Prevalence and Antimicrobial Resistance Pattern of Salmonella Isolates from Apparently Healthy Slaughtered Cattle in Ethiopia

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ABSTRACT

The prevalence and antimicrobial resistance pattern of Salmonella isolates was determined from apparently healthy slaughtered cattle at Debre Zeit (Ethiopia). A total of 323 cattle were examined for the presence of Salmonella in faeces, mesenteric lymph nodes, abdominal and diaphragmatic muscles. Salmonellae were cultured from 23 (7.1%) of the animals. Salmonellae were isolated from 2 (3.1%) and 3 (4.5%) of 65 pooled faecal and mesenteric lymph node samples, respectively. Nine (2.8%) abdominal muscle and 10 (3.1%) diaphragmatic muscle samples (n=323 of each) were contaminated by Salmonella. About 60% of the serovars identified in the abdominal and diaphragmatic muscles were also detected from faeces and mesenteric lymph node samples. The five different serovars isolated were Salmonella mishmarhaemek (48%), S. typhimurium (20%), S. enteritidis (12%), S. guildford (12%) and S. dublin (48%). The antimicrobial resistance profiles of 25 of the Salmonella isolates with 17 antimicrobials showed that 52% (13/25) of them were resistant to three or more antimicrobials. Both strains of Salmonella (S. mishmarhaemek and S. typhimurium) showed multiple resistance to ampicillin, sulfamethoxazole and ticarcillin.

Keywords: antibiotics, cattle, drug resistance, epidemiology, Salmonella, serovars

Abbreviations: BPLS, Brilliant green-phenol red-lactose-sucrose agar; BPW, buffered peptone water; FVM, Faculty of Veterinary Medicine; ISO, International Organization for Standardization; NCCLS, National Committee for Clinical Laboratory Standards; PT, phage type; RV, Rappaport-Vassiliadis; SC, selenite-cystine; MIC, minimal inhibitory concentration

INTRODUCTION

Foods of animal origin are considered to be the major source of food-borne salmonellosis. The primary reservoir of *Salmonella* is the intestinal tract of infected animals and humans. It is known that diarrhoeal diseases are one of the leading causes of morbidity and mortality, especially among children in developing countries (Motarjemi *et al.*, 1995). However, the very limited scope of studies and lack of a

surveillance network for salmonellosis in most of these countries make it difficult to assess the actual magnitude of this zoonotic disease. In addtion, under-reporting of cases and the presence of other diseases considered to be of a higher priority may have overshadowed the problem of salmonellosis in some developing countries (Acha and Szyfres, 1989; Oosterom, 1991). Salmonella infection in adult animals is usually limited to a healthy carrier state. However, stress associated with transport of animals from rearing farms to abattoirs augments the shedding of salmonellae, while crowding and prolonged lairage in abattoir pens predisposes the animals to infection. The process of removing the gastrointestinal tract is regarded as one of the most important sources of contamination of carcases and organs with Salmonella at abattoirs (Samuel et al., 1980; Adesiyun and Oni, 1989).

Previous studies conducted in Ethiopia on Salmonella in animals, animal products and humans indicated the presence of a number of serovars (Mache and Mengistu, 1998; Molomo, 1998; Molla et al., 1999a; Nyeleti, 1999). An increase in the resistance of Salmonella to commonly used antimicrobials has been noted in both the public health and veterinary sectors (Tauxe, 1991; D'Aoust et al., 1992). The issue of antibiotic resistance is more complex in developing countries (Leegard et al., 1996) like Ethiopia than in developed countries, since Salmonella are not routinely cultured and their resistance to the antibiotics commonly used in both human and veterinary medicine is seldom assessed in these countries. The increasing proportion of Salmonella strains isolated from human cases of salmonellosis that are resistant to one or more antimicrobials has been associated with the widespread use of antimicrobial agents in food animal production. Antimicrobials are extensively used as feed additives in food animals, in addition to being employed to treat Salmonella and other bacterial infections. This is a matter of concern to public health since there is a considerable potential for the transmission of singly and/or multiply resistant Salmonella strains to humans through the food chain (Tauxe, 1991; Gay et al., 1994; Gustafson and Bowen, 1997; Duffy et al., 1999). Mache and Mengistu (1998) and Molla and colleagues (1999b) reported a high level of antibiotic resistance in foodborne Salmonella isolates from raw minced beef, poultry and human stool samples in Addis Ababa (Ethiopia). The present study was undertaken to determine the prevalence of salmonellae, to identify the common serovars and to assess the antimicrobial resistance profiles of Salmonella isolates from apparently healthy slaughtered cattle at Debre Zeit, Ethiopia.

