIM, diaphragmatic muscle

[b]Amp, ampicillin; Amc, amoxicillin/clavulanic acid; Cef, cephalothin; Chl, chloramphenicol; Spt, spectinomycin; Str, streptomycin; Sul, sulfisoxazole; Sxt, sulfamethoxazole/trimethoprim; Tet, tetracycline; Tmp, trimethoprim

Salmonella isolates were detected at the Addis Ababa abattoir, whereas isolates from the Modjo abattoir were susceptible to the 24 antimicrobials tested. All *Salmonella* isolates were susceptible to the antimicrobial effects of amikacin, apramycin, carbadox, ceftriaxone, ceftiofur, cefoxitin, ciprofloxacin, florfenicol, gentamicin, kanamycin, nalidix acid, neomycin, nitrofurantoin and tobramycin. Resistance to the remaining 10 antimicrobials varied between 4.6% and 18.2%. Resistance to ampicillin, sulfisoxazole, streptomycin and cephalothin (each 18.2%) was common among the isolates, followed by tetracycline (13.6%).

Salmonella typhimurium isolates showed resistance to ampicillin and cephalothin, sulfisoxazole, streptomycin, sulfamethoxazole-trimethoprim and trimethoprim, amoxicillin/clavulanic acid, chloramphenicol, spectinomycin and tetracycline. However, *S. reading* isolates showed resistance to streptomycin, sulfisoxazole and tetracycline only (Table 3). All the resistant strains of *S. typhimurium* and *S. reading* showed a multidrug resistance pattern. *S. typhimurium* PT 193 demonstrated a multidrug resistance pattern to up to 9 different antimicrobials (AmpAmcCefChlSptStrSulSxtTmp). A resistance pattern to AmpCef and StrSulTet was also observed. *Salmonella reading* isolates were multiply resistant to StrSulTet (Table 3).

Discussion

The occurrence of *Salmonella* was higher in sheep (11.5%) as compared to goats (3%). The variation in the occurrence of *Salmonella* between sheep and goats might be due to differences in feeding behaviour, rearing areas and management in the two species. In our study multiple ($n = 6$) samples were included from the same animal, which could possibly increase the chance of detecting a positive animal. Our finding was consistent with previous reports of 2–51.5% prevalence of *Salmonella* in sheep (Abdel-Ghani *et al*., 1987; Sierra *et al*., 1995). The results, however, were higher than those in previous studies (6.4%) of Woldemariam and colleagues (2005) at the Elfora Debre Zeit abattoir (Ethiopia) and other workers elsewhere (Kumar *et al*., 1973; Nabbut and Al-Nakhli, 1982; Sharma *et al*., 1996). The difference in the prevalence reported could be due to differences in study sites (abattoirs) and animal populations. In these studies, only one or two sample types were examined and a higher percentage prevalence would probably have been obtained if other organs had been examined.

Of the sample types taken from each animal during the study period, the mesenteric lymph nodes and faecal samples proved to be useful indicators of infection, as most of the sheep and all *Salmonella*-positive goats were detected on the basis of those samples. The liver, spleen, abdominal and diaphragmatic samples did not appear to harbour *Salmonella* on most occasions. This indicates that the organisms did not spread beyond the lymph nodes or if they did they were in numbers too small to be detected by the method used (Samuel *et al*., 1981; Nabbut and Al-Nakhli, 1982). The detection of *Salmonella* in 4.8% and 2% of the faecal samples of sheep and goats, respectively, in this study supports earlier observations (Nabbut and Al-Nakhli, 1982; Abdel-Ghani *et al*., 1987; Woldemariam *et al*., 2005). Usually, healthy carriers intermittently excrete only a few salmonellae, unless they undergo some kind of stress, for example during transport or holding in the lairages prior to slaughter (Samuel *et al*., 1981).

The carrier rate of goats in mesenteric lymph nodes was low (only 2% compared to 7.7% in sheep). Carrier sheep and goats could serve as sources of *Salmonella* contamination during and after transportation to a slaughterhouse, increasing the risk that the meat will be contaminated during the evisceration process and after slaughter. When lymph nodes from infected animals are incised during meat inspection, a substantial reservoir of *Salmonella* would be exposed and be transferred to other parts of the carcasses via equipment or personnel (Samuel *et al*., 1981). The finding of a high proportion of infected sheep (7.7%) harbouring *Salmonella* in their mesenteric lymph nodes indicates the existence of a substantial risk of cross-contamination during slaughtering, subsequent handling, storage and distribution of the carcasses.

The low prevalence of *Salmonella* in liver and spleen of sheep in this study supports other findings (Kumar *et al*., 1973; Nabbut and Al-Nakhli, 1982), indicating that localization of the organisms in these organs is most likely minimal. The liver and spleen are usually free of *Salmonella* at slaughter, but the surfaces can become contaminated during processing. The ultimate source of this contamination is likely to be the *Salmonella* present in the gastrointestinal tract and mesenteric lymph nodes either of the same animal or of other animals slaughtered on the same day (Samuel

et al., 1981; Nabbut and Al-Nakhli, 1982). In the present study, *Salmonella* was isolated in 1.9% of the abdominal muscle of sheep while it was not detected in any of the diaphragmatic muscle of sheep or diaphragmatic and abdominal muscles of goats, suggesting a low contamination level of red meat of sheep and goats during slaughtering.

