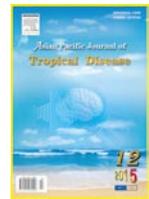




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Prevalence and antimicrobial resistance profiles of *Salmonella* isolates in apparently healthy slaughtered food animals at Maiduguri central abattoir, Nigeria

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ABSTRACT

Objective: To determine the prevalence and antimicrobial resistance profiles of *Salmonella* isolates in the mesenteric lymph nodes of apparently healthy slaughtered food animals at Maiduguri central abattoir, Nigeria.

Methods: A total of 154 lymph nodes (cattle-54, camel-22, sheep-12, goats-66) were collected from slaughtered animals and analysed using standard microbiological and biochemical methods.

Results: An overall prevalence of 39.0% [95% confidence interval (CI): 31.3–46.7] was obtained. The prevalence rate across studied species ranged from 24.2% (95% CI: 13.9–34.5) in goats to 61.1% (95% CI: 48.1–74.1) in cattle. There was statistically significant association between *Salmonella* infection and species of food animals ($P < 0.05$). Males had a high prevalence of 44.7% (95% CI: 28.9–60.5) as compared with females (37.1%; 95% CI: 28.3–45.9) ($P > 0.05$). Younger animals had slightly higher prevalence (44.0%; 95% CI: 24.5–63.5) compared with adults (38.0%; 95% CI: 29.6–46.4) ($P > 0.05$). All isolates showed marked susceptibility to ciprofloxacin, cotrimoxazole and chloramphenicol. Whereas, high resistance patterns to ampicillin, kanamycin and streptomycin, and moderate resistance patterns to kanamycin and tetracycline were observed from camels.

Conclusions: *Salmonella* is high in the mesenteric lymph nodes of apparently healthy slaughtered food animals in Maiduguri. Therefore, it is recommended that further studies should be carried out to identify the serotypes and phage typing of the isolates, and hazard analysis and critical control point should be applied in handling of meat and meat products to avoid the risk of foodborne salmonellosis as well as appropriate use of antibiotics like ciprofloxacin in food animals.

1. Introduction

Salmonellosis, an enteric disease of humans and animals caused by a bacteria of the genus *Salmonella*, is one of the most important zoonotic diseases distributed worldwide and poses a major public health challenge[1-3]. *Salmonella* primarily inhabits the gastrointestinal tract of both domestic and wild animal species including birds, thus excreting the organism in their faeces resulting in the contamination of food, water and the environment[4]. Over 2600 *Salmonella* species are associated with

varying degrees of virulence and can affect a wide host range with the exception of few *Salmonella* serovars that are host-specific like *Salmonella* Typhimurium in humans and *Salmonella* Gallinarum in chickens[5-7]. *Salmonella* Typhimurium and *Salmonella* Enteritidis are the major causes of human salmonellosis in most developed countries and other serotypes in different geographical areas might also be associated with human salmonellosis[8-10]. Most cases of human salmonellosis are foodborne and few are acquired via direct or indirect animal contact at homes, veterinary clinics or public settings. Several studies have implicated animals as major source of human infection with antimicrobial resistant *Salmonella*[11,12]. *Salmonella* infection in adult animals is usually limited to a healthy carrier state[13]. Overcrowding and prolonged stay in the abattoir lairage were known to predispose animals

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to *Salmonella* infection and in addition, the stress associated with the transportation of these animals from farms to abattoirs increases the shedding of salmonellae[13]. There are several ways of *Salmonella* contamination of carcasses in the slaughterhouse. Fecal materials serve as a major source of contamination and this could reach animal carcasses through direct deposition as well as by indirect contact through contamination with clean carcasses, equipment and butchers during the slaughtering process[14,15]. Also, the process of removing the gastrointestinal tract during the slaughtering process has been regarded as one of the most important sources of contamination of carcasses and organs with *Salmonella* at abattoirs[16].

Other potential source of *Salmonella* in animals is the lymphatic system, specifically the lymph nodes[17]. One of the functions of lymphatic system is the sequestering of bacteria, viruses and other foreign bodies via filtering mechanism, which are subsequently destroyed by lymphocytes[17]. Several studies had reported bacteria isolated from the lymph nodes of food animals at slaughter.