MATERIALS AND METHODS

Sample collection and processing

Samples were collected aseptically during slaughtering operations from 323 adult, apparently healthy, cattle that were slaughtered on Mondays and Thursdays at a small abattoir at the Faculty of Veterinary Medicine (FVM) at Debre Zeit, 45 km east of Addis Ababa during the period October 1999 to March 2000. The animals brought for slaughter came immediately from a feedlot at the FVM, which is about 3 km away from the abattoir. They had been bought from Nazareth cattle market, located 55 km

east of Debre Zeit. The animals stay for a maximum of one month in the feedlot. They were brought to the abattoir on the hoof during the late afternoon, 10 to 12 h before slaughter. Samples of approximately 100 g of muscle, 30–45 g of lymph nodes and 25 g of faeces were obtained from each animal. The faecal samples were collected directly from the rectum during slaughtering and placed into wide-mouthed sterile bottles. Diaphragmatic and abdominal muscle samples were aseptically cut into small pieces, put in sterile plastic bags with buffered peptone water (BPW) (Merck, Darmstadt, Germany), homogenized with a stomacher (Seward Stomacher 400, London, UK) and tested separately. Mesenteric lymph nodes were processed by trimming the fascia and fat from the lymph nodes before mixing and pre-enrichment. Faecal (2–3 g) and mesenteric lymph node (5 g) samples from 5 animals were pooled for the initial testing, but the individual samples were tested for the presence of *Salmonella* when the pooled samples had tested positive.

Isolation and identification of Salmonella

Techniques recommended by the International Organization for Standardization (ISO 6579, 1998) and Quinn and colleagues (1994) were used to isolate and identify the *Salmonella*. Briefly, the following procedures were employed.

Pooled faecal samples (containing 5 individual samples): About 2–3 g samples of faeces were added to 10 ml of Rappaport–Vassiliadis (RV) enrichment broth (Merck), and a further sample (2–3 g) was added to 10 ml of selenite-cystine (SC) broth (Difco, Detroit, MI, USA) and incubated at 42°C and 37°C, respectively, for 24 h (Fedorka-Cray *et al.*, 1998). This was followed by streaking the broths on two different plates of brilliant green–phenol red–lactose–sucrose agar (BPLS) and MacConkey agar (Merck) and incubation at 37°C for 24–48 h.

Pooled samples of mesenteric lymph nodes (containing 5 individual samples): 25 g of the sample was added to 225 ml of buffered peptone (BPW) (Merck) and incubated at 37°C for 16–20 h. About 0.1 ml of the culture was then transferred into 10 ml of RV broth and incubated at 42°C for 24 h, and 1 ml of the same culture was transferred to 10 ml of SC broth and incubated at 37°C for 24 h. On the third day, the broths were separately streaked on selective agar media (BPLS and MacConkey), as above, and incubated at 37°C for 24–48 h.

Abdominal and diaphragmatic muscles: 25 g of abdominal and diaphragmatic muscle samples from each of the 323 animals were separately pre-enriched in 225 ml of BPW and treated in a similar manner to the lymph node samples.

The plates were examined for growth of *Salmonella* colonies. If growth was slight, or if no typical colonies of *Salmonella* were present on agar plates, they were re-incubated at 37°C for another 18–24 h. About five suspected colonies were selected from each plate, streaked on to nutrient agar (Merck) and incubated at 37°C for 24 h. Suspect colonies

were tested biochemically using standard methods (Quinn *et al.*, 1994) and putative *Salmonella* isolates were examined for agglutination using polyvalent I and II anti-*Salmonella* sera (SIFIN, Berlin, Germany). *Salmonella* serotyping and phage typing were done at the OIE Reference Laboratory for Salmonellosis of Health Canada in Guelph, Ontario, Canada.

