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Prevalence of salmonella serotypes in Sokoto abattoir effluents and vegetables cultivated around the abattoir

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ABSTRACT

This study was conducted to determine the relationship between Salmonella serotypes isolated from Sokoto abattoir effluents, discharged directly into nearby water bodies and Salmonella isolated from raw consumed vegetables cultivated and sold around the abattoir area known as 'kara' Market. A total of 250 samples were collected, 100 from five different points of the abattoir draining system and 150, from raw consumed vegetables (50 onions, 50 lettuce and 50 tomatoes) cultivated and sold in the abattoir area. The samples were analyzed for the presence of Salmonella serotypes by biochemical tests. Salmonella suspected isolate were confirmed by serotyping using commercially prepared O and H polyvalent antigens (Salmonella Sero Quick group Kit) obtained from Staten's Serum Institut (SSI) Copenhagen, Denmark. This study has shown the occurrence and similarities between Salmonella serotypes in both abattoir effluents and vegetables with high frequency of occurrence of *Salmonella* Typhimurium (19.5%), *S.* Enteriditis (15.3%) and other Salmonella serotypes isolated are *S.* Typhi (13.9%), S. Paratyphi A (8.3), *S.* Paratyphi C (13.9%), *S.* Derby (9.7%), *S.* Newport (6.9%) and *S.* Paratyphi B (12.5%). This study shows that *S.* Typhimurium*, S.* Enteritidis and other Salmonella serotypes from intestinal contents of slaughtered animals contaminates nearby water bodies and consequently vegetables cultivated in the area and this may account for frequent outbreak of gastroenteritis in the town.

Keywords: Prevalence, salmonella serotypes, Sokoto abattoir effluents, vegetables.

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INTRODUCTION

Salmonella causes a disease; salmonellosis and it is responsible for significant morbidity and mortality in both humans and animals and has a substantial global socioeconomic impact. Salmonella infections in humans can range from a self-limited gastro-enteritis usually associated with non-typhoidal Salmonella (NTS) to typhoidal fever with complications such as a fatal intestinal perforation. Non-typhoidal Salmonella is one of the principal causes of food poisoning worldwide with an estimated annual incidence of 1.3 billion cases and 3 million deaths each year (Torpdahl, 2007). Typhoid fever, which is caused mainly by *S. typhi*, continues to be a major problem in developing countries. Recently, it has been estimated that globally there are more than 22 million cases of typhoid fever each year with more than 200,000 deaths, however, the true magnitude is difficult to quantify because the clinical picture is confused with many other febrile illnesses and most typhoid endemic areas lack facilities to confirm the diagnosis (Carrique-Mas and Davies, 2008). Members of the genus Salmonella are ubiquitous pathogens found in humans and livestock, wild animals, reptiles, birds and insects. Salmonellae are gram-negative, non-spore forming, facultative anaerobic bacilli, and 2µm to 3µm by 0.4µm to 0.6 µm in size. Like other members of the family *Enterobacteriaceae*, they produce acid on glucose fermentation; reduce nitrates to nitrite, and do not produce cytochrome oxidase (Brenner, 2000). Most organisms except *S. gallinarum* are motile by peritrichous flagella. The differential 5 metabolism of sugars can be

used to distinguish some Salmonella serotypes, e.g., most do not ferment lactose. *S. typhi* is the only organism that does not produce gas in sugar fermentation. Salmonella are non-capsulated except *S. typhi*, *S. paratyphi* C and some strain of *S. dublin* (Brenner, 2000). The survival of Salmonellae in the abattoir effluents may pose a potential health hazard and contribute to the re-inoculation of the pathogen in the environment (Rabah et al., 2010). The increase of contamination in natural water has intensified the detection frequency and persistence of pathogenic microorganismsin areas affected by sewage discharge. Waste from slaughtering ground in the abattoir may contaminate hand-dug well which serve the dual purpose of drinking water for the butchers and other worker in the abattoir, and become a sources of infection and contamination, attracting domestic and wide carnivores, rodents and flies, which are vector of diseases when organic matter exceeds the capacity of the microorganisms in water that break down and recycle the organic matter, it leads to eutrophication and encourages rapid growth of bacteria (Akan et al., 2010). Surrounding Sokoto abattoir is a fadama farmland area used for farming of various vegetables which could be contaminated by abattoir wastes. There has been frequent outbreak of gastroenteritis in Sokoto and increase number of people presenting typhoid fever case in various Hospitals across the city. Isolation of Salmonella serotypes from the abattoir effluents and vegetables cultivated around the abattoir could help to establish the role played by the abattoir in the Salmonellae contamination of vegetables cultivated in the area.