Antimicrobials are now increasingly used as treatment remedies for bacterial diseases like *Salmonella* as well as feed additives in food animals[13]. Recently, the indiscriminate use of these antimicrobials in food producing animals had resulted in the emergence of antimicrobial-resistant *Salmonella* strains. This presents a public health concern, because of the potential risk of these resistant strains transmitted through the food chain to humans. The increase in resistance of *Salmonella* to commonly used antimicrobials had been reported in both the human and veterinary field[18-21]. *Salmonella* infection still remains a complex issue in most developing countries like Nigeria, because it is not routinely cultured and its resistance profiles to commonly used antibiotics in both veterinary and public health are not frequently assessed[13].

To the best of our knowledge, there is a dearth of data and information regarding the prevalence and isolation of *Salmonella* from the mesenteric lymph nodes of apparently healthy slaughtered food animals in Maiduguri, Northeastern Nigeria. This informed the need to carry out this research. Therefore, the aim of this study is to determine the prevalence and antibiotic resistance patterns of *Salmonella* isolated from the mesenteric lymph nodes of apparently healthy carcasses that have been branded fit and passed for human consumption in Maiduguri, Northeastern Nigeria.

2. Materials and methods

2.1. Study area

Borno State is located in the corner of the Northeastern region of Nigeria and lies approximately between latitude 10.20 N and 13.40 N and longitude 9.80 E and 14.40 E. Maiduguri is the capital and largest city of Borno State and lies around 11°05' N latitude and 13°05' longitude and at about 350 m above sea level (http://www.unimaid.edu.ng/About_Maid.aspx). Maiduguri is located within the Sahel Savannah zone and occupies an area of 50 778 km². It occupies the greater part of the Chad Basin and shares border with

Republics of Niger to the north, Chad to the northeast, Cameroon to the east and Yobe State to the west. The climate is hot and dry for a greater part of the year with rainy season from June to September in the northern part and May to October in the southern part with a mean annual rainfall and temperature of about 650 mm and 32 °C respectively. The hottest periods of the year are the months of March and April with temperatures ranging from 30 °C–40 °C[22].

2.2. Sample collection and processing

A total of 154 mesenteric lymph nodes comprising 38 males and 116 females, were collected from apparently healthy slaughtered camels ($n = 22$), cattle ($n = 54$), sheep ($n = 12$) and goats ($n = 66$). Samples of lymph nodes were collected aseptically into sterile plastic bags during slaughtering operations at the Maiduguri central abattoir located at the Maiduguri Metropolitan Council. The samples were labeled and immediately transported ice cooled within 8 h of collection to the Veterinary Public Health Research Laboratory, Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri for further processing and analysis. This was kept in the refrigerator at 4 °C until cultured within 18 h of collection. The animals brought for slaughter which came immediately from Maiduguri livestock market (Kasuwan Shanu) were usually drove to the abattoir by trucks/trailers or on the hoof 12 to 24 h before slaughtering. The source of these animals was unknown, as some were brought and sold from as far as neighbouring Cameroon, Chad and Niger Republics. These animals were left in the lairage for about 3–4 days before slaughter. Samples of approximately 30–45 g of lymph nodes were obtained randomly from each animal. The lymph nodes were processed by trimming the fascia and fat using sterile scissors and forceps before mixing and pre-enrichment. The remaining fat encased node was seared with red-hot spatula. The lymph nodes were later dipped in 95% ethyl alcohol and flamed.

2.3. Bacteriological analysis

About 2 g of small pieces of the lymph nodes were removed from the capsule and inoculated into 10 mL of sterile selenite broth (Oxoid®) for enrichment and incubated aerobically for 24 h at 37 °C. Subcultures were then made from each broth culture by streaking onto MacConkey agar (Oxoid®), *Salmonella-Shigella* agar (Oxoid®), and xylose lysine deoxycholate agar (Oxoid®). These cultured plates were incubated at 37 °C for 48 h. These plates were then examined based on cultural and morphological characteristics conforming with the presence of typical colonies of presumptive *Salmonella* appearing colourless and non-lactose fermenting on MacConkey agar, transparent colonies with black centre on *Salmonella-Shigella* agar, dome-shaped colonies with central black spot on xylose lysine deoxycholate agar. All these were selected as presumptive *Salmonella* colonies and subjected to further Gram-staining and biochemical tests as described by Barrow and Feltham[23].