For serotyping, the somatic (O) antigens of the *Salmonella* isolates were determined by slide agglutination tests as described by Ewing (1986). The flagellar (H) antigens were identified using a microtechnique (Shipp and Rowe, 1980). The antigenic formulae of Le Minor and Popoff (1992) were used to name the serovars. The standard phage typing technique described by Anderson and Williams (1956) was employed throughout. Strains that did not conform to any recognized phage type were considered to be atypical. Strains that did not react with any of the typing phages were considered to be untypable. *Salmonella enteritidis* strains were phage typed with typing phages obtained from the Central Public Health Laboratory, Colindale, London, UK (Ward *et al.*, 1987). 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 also obtained from the Central Public Health Laboratory, UK.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the 25 isolates of *Salmonella* was performed in the Food Microbiology Laboratory, Laboratory Services Division, Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada, using SensititreTM (Trek Diagnostics, Inc., Westlake, OH, USA) microtitre plates to determine the minimal inhibitory concentration (MIC) as per the manufacturer's instructions. The National Committee for Clinical Laboratory Standards (NCCLS, 1990) guidelines were followed throughout the testing procedure. Table I shows the antimicrobials employed, their symbols, concentrations and break points.

RESULTS

Of the 323 cattle slaughtered at the small-scale slaughterhouse of the FVM, 23 (7.1%) were positive for the presence of *Salmonella*. Salmonellae were detected in all types of samples but with different frequencies (Table II). Two (3.1%) and 3 (4.6%) of the 65 pooled faecal and mesenteric lymph node samples, respectively, were *Salmonella* positive. Examination of the individual samples that constituted the pooled positive samples showed that 2 and 4 of the faecal and mesenteric lymph node samples, respectively, were culture-positive for *Salmonella*.

A total of 25 Salmonella isolates, consisting of five different serovars were detected (Table II). The dominant serovar was S. mishmarhaemek (48%), followed by S. typhimurium (20%), S. enteritidis (12%), S. guildford (12%) and S. dublin (8%). Salmonella mishmarhaemek and S. guildford were reported for the first time in

TABLE I Antimicrobials used, their symbols, concentrations and break points (NCCLS, 1990)

			Break points ^b (µg/ml)		
Antimicrobial	Symbol	Concentrations ^a (μg/ml)	R	I	S
Amikacin	AMI	4–32	≥64	32	≤16
Amoxicillin/clavulanic acid	AUG	0.5/0.25 - 32/16	≥32	16	€8
Ampicillin	AMP	2–64	≥32	16	€8
Apramycin	APR	2–16	≥32	16	€8
Ceftiofur	TIO	0.5–16	≥8	4	€2
Ceftriaxone	AXO	0.25-16	≥64	32	€8
Cephalothin	CEP	1–32	≥32	16	€8
Chloramphenicol	CHL	4–32	≥32	16	€8
Ciprofloxacin	CIP	0.015-2	≥4	2	≤1
Gentamicin	GEN	0.25-16	≥16	8	≤4
Kanamycin	KAN	16-64	≥64	32	≤16
Nalidixic acid	NAL	4–64	≥32		≤16
Streptomycin	STR	32-256	≥64		≤32
Sulfamethoxazole	SMX	128-512	≥512		≤256
Tetracycline	TET	4–64	≥16	8	≤4
Ticarcillin	TIC	2-128	≥128	32	≤16
Trimethoprim-sulfamethoxazole	SXT	0.12/2.4-4/76	≥4/76		≤2/138

^aThese were chosen to detect incremental changes in resistance based on data from the previous two years; the ranges may be outside the breakpoint values

Ethiopia. *Salmonella dublin* and *S. guildford* were isolated only from muscle samples. The phage types (PT) of the *S. typhimurium* isolates were PT 2, PT 79 and PT atypical. All the phage types of *S. enteritidis* were atypical.

Resistance to three or more antimicrobials was detected in 52% (13/25) of the Salmonella isolates (Table III). However, of the five different Salmonella serovars detected, only strains of S. mishmarhaemek and S. typhimurium were found to be resistant. All the resistant strains had multiple resistance (Table III). A PT 2 strain was the only resistant strain of S. typhimurium (Table III). Eleven of the 17 (64.71%) antimicrobials were effective against all the Salmonella isolates tested, with the exception of one serovar of S. mishmarhaemek, which showed intermediate resistance to cephalothin.