MATERIALS AND METHODS

Study area

Sokoto metropolis is located on latitudes 10° N and 14° 50′ N and longitudes and 7° E, east of the equator, in the extreme northwest of Nigeria. It covers an area of approximately 2,823,237 square kilometers. The last National census reported the state population to be 3,696,999 (NPC, 2006) with annual rainfall of between 500 and 1300 mm, humidity varies from 10 to 90% (Sokoto profile, 2009). The state is endow with livestock resources with an estimate of 3 millions cattle, 3 million sheep, 5 million goats, 4600 camels, 52000 donkeys and host of other species of local and exotic poultry species (MOCIT, 2002; Mamman, 2005). Sokoto abattoir is located at the outskirt of the city off western by-pass road in the area commonly known as ´kasuwandaji´. The abattoir is situated on a highland which makes easy for the effluents to drain directly in to nearby farm lands and during the raining season washed directly to the nearby river Rima which is used for irrigation and domestic purposes in cold and hot dry seasons (Mamman et al., 2000).

Samples collection

A total of 250 samples were collected, 100 from five different points of the abattoir draining system and 150 from raw consumed vegetables (50 onions, 50 lettuce and 50 tomatoes) cultivated and sold in the abattoir area.

Abattoir effluents sample collection

Five ml of abattoir effluents were aseptically collected from different points in the abattoir draining system and receiving water bodies with sterile gloves into sterile bijou bottles. Sample collection took place during normal working hours (8:00 am to 12:00 noon), in the morning once a week for a period of four months (between August to September, 2011 and February to March, 2012) (Rabah et al., 2010).

Vegetables samples collection and processing

Fresh vegetables (tomatoes, lettuces and onions) were collected in separate sterile polythene bags and labeled accordingly from cultivation lands around the abattoir and some selling points in the ‘Kara’ Market.

Onion flakes

10 g was weighed and homogenized with 90 ml of sterile distilled water then serial dilution of each of the samples was made and 0.1 ml from 10^4 dilutions was used for inoculation into Selenite-F-broth for enrichment and incubated at 35°C for 24 h. The broth cultures were aseptically inoculated onto Salmonella-Shigella agar plates for the isolation of Salmonellaeand the plates were incubated at 37°C for 24 h (Cheesbrough, 2006).

Tomatoes

10 g sample was weighed into sterile blending container. Sterile buffered peptone water was added and blends for 2 min and allowed to stand for 60 min at room temperature with container securely capped. Enough Selenite-F-broth was added to allow the tomato to float. The tomato and broth were placed into a sterile beaker for support during incubation and it was allowed to stand for 60 min at room temperature then incubated at 35°C for 24 h using standard procedure (Cheesbrough, 2006).

Lettuce

10 g was weighed into a sterile beaker and homogenized using chopper in sterile 90 ml of distilled water. Lactose broth was added. The beaker was allowed to stand at room temperature for 60 min and incubated at 35°C for 24 h (Cheesbrough, 2006).

Sample enrichment

Effluents, water, tomatoes, lettuce and onion samples were first enriched in selenite-F-broth for 24 h and then the resulting broth was inoculated on Salmonella-Shigella agar and incubated at 34°C for 48 h (Santos et al., 2007).

Selective plating

The broth cultures from enriched samples were aseptically inoculated on Salmonella-Shigella agar plates for the isolation of Salmonellae. The plates were incubated at 37°C for 18 h (Santos et al., 2007).

Preservation of strains

Isolates were preserved in slant bottles of nutrient agar at 4°C

Table 1. Prevalence of Salmonella serotypes in abattoir effluents, receiving water bodies and vegetables using biochemical tests.

Samples	Number of samples	No. of positive samples	Percentages (%)
Abattoir effluents	50	21	42.0
Water bodies	50	18	36.0
Vegetables	150	33	22.0
Total	250	72	100

before biochemical and serotyping was carried out (Charles, 1976).

Biochemical characterization of the Isolates

After isolation of colony from Salmonella-Shigella agar and in order to detect Salmonella strains biochemical tests such as Motility test, H_2S production acid and gas production from glucose, Manitol, maltose and sorbitol; acid production from adonitol, sucrose, Salicin and Lactose tests were carried out. Indole, Methyl-Red test, VogesProskauer test, oxidase test, citrate test, lysine decarboxylase tests, growth with KCN phenylalanine and tryptophan deaminase tests and Gelatin hydrolysis tests were conducted according to standard procedure (Cheesbrough, 2006).