Of the nine different *Salmonella* serovars identified in sheep and goats, some of the serovars (*S. typhimurium*, *S. poona*, *S. heidelberg*, *S. reading* and *S. enteritidis*) were also reported previously (Woldemariam *et al*., 2005). *Salmonella typhimurium* was the most prevalent serovars in this study. This finding was consistent with previous reports (Kumar *et al*., 1973; Nabbut and Al-Nakhli, 1982; Sierra *et al*., 1995). In Ethiopia, *S. typhimurium* has been recovered in bovine and camel mesenteric lymph nodes and beef cuts (Alemayehu *et al*., 2003; Molla *et al*., 2004). Different *Salmonella* serovars have been previously identified from sheep and goats (Woldemariam *et al*., 2005) in Ethiopia. However, from those serovars identified previously, only *S. typhimurium* was detected again among the nine serovars isolated in the present study. It is possible that those serovars could be present in Ethiopian sheep and goats in such low levels that our present sample size was not large enough to detect them or they might be restricted to a certain geographical areas and sheep and goat populations of the country. It is known that the presence and distribution of *Salmonella* serovars can vary geographically from region to region. While some serovars remain prevalent over many years, others emerge or decreased over time. The rapid international trade in agricultural, aquaculture and food products has also facilitated the introduction of new *Salmonella* serovars into importing countries (D'Aoust, 1994).

Antimicrobial-resistant *Salmonella* isolates from animals and human sources have been reported in Ethiopia (Gebre-Yohannes *et al*., 1987; Mache *et al*., 1997; Alemayehu *et al*., 2003; Molla *et al*., 2003). In our study 31.8% of the *Salmonella* isolates from sheep and goats were multiply resistant to antimicrobial drugs commonly used to treat bacterial infections in animals and humans in Ethiopia. This could be attributed primarily to the indiscriminate use and misuse of antimicrobials both in the veterinary and human health sectors (Leegard *et al*., 1996; Molla *et al*., 2003). In Ethiopia, people have easy access to antimicrobials and can purchase them without prescription. In this study, all the resistant strains of *S. typhimurium* (62.5%) exhibited multidrug resistance. Gebre-Yohannes and colleagues (1987) also reported that *S. typhimurium* strains isolated from hospitalized human patients in Addis Ababa were resistant to a number of antimicrobials. In the present study *S. reading* strains were resistant to streptomycin, sulfisoxazole and tetracycline.

Salmonella typhimurium phage type 193 is a notable multidrug resistance strain responsible for outbreaks in humans in the late 1980s and early 1990s, mainly in Europe (Gebreyes and Altier, 2002). This phage type was among the most common multidrug-resistant strains isolated from sheep and goats in this study. This phage type exhibited a multidrug resistance to up to 9 antimicrobials (AmpAmcCefChlSptStrSulSxtTmp). Agricultural use of subtherapeutic doses of antimicrobial drugs could select for bacterial strains harbouring plasmids with multiple resistance genes. The emergence and prevalence of multiply resistant *Salmonella* in food animals can seriously compromise public health (D'Aoust *et al*., 1992). The existence of *Salmonella* in Ethiopian sheep and goats in general and the detection of multidrug-resistant and pathogenic *Salmonella* serovars such as *S. typhimurium*, *S. heidelberg*, *S. enteritidis* and *S. reading* in particular emphasize the threat to public health.

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Occurrence et résistance antimicrobienne de sérotypes de *Salmonella* chez des moutons et des chèvres apparemment en bonne santé abattus en Éthiopie centrale

Résumé – La présente étude a été entreprise afin de déterminer le profil de l'occurrence, de la distribution et de la résistance antimicrobienne de sérotypes de *Salmonella* chez des moutons et des chèvres apparemment en bonne santé abattus en Éthiopie centrale. 1224 échantillons au total consistant en fèces, ganglions lymphatiques mésentériques, foie, rate, muscles abdominaux et diaphragmatiques ont été recueillis de 104 et 100 moutons et chèvres respectivement. *Salmonella* a été isolé chez 12 sur 104 (11.5%) moutons et chez 3 sur 100 (3%) chèvres. Sur le total de 624 et 600 échantillons de moutons et chèvres examinés, 18 (2.9%) et 4 (0.7%) respectivement ont été positifs à *Salmonella*. Les 22 isolats de *Salmonella* appartenaient à 9 sérotypes différents. Les sérotypes fréquemment isolés ont été *S. typhimurium*, suivis de *S. heidelberg*, *S. reading*, *S. give* et *S. poona*. Sept des 22 isolats (31.8%) se sont avérés multirésistants à divers agents antimicrobiens.

Presencia y resistencia antimicrobiana de serovares de *Salmonella* en ovejas y cabras aparentemente sanas sacrificadas en Etiopía Central

Resumen – El presente estudio se llevó a cabo para determinar la incidencia, distribución y pauta de resistencia antimicrobiana de serovares de *Salmonella* en ovejas y cabras aparentemente sanas sacrificadas en el centro de Etiopía. Se recogieron un total de 1224 muestras de heces, ganglios linfáticos mesentéricos, hígado, bazo, y músculos abdominales y diafragmáticos, a partir de 104 ovejas y 100 cabras. Se aisló *Salmonella* en 12 de las 104 ovejas (11.5%) y en 3 de las 100 cabras (3%). Del total de las 624 y 600 muestras examinadas de ovejas y cabras respectivamente, 18 (2.9%) y 4 (0.7%) respectivamente dieron positivas para la *Samonella*. Los 22 aislados de *Salmonella* pertenecían a 9 serovares distintos. Los serovares aislados más frecuentes fueron *S. typhimurium*, seguido por *S. heidelberg*, *S. reading*, *S. give*, y *S. poona*. Siete de los 22 aislados (31.8%) fueron resistentes farmacológicamente a varios antimicrobianos.