^bR = resistant; I = intermediate; S = susceptible

TABLE II Salmonella serovars isolated by source

	Number	of samples		
Source	Examined	Positive (%)	Serovar (number)	
Faeces	323	2 (0.6)	S. mishmarhaemek (2)	
Mesenteric lymph node	323	4 (1.2)	S. typhimurium (3) S. enteritidis (1)	
Abdominal muscle	323	9 (2.8)	S. mishmarhaemek (5) S. typhimurium (1) S. guildford (2) S. dublin (1)	
Diaphragmatic muscle	323	10 (3.1)	S. mishmarhaemek (5) S. typhimurium (1) S. enteritidis (2) S. guildford (1) S. dublin (1)	

TABLE III Resistance pattern of *Salmonella* isolates

	Number	r of <i>Salmone</i>	ella isolates		
Serovar	Tested	Resistant	Multiple resistance	Antimicrobial type ^a	
S. mishmarhaemek	12	12	12:		
			11 1	AMP, SMX, TIC AMP, SMX, TIC, CEP ^b	
S. typhimurium	5	1	1	AMP, SMX, TIC, SXT, TET, STR	
S. enteritidis	3	=	=		
S. guildford	3	=	=		
S. dublin	2	=	=		
Total	25	13	13		

^aFor key to abbreviations see Table I

^bIntermediate resistance to CEP

DISCUSSION

The prevalence of Salmonella infection in apparently healthy cattle at abattoirs has been reported to range from 0.3% to 11.6% (Gay et al., 1994). However, even though there have been many studies on Salmonella infection in apparently healthy slaughtered cattle, there has been no uniformity with respect to the materials examined or the sampling and cultural techniques. Consequently, the results may not be comparable (Wray and Davies, 2000). In our study, 0.6% of the faecal samples from slaughtered cattle were culture-positive for Salmonella. This is comparable with previous studies undertaken elsewhere (Opuda-Asibo et al., 1990; Gay et al., 1994). Williams and Bellhouse (1978) found that 2% of faecal samples from healthy adult cattle slaughtered at an abattoir in the United Kingdom contained Salmonella. Miller (1971), on the other hand, reported that 6.7% of faecal samples taken from apparently healthy cattle in Botswana cultured positive for Salmonella. The low estimate in the present study might be due to a low carrier rate for Salmonella among the cattle in the study population. It may also be related to the fact that the faecal samples were directly inoculated into RV and SC broth, without pre-enrichment. However, pre-enrichment is not routinely done on animal faeces for the isolation of Salmonella in clinical diagnostic laboratories (Quinn et al., 1994). Fedorka-Cray and colleagues (1998) detected more positive samples of Salmonella from cattle in the United States that had been kept for a longer time in a feedlot, and this may indicate that more animals become infected with, or shed, the organism when the animals are housed together over a considerable time. In this study, the animals stayed in the feedlot for not longer than a month, which may have limited transmission of the organisms among the animals. The absence of concentrated feed or of ingredients of animal origin that might be contaminated by salmonellae, being used as cattle feed, and the low and intermittent faecal shedding by carrier cattle could also explain the low recovery rate of Salmonella from the study animals. House and Smith (1998) reported that, in order to define the true infection status of apparently healthy cattle, it is necessary to perform multiple cultures over a 3- to 6-month period to distinguish convalescent animals from animals that are chronically infected with Salmonella and passive carriers.