2.4. Antimicrobial susceptibility testing

The antibiotic susceptibility and resistance patterns of all the *Salmonella* isolates to routine antibiotics were determined using the modified Kirby-Bauer disc diffusion method on Mueller- Hinton agar (Oxoid®). The following antimicrobial disks (Difco, concentrations in µg) were used: chloramphenicol (30 µg), ceftriaxone (30 µg), ampicillin (30 µg), cotrimoxazole (30 µg), ciprofloxacin (30 µg), vancomycin (30 µg) and tetracycline (30 µg). The zones of inhibition were measured in diameters around each disk and interpreted based on the guidelines outlined by the Clinical and Laboratory Standards Institute[24].

2.5. Statistical analysis

The raw data were initially analysed in Microsoft Office Excel 2007 to obtain percentages and proportions of infection. The data were finally imported into GraphPad Prism® Version 6.01 for Windows (GraphPad Software, Inc., San Diego, California, USA) to determine the prevalence of infection, 95% confidence intervals (CI) on the estimates and statistically significant association of variables tested. A $P < 0.05$ was considered statistically significant.

3. Results

A total of 154 samples of mesenteric lymph nodes from apparently healthy slaughtered animals were collected and tested for *Salmonella*. A total of 54 (35.1%) of these were from cattle, 22 (14.3%) were camel, 12 (7.8%) were sheep and 66 (42.9%) were goats (Table 1). Of these, 60 (39.0%; 95% CI: 31.3–46.7) were positive for *Salmonella* (Table 1). Species-wise prevalence revealed a high prevalence of 61.1% (95% CI: 48.1–74.1) in cattle as compared with other species ($P < 0.05$) (Table 1). While sheep, camel and goat had 33.3% (95% CI: 6.6–60.0), 31.8% (95% CI: 12.3–51.3) and 24.2% (95% CI: 13.9–34.5), respectively.

Table 1

Species-wise prevalence of *Salmonella* isolated in the mesenteric lymph nodes of apparently healthy slaughtered animals in Maiduguri central abattoir, Northeastern Nigeria.

Species	Examined number	Positive number	Prevalence [% (95% CI)]
Cattle	54	33	61.1 (48.1–74.1)*
Camel	22	7	31.8 (12.3–51.3)
Sheep	12	4	33.3 (6.6–60.0)
Goat	66	16	24.2 (13.9–34.5)
Overall	154	60	39.0 (31.3–46.7)

*: Statistically significant difference compared with other animal species ($P < 0.05$).

Based on sex, 38 (24.7%) of the samples were males, while 116 (75.3%) were from female animals (Table 2). Overall males had a high prevalence of *Salmonella* (44.7%; 95% CI: 28.9–60.5) as compared with the females (37.1%; 95% CI: 28.3–45.9) ($P > 0.05$) (Table 2). A total of 33 (61.1%) samples tested positive for *Salmonella* out of the total 54 samples examined in cattle. These comprised 12 (100.0%) from males and 21 (50.0%) from females. Seven (31.8%) samples out of the 22 samples tested were positive

in camels. The positive samples comprised 5 (50.0%) males and 2 (16.7%) females. Of the 12 samples examined in sheep, 4 (33.3%) were positive (Table 2). These comprised 0 (0.0%) males and 4 (40.0%) females. Sixteen (24.2%) samples from goat were positive out of the 66 samples tested. All the 16 (30.8%) positive samples were from the females. However, none of the 14 samples from males were positive (Table 2).

Table 2

Sex-wise prevalence of *Salmonella* isolated in the mesenteric lymph nodes of apparently healthy slaughtered animals in Maiduguri central abattoir, Northeastern Nigeria.

Species	Examined No.	No. positive/No. total (%)	
		Male	Female
Cattle	54	12/12 (100.0)	21/42 (50.0)
Camel	22	5/10 (50.0)	2/12 (16.7)
Sheep	12	0/2 (0.0)	4/10 (40.0)
Goat	66	0/14 (0.0)	16/52 (30.8)
Overall	154	17/38 (44.7)	43/116 (37.1)