Serotyping of isolates

The preliminary identified isolates by biochemical tests as Salmonellae were further subjected to serological test according to Kauffmann-White scheme using slide agglutination test. The polyvalent somatic (O), flagellar (H) Salmonellaantisera used were obtained from Staten's Serum Institut (SSI), Copenhagen, Denmark. Suspected Salmonella were cultured on nutrient agar for 24 h at 37°C according to manufacturer's instruction. Salmonella Sero-Quick group kit contain eight (8) antisera for serotyping of Salmonella to their Sero-group levels A to G (A, B, C_1, C_2, D, E, F and G). The kit was use in the following order: 0.02 ml of normal saline was first placed on a clean glass slide, then a loop of test organism was emulsified in the normal saline, presence of agglutination indicate that the colony is rough and cannot be serotyped, if no agglutination then 0.02 µl of D-antisera is added, if no agglutination then B-antisera is applied continuously until agglutination is obtained. Strains that are positive in D-antisera are further tested with the Vi antibodies to confirm for capsular antigen. When a positive reaction appear the organism belong to sero-group indicated on the bottle and no further test is necessary e.g. When the test of D, B and C were negative but the test of E is positive, Salmonella strains belong to sero-group.

RESULTS

All salmonella suspected isolates showed white colonies with black center on Salmonella-Shigella agar (S. S. agar). Gram staining of the colonies revealed gram negative straight, non-spore forming rods, lactose negative; acid and gas from glucose, mannitol, maltose, and sorbitol; no acid from adonitol, sucrose, salicin, lactose, ONPG test negative (lactose negative), indole test negative, methyl red test positive, Voges-Proskauer test negative, citrate positive (growth on Simmon's citrate agar), lysine decarboxylase positive, urease negative, ornithine decarboxylase positive, H_2S produced from thiosulfate, do not grow with KCN, phenylalanine and tryptophan deaminase negative, gelatin hydrolysis negative. A total of 72 Salmonella isolates were recovered from biochemical tests, 21 from abattoir effluents, 18 from receiving water bodies and 33 from vegetables cultivated around the abattoir table (Table 1). The results of serotyping show that *Salmonella* Typhimurium is the most commonly isolated with prevalence of 19.4%, this was followed by *S.* Enteritidis ranked the second with prevalence of 15.3%, then followed by *S.* Typhi (13.9), S. Paratyphi B (8.3%), *S.* Paratyphi C (13.9%), *S.* Derby (9.7%) and *S.* Newport (6.9%) (Table 2).

DISCUSSION

Results from this study shows that wastewater from the abattoir used for irrigation has been frequently contaminated with Salmonella. Out of 72 isolates serotyped from abattoir effluents, receiving water bodies and vegetables cultivated in the area (Table 3), *S.* Typhimurium was the predominant isolates with 14 (19.4%) and this agrees with the findings of Santos et al. (2001) who obtained prevalence of *Salmonella* Typhimurium at 13.5% from abattoir effluents (Nafaranda et al., 2006) obtained 12.3% receiving water bodies and 13.2% from vegetables irrigated with wastewater from Gwagwalada abattoir, Nigeria. The isolation of similar Salmonella serotypes from vegetables is an indication of abattoir effluents contamination of water used for irrigation. *Salmonella* Typhimurium in this study occurred at 14 (19.3%) while (Smith-Palmer et al., 2003) reported a prevalence of 12.6%. The higher prevalence in this study could be as a result of proximity of the abattoir to water bodies and farmlands.

Therefore the presence of Salmonellae in the vegetables cultivated in the abattoir area is however of public health concern. Center for disease control and prevention (CDC) reported Salmonella as the most prevalent bacterial diarrheal pathogen followed by *Shigella* sp., *Camphylobacter* sp. and *E. coli*. Wedel et al. (2005) reported that cattle are the reservoir of Salmonellae that may be transmitted to humans with resultant illness. In the present study, Salmonella serotypes isolated includes *Salmonella* Enteriditis*, S.*

Table 2. Prevalence of Salmonella serotypes in samples collected from abattoir effluents, receiving water bodies and vegetables cultivated around the abattoir using slide agglutination.

Salmonella isolates	Number of samples (%)	Sero-group
S. Enteritidis	11 (15.3)	G
S. Typhimurium	14 (19.4)	G
S. Typhi	10 (13.9)	D
S. Derby	7 (9.7)	F
S. Paratyphi A	6 (8.3)	A
S. Paratyphi C	10(13.9)	C
S. Paratyphi B	9 (12.5)	B
S. Newport	5 (6.9)	E
Total	72 (100)	

Table 3. Mean values and standard deviation of Salmonella serotypes in samples collected from abattoir effluents, receiving water bodies and vegetables cultivated around the abattoir.

Salmonella isolates	Effluents*	Receiving water bodies*	Vegetables*
S. Enteritidis	40 ± 8	28 ± 2	32 ± 3
S. Typhimurium	28 ± 6	33 ± 3	32 ± 5
S. Typhi	36 ± 7	40 ± 7	40 ± 7
S. Paratyphi A	37 ± 0	48 ± 1	52 ± 2
S. Paratyphi C	65 ± 3	37 ± 0	56 ± 3
S. Paratyphi B	28 ± 6	28 ± 4	25 ± 0

*Results are presented in mean ± standard deviation.