The lack of stress before slaughter would probably decrease the number of animals shedding *Salmonella* and the number of *Salmonella* being shed by each animal. In a study to determine the role of transport stress on faecal shedding of salmonellae, Puyalto and colleagues (1997) found that, whereas 5% of a group of cattle were shedding faecal *Salmonella* before transport, this became 11.3% after transport. The mesenteric lymph node samples from two of the faecal-positive cattle were negative; this may suggest that the organism had not reached the lymphatic system and invaded the mesenteric lymph nodes or may have been caused by intermittent excretion of the organism in faeces without a temporal relationship to the presence of *Salmonella* in the mesenteric lymph nodes. The prevalence of *Salmonella* in the mesenteric lymph nodes of the study cattle was lower (1.2%) than that reported by Samuel and colleagues (1979, 1980). The relatively high prevalence (76%) reported by those authors may reflect the fact that most of the animals they studied had been held for at least 4 days in the lairage before slaughter and had travelled over 200 km, or they may have originated

from a population with a high proportion of cattle carrying *Salmonella*. The group studied by Samuel and colleagues (1979) that had been held for the shortest time before slaughter (1 day) yielded the lowest number of infected animals and no *Salmonella* were found in the mesenteric lymph nodes from that group. Culture of *Salmonella* from mesenteric lymph nodes is the principal procedure used in identifying animal carriers of *Salmonella* (Acha and Szyfres, 1989). However, in the present study, there was no significant difference in the recovery rate of *Salmonella* from faeces or mesenteric lymph nodes. On balance, the low prevalence of *Salmonella* in the mesenteric lymph nodes and faeces suggests a low prevalence of *Salmonella* in healthy slaughtered cattle in the study area.

According to D'Aoust (1989), the Salmonella contamination rate of beef carcases varies from 0.1% to 21.5%, with a median of 3.3%. Regarding carcase contamination, it should be noted that about 3% of the muscle samples in our study were positive for Salmonella. The detection of about 60% of the servors in abdominal and diaphragmatic muscles, as well as in the faeces and mesenteric lymph node samples, suggests that the process of evisceration could be the main source of carcase contamination. In a study undertaken in Zaria abattoir (Nigeria), the frequency of isolation of Salmonella from the dressing area was 7.3% (Adesiyun and Oni, 1989). Thus, the abattoir dressing area can serve as a source of carcase contamination. Cross-contamination can also occur during the skinning process as a result of poor hygienic conditions. Another probable source of contamination is infected abattoir personnel. Nyeleti (1999) reported that 6% (18/300) of stool samples from abattoir personnel in Addis Ababa were positive for Salmonella and 61.1% of the serovars therein were also isolated from samples from slaughtered cattle. This may be interpreted as evidence of a link between contamination of cattle carcases and people. In general, poor disinfection of knives and other equipment, poor personal hygiene of the plant personnel and poor sanitation at the abattoir might contribute to the contamination of carcases.

The commonest serovar identified in our study was *S. mishmarhaemek* followed by *S. typhimurium*. Nyeleti (1999) reported that *S. dublin* and *S. anatum* were the most common serovars isolated from healthy cattle slaughtered in Addis Ababa abattoir. A study carried out by Molla and colleagues (1999a) showed that *S. dublin* was the prevailing serovar, followed by *S. typhimurium*, in minced beef samples in Addis Ababa. These findings suggest that several serovars of *Salmonella* that may cause significant animal and human illness occur in Ethiopia.

Some of the salmonellae showed resistance to antimicrobials that are extensively used in veterinary and human medicine in Ethiopia. Antimicrobial-resistant isolates of *Salmonella* from animal and human sources have been reported in various countries in Africa (Hummel, 1979; Hadfield *et al.*, 1985; Leegard *et al.*, 1996). Molla and colleagues (1999b) reported that isolates of *Salmonella* from minced beef samples in Addis Ababa were resistant to commonly used antimicrobials, including ampicillin, furazolidone, nitrofurantoin, streptomycin and trimethoprim-sulfamethoxazole. The high level of antimicrobial resistance in *Salmonella* isolates in sub-Saharan Africa, including Ethiopia, is probably due to the indiscriminate and inappropriate use of commonly available antimicrobials, in both veterinary and public health practices.