Based on age, 25 (16.2%) of the samples were younger animals and 129 (83.8%) were adults (Table 3). Overall younger animals had a slightly higher prevalence of *Salmonella* (44.0%; 95% CI: 24.5–63.5) compared with older animals (38.0%; 95% CI: 29.6–46.4) ($P > 0.05$) (Table 3). Of the 54 samples examined in cattle, 9 (75.0%) and 24 (57.1%) samples were tested positive for *Salmonella* in younger and adult animals respectively. Seven (31.8%) samples were tested positive out of the total 22 samples examined in camel. These comprised 2 (40.0%) and 5 (29.4%) in young and adult animals respectively (Table 3). The 12 samples tested in sheep comprised 2 younger and 10 adult animals. Four (40.0%) of the 10 adult animals were positive, whereas all the two younger animals were tested negative for *Salmonella*. Of the 16 (24.2%) positive samples from goat, 0 (0.0%) were from younger animals and all the positive samples were from adult goats.

Table 3

Age-wise prevalence of *Salmonella* infection in the mesenteric lymph nodes of slaughtered healthy animals in Maiduguri central abattoir, Northeastern Nigeria.

Species	Examined No.	No. positive/No. total (%)	
		Young	Adult
Cattle	54	9/12 (75.0)	24/42 (57.1)
Camel	22	2/5 (40.0)	5/17 (29.4)
Sheep	12	0/2 (0.0)	4/10 (40.0)
Goat	66	0/6 (0.0)	16/60 (26.7)
Overall	154	11/25 (44.0)	49/129 (38.0)

The isolation rate was highest in cattle with 61.1% (Table 4). The antimicrobial susceptibility and resistance patterns of the isolates indicated the highest resistance patterns for ampicillin (100%), kanamycin (100%), streptomycin (97%), nitrofurantoin (91%) and tetracycline (85%) in cattle; ampicillin (100%), kanamycin (100%), streptomycin (100%) and nitrofurantoin (75%) in sheep; streptomycin (100%), kanamycin (94%), ampicillin (88%) and tetracycline (75%) in goat and tetracycline (57%) and kanamycin (57%) in camels (Table 4). While all the isolates tested from the different species of animals showed marked susceptibility to ciprofloxacin, ceftriaxone, cotrimoxazole, chloramphenicol and gentamicin (Table 4).

Table 4

Frequency of antibiotic resistance patterns of *Salmonella* isolates from mesenteric lymph nodes of apparently healthy slaughtered food animals in Maiduguri, Northeastern Nigeria. *n* (%).

Source	No. of isolates	No. of resistant isolates									
		AMP	KAN	NT	TET	STR	CIP	CEFT	COT	CHL	GEN
Cattle (<i>n</i> = 54)	33 (61.1)	33 (100)	33 (100)	30 (91)	28 (85)	32 (97)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Camel (<i>n</i> = 22)	7 (31.8)	2 (29)	4 (57)	2 (29)	4 (57)	2 (29)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sheep (<i>n</i> = 12)	4 (33.3)	4 (100)	4 (100)	3 (75)	2 (50)	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Goat (<i>n</i> = 66)	16 (24.2)	14 (88)	15 (94)	10 (63)	12 (75)	16 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

AMP: Ampicillin; KAN: Kanamycin; NT: Nitrofurantoin; TET: Tetracycline; STR: Streptomycin; CIP: Ciprofloxacin; CEFT: Ceftriaxone; COT: Cotrimoxazole; CHL: Chloramphenicol; GEN: Gentamicin.