Typhimurium, *S.* Typhi*, S.* Paratyphi A*, B and C, S.* Newport and *S.* Derby. Contamination of vegetables could transmit Salmonella serotypes to farm workers and nearby settlers as they occasionally drink this water and vegetable consumers. Previous report by Weinberger and Keller (2005) reported high prevalence of Salmonellosis in among children living in the areas where waste water irrigated vegetables are cultivatedcompare with those from an area that did not practice sewage irrigation. It was also reported that Serogroup B and C were the most frequently isolated. Spread of cholera case was stopped when irrigation of vegetables was forbidden by authorities in Santiago (Chile) (Melloul et al., 2001).

Conclusion

This work confirmed that Sokoto abattoir effluents frequently contaminate nearby water bodies which are used for irrigation and poor hygienic practices in this abattoir could be responsible for the introduction of enteric pathogens to receiving surface water. Thus, there is an urgent need to put in place effluent treatment facilities to treat wastes from abattoirs in study area or re-location of the abattoir to a place away from cultivation farmlands.

REFERENCES

Akan JA, Abdulrahman FI, Yusuf E, 2010. Physical and chemical parameter in abattoir wastewater sample, Maiduguri metropolis, Nigeria. Pac J Sci Tech, 11(1):640-648.

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Waminathan B, 2000. *Salmonella* nomenclature. J Clin Microbiol, 38:2465-2467.

Carrique-Mas JJ, Davies RH, 2008. Sampling and bacteriological detection of *Salmonella* in poultry and poultry premises: A review. Rev Sci Tech Off Int Epiz, 27(3):665–677.

Charles W, Spyros DK, Robert BY, 1976. *Enterobacterceae* and *Pseudomonads aerogenosa* recovered from vegetables salad. Appl Environ Microbiol, 31:453-453.

Cheesbrough M, 2006. District laboratory practice in tropical countries, Microbiological tests. Chapter 7. In: Cheesbrough M, Ed. pt 2. 2nd Ed. Cambridge: Cambridge University Press. pp: 9-267.

Mamman AB, 2005. Transport Aspects of Livestock Marketing at the Achida and Sokoto Kara Market. Paper prepared under a network supported by the UK Department of International Development (DFID), Sokoto.

Mamman AB, Oyebanje JO, Peters SW, 2000. Nigeria: A people united, A future assured. (Survey of States), Vol 2. Gabumo Publishing Co. Ltd, London.

Melloul AA, Hassani L, Rafouk L, 2001. Salmonella contamination of vegetables irrigated with untreated waste water. World J Microbiol Biotechnol, 17:207-209.

MOCIT, 2002. Guide to Sokoto state's Economic Potentials. Commerce Department, Ministry of Commerce, Industry and Tourism, Sokoto State, pp: 4-18.

Nafaranda WD, Yaji A, Kubkomawa HI, 2006. Impact of abattoir wastes on aquatic life: A case study of Yola abattoir. Global J Pure Appl Sci, 12:31-33.

NPC, 2006. National Population Commission, Nigeria.
Rabah, AB, Oyeleke SB, Manga SB, Hassan LG, Ijah UJJ, 2010. Microbiological and physic- chemical assessment of soil contaminated with abattoir effluents in Sokoto metropolis, Nigeria. Sci World J, 5(3):1-4.
Santos FB, Dsouza DH, Jaykus L, Ferket PR, Sheldon BW, 2007. Genotypes, serotypes, and antibiotic resistance profiles of *Salmonella* isolated from commercial North Carolina turkey farms. J Food Prot, 70:1328-1333.
Santos RL, Zhang S, Tsolis RM, Kingsley RA, Adams LG, Baumler AJ, 2001. Animal models of *Salmonella* infections: enteritis versus typhoid fever. Microb Infect, 3(14-15):1335-1344.
Smith-Palmer A, Stewart WC, Mather H, Greig A, Cowden JM, Reilly WJ, 2003. Epidemiology of *Salmonella enterica* serovars Enteritidis and Typhimurium in animals and people in Scotland between 1990 and 2001. J Vet Rec, 153:517-520.
Torpdahl M, Sorensen G, Lindstedt BA, Nielsen EM, 2007. Tandem repeat analysis for surveillance of human *Salmonella* Typhimurium infections. Emerging Infect Dis, 13:388-395.
Wedel SD, Bender JB, Leano FT, Boxrud DJ, Hedberg C, Smith KE, 2005. Antimicrobial drug susceptibility of human and animal *Salmonella* Typhimurium, Minnesota, 1997-2003. Emerg Infect Dis, 11:1899-1906.
Weinberger M, Keller N, 2005. Recent trends in the epidemiology of non-typhoid *Salmonella* and antimicrobial resistance: the Israeli experience and worldwide review. Curr Opin Infect Dis, 18(6):513-521.