This high percentage of Salmonella isolates of bovine origin with multiple resistance to the commonly used antimicrobials could pose a significant public health risk. In Ethiopia, there is limited access to the newer cephalosporins and fluroquinolone antimicrobials that may be effective against the salmonellae. Also, the high cost would limit the use of these antimicrobials. According to Mache and Mengistu (1998), 45 Salmonella strains isolated from 700 human diarrhoeal samples collected in Addis Ababa showed resistance to tetracycline, ampicillin, cephalothin, trimethoprimsulfamethoxazole, kanamycin and chloramphenicol, which are commonly used for the treatment of various bacterial infections in humans in Addis Ababa. The isolation of Salmonella with multiple resistance may reflect the frequent association of resistance to sulfonamides, tetracyclines, streptomycin and ampicillin in isolates, as described by Bennet (1980). The emergence and prevalence of Salmonella with multiple resistance in food animals can seriously compromise public health (D'Aoust et al., 1992). Increased spread by the conjugative plasmids that mediate antibiotic resistance is worrisome since it compromises the efficacy of valued antibacterial agents (ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole) for the treatment of systemic salmonellosis and other bacterial infections in humans. The lack of resistance of Salmonella isolates to the less commonly used antimicrobials (fluroquinolones, third-generation cephalosporins and broad-spectrum aminoglycosides) was to be expected in our study, since these drugs have not been used extensively in veterinary practice in Ethiopia.

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Prévalence et caractéristiques de la résistance antimicrobienne d'isolats de Salmonella provenant de bétail abattu apparemment en bonne santé en Éthiopie

Résumé – La prévalence et les caractéristiques de la résistance microbienne d'isolats de Salmonella ont été déterminés chez du bétail abattu apparemment en bonne santé à Debre Zeit (Éthiopie). Un nombre total de 323 bovins a été examiné pour déterminer la présence de Salmonella dans le fèces, dans des ganglions lymphatiques mésentériques et dans des muscles abdominaux et diaphragmatiques. Des salmonelles ont été isolées de 2 (3,1%) et de 3 (4,5%) respectivement des 65 échantillons de ganglions lymphatiques fécaux et mésentériques mis en commun. Neuf (2,8%) des échantillons du muscle abdominal et 10 (3,1%) des échantillons du muscle diaphragmatique se sont avérés contaminés par Salmonella. Environ 60% des sérotypes identifiés dans les muscles abdominaux et diaphragmatiques ont également été retrouvés dans des échantillons de ganglions lymphatiques du fèces et mésentériques. Les cinq sérotypes différents isolés ont été Salmonella mishmarhaemek (48%), S. tiphimurium (20%), S. enteritidis (12%), S. guildord (12%) et S. dublin (48%). Les profils de résistance antimicrobienne de 25 des isolats de Salmonella testés avec 17 agents antimicrobiens ont mis en évidence que 52% (13/25) d'entre eux étaient résistants à trois agents antimicrobiens ou plus. Les deux souches de Salmonella (S. mishmarhaemek et S. typhimurium) ont présenté une résistance multiple à l'ampicilline, au sulfaméthoxazole et à la ticarcilline

Prevalencia y resistencia a los antibióticos de cepas de Salmonella aisladas a partir de vacas aparentemente sanas sacrificadas en Etiopía

Resumen – En este estudio se determinaron la prevalencia y la resistencia a los antibióticos de las cepas de Salmonella aisladas a partir de vacas aparentemente sanas sacrificadas en Debre Zeit (Etiopía). Se buscó la presencia de Salmonella en las heces, en los nódulos linfáticos mesentéricos y en los músculos abdominales y diafragmático en un total de 323 vacas. Se aislaron salmonelas en 23 animales (7,1%). Las salmonelas se aislaron en 2 muestras (3,1%) de heces y en 3 muestras (4,5%) de nódulos linfáticos mesentéricos de un total de 65 muestras. Nueve muestras (2,8%) de músculos abdominales y 10 (3,1%) de músculo diafragmático (n = 323) estaban contaminadas con Salmonella. Alrededor del 60% de los serotipos identificados en los músculos abdominales y diafragmático también se detectaron en las muestras de heces y de nódulos linfáticos mesentéricos. Los cinco serotipos aislados fueron: Salmonella mishmarhaemek (48%), S. typhimurium (20%), S. enteritidis (12%), S. guildford (12%) y S. dublin (8%). Los antibiogramas de 25 de las cepas aisladas de Salmonella frente a 17 antibióticos mostraron que el 52% (13/25) de ellas eran resistentes a tres o más antibióticos. Las dos cepas de Salmonella resistentes (S. mishmarhaemek y S. typhimurium) mostraron una resistencia múltiple frente a la ampicilina, el sulfametoxazol y la ticarcilina.