4. Discussion

It has been established that the mesenteric lymph nodes of animals that had travelled long distances or held for several days in lairages may predispose them to harbouring and shedding of *Salmonella* and consequently contaminating their carcasses during slaughtering and evisceration processes[13]. In the present study, the overall prevalence of *Salmonella* from apparently healthy slaughtered animals was 39.0%, of which 61.1%, 31.8%, 33.3% and 24.2% were from cattle, camel, sheep and goats respectively. These findings of respective prevalence of 33.3% and 24.2% in sheep and goats, are in agreement with the study of D' Aoust, who reported that the prevalence of *Salmonella* in these species ranged between 2%–51.5% and 1%–18.8% in sheep and goats respectively[25]. However, the prevalence in the present study was slightly higher in goats (24.2% vs. 18.8%). Woldemariam *et al.* reported respective prevalences of 2.8% and 9.8% in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia[26]. Nabbut and Al-Nakhli also reported the prevalences of 14.7% and 18.3% in slaughtered sheep and goats respectively in Riyadh public abattoir[27]. This is lower compared with our present study. Others reported the prevalence of *Salmonella* in the mesenteric lymph nodes of sheep and goats as 7.7%–2.0% and 0.0%–11.7% respectively[26]. The prevalence of 61.1% in cattle reported in this study was considerably higher compared with 1.4% reported by Alemayehu *et al.*[13]. A prevalence of 31.8% was reported in camels. This is not in line with the findings by Molla *et al.* who reported 15.9% prevalence in apparently healthy slaughtered camels in Ethiopia[28]. Wernery also reported the prevalence of < 5% of *Salmonella* in camels in the United Arab Emirates, which is considerably lower than 31.8% we found in this study[29]. This study reported a relatively higher carrier state of *Salmonella* in all the studied species as compared with other studies in Nigeria and elsewhere. The reasons for these differences could be associated with the fact that these animals were kept in crowded lairages/pens for 3–4 days at the abattoir, which is known to facilitate the shedding and transmission of *Salmonella* organism among them[13,30]. It could also reflect the fact that these animals had travelled over long distances or might have originated from a population with a high proportion of animals with high *Salmonella* carrier state[13]. Keeping animals in the abattoir lairages/pens for some days is associated with stress and this could induce high *Salmonella* infection rates among these animals[13,31]. The differences in the reported prevalence could also be associated with the sampling procedures, sample size, bacteriological methods employed or differences in the occurrence and distribution of *Salmonella* in different study populations under different prevailing conditions[31]. In addition, Nabbut and Al-Nakhli reported that the enrichment method compared with the direct plating method reveals more infected lymph nodes[27]. Consequently, the observed high prevalence reported in the present study could be linked with the bacteriological techniques employed for the detection

of *Salmonella* organism. It should be borne in mind that the current study was conducted in a municipal abattoir that may have poor sanitary and hygienic standards as compared with other abattoirs.

Males had a non-significant high prevalence compared with females in this study as are younger animals compared with the adults. The difference in the sample size for both sexes could probably explain the high prevalence in males. The findings of higher prevalence in younger animals are expected, as younger animals have weak immune system and therefore are vulnerable to severe *Salmonella* infection compared with other age brackets. It could also be associated with the sampling size as most of the sampled animals in this study were adults and only few younger ones were sampled.

The current study reveals a high resistant profile of the *Salmonella* isolates to routinely used antibiotics in the study area including ampicillin, kanamycin, streptomycin, nitrofurantoin and tetracycline with significant varying resistant rates. This is consistent with the reports of other researchers[11,12]. The high resistance exhibited by the isolates could be due to the indiscriminate and inappropriate use of these routine antimicrobials in both human and veterinary medicine in Nigeria. Our study also revealed that all the *Salmonella* isolates recovered were susceptible to ciprofloxacin, ceftriaxone, cotrimoxazole, chloramphenicol and gentamicin. This is comparable with previous reports on *Salmonella* isolates recovered from animal and human sources in Nigeria and elsewhere[12]. It should be noted however, that these antibiotics were not routinely used for the treatment of varying bacterial diseases in both humans and animals in the country. This highlights the advantage of the rational use of antimicrobials in the study area. Isolates from camels exhibited some moderate resistance profiles especially to kanamycin and tetracycline, indicating lower prevalence of antibiotic resistant *Salmonella* in this species as compared with sheep, goat, cattle and to some extent pigs[11]. This is probably due to the lower therapeutic usage of these antibiotics in camels compared with the other species. During the last decades, the continued development of antibiotic resistance by bacteria had become a major public health concern worldwide.

In conclusion, this study reveals high prevalence of *Salmonellae* in the mesenteric lymph nodes of cattle, camel, sheep and goats as well as high resistance profiles exhibited by most of the isolates to commonly used antimicrobials. This highlights the poor hygienic standards practiced in Maiduguri municipal abattoir. Therefore, meat from these animals might serve as potential source of foodborne salmonellosis to consumers in Maiduguri. It is recommended therefore, that further studies should be carried out to identify serotypes, and hazard analysis and critical control point should be applied in handling of meat and meat products from these animals in the abattoir to avoid the risk of salmonellosis resulting from consumption of meat contaminated with *Salmonella* as well as appropriate use of antibiotics like ciprofloxacin in both human and veterinary practices.

Conflict of interest statement

We declare that we have no conflict of interest.

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