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PREVALENCE AND DISTRIBUTION OF *SALMONELLA* SEROVARS FROM WATER SAMPLES IN CATTLE REARING ENVIRONMENT OF JOS AND ENVIRONS, NIGERIA

Zainab Tamba[1*], Yunusa Adamu Wada[1], Iliya Shehu Ndams[1], Sonnie Joshua Oniye[1], Jacob Kwada Paghi Kwaga[2], Veronica Cibin[3]

[1]Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University, Zaria; [2]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine. Ahmadu Bello University, Zaria; [3]Reference Laboratory for *Salmonella* Instituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

*Correspondence: salehzee1@gmail.com; +2348037280014

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ABSTRACT

Salmonellosis is a zoonotic disease spread through food that is a global public health concern. The prevalence of *Salmonella* spp. and its serovars distribution in water sources within cattle-rearing habitats in Jos and environs were investigated in this cross-sectional study. A total of 150 water samples were obtained from several nomadic communities from diverse sources (pipe-borne, borehole, well, and stream) across six Local Government Areas (LGAs), namely Jos North, Jos South, and Jos Southeast, Barkin Ladi, Bassa, and Toro were used for the study. The International Standard Organization (ISO) 6579:2002/Amd1:2007 protocol was used for isolation. Serological confirmation of suspected *Salmonella* colonies was performed at the Office International des Epizooties (OIE)/Italian Reference Laboratory for *Salmonella* in Italy. The prevalence of *Salmonella* serovars, water sources, and locations were all correlated using a multivariate principal component analysis (PCA). The overall *Salmonella* spp prevalence obtained was 12.00 %. The serovars detected include *S.* Kentucky (2: 1.33%), *S.* Lille (5: 3.33%), *S.* Lagos (10: 6.67%), and *S.* Gatineau (1: 0.67%). The serovars were more prevalent in stream water (9: 15.52%) and well water (8: 10.00%), but none were found in borehole water. *Salmonella* serovars' highest prevalence and distribution were found in Bassa LGA and Jos South, while none were found in Jos. The four (4) different *Salmonella* serovars detected are known to be pathogenic and pose serious zoonotic concerns to humans, and this highlights the need for immediate public health intervention to avert salmonellosis outbreaks.

Keywords: Salmonella, Water contamination, Zoonosis, Cattle-rearing environment, Jos

INTRODUCTION

Water is a universal solvent that covers roughly seventy-one percent of the earth's surface and on which humans and animals rely. Drinking water should, in theory, be safe and acceptable to everybody, but in most developing nations, access to potable water is a major issue, especially given the massive health burden posed by dirty water and its sources (1, 2).

Globally, it is estimated that 2.2 billion people lack access to safely managed drinking water services, with most of these individuals living in countries with severe water shortages (3). Unfortunately, eighty percent of wastewater runs back into the ecosystem via multiple routes without being cleaned or reused (4), causing human disease and mortality. For example, 297,000 children under five years die from water-borne infections caused by contaminated drinking water (5). When drinking water becomes contaminated, it becomes a source of disease outbreaks such as cholera, dysentery, diarrhoea, and Salmonellosis (5).

Salmonella is a Gram-negative, short rod bacterium belonging to the Enterobacteriaceae family. *Salmonella* can be found in wells, lakes, streams, ponds, rivers, springs, and storage reservoirs, among other places. However, the faeces of diseased humans or animals can pollute these water sources (6). *Salmonella* can be isolated from domestic and wild animals and the environment and cause diseases in humans, constituting a public health threat. *Salmonella* infection in humans has been linked to various illnesses, including typhoid fever, septicaemia, localised infections of different bodily tissues, and enterocolitis. Salmonellosis causes fever, stomach pain, diarrhoea, dehydration, and weight loss in animals (6).

In Nigeria, *Salmonella* spp. has been isolated from several water sources. In Kano State, for example, Abakpa *et al*. (7) recovered *Salmonella* spp from 3.4 percent of irrigation water sources, while Ekelozie *et al*. (80) isolated *Salmonella* spp. from 16 percent of stream water samples, 10 percent from wells, and 4% from borehole sources in Anambra State, Nigeria. Furthermore, *Salmonella* spp. was isolated at 16 percent from a stream, 10% from a well, and 4% from a borehole (8). Increased population density, lack of proper sanitation, and poor hygiene increase the potential of pathogen contamination of these drinking water sources (1). In a cow-rearing environment, herders perform wide livestock production, where the cattle graze on grassland and come into touch with humans, domestic livestock, and wildlife, all of whom share a shared water source (9).

A vast spectrum of bacterial pathogens can be found in cattle manure. Surface runoff is how these bacteria get into water bodies. Rainfall-runoff, land application of manure from livestock ranches, and anthropogenic activities such as waste dumping into surface waters are also primary sources (streams and rivers). Also, contamination of wells with surface water when wells are not covered after heavy rain (9). As a result, the goal of this study was to isolate *Salmonella* serovars from water samples in Jos and its environs and determine their distribution and prevalence.

MATERIALS AND METHODS

Study Area

The research was carried out at Jos-Plateau and its environs, with GPS coordinates of latitude 10° 40'0" North and 9° 20'0" North of the Equator, and Longitude 9° 40'0" East and 8° 40'00" East of the Equator (Figure 1). Jos-Plateau covers a total area of 26,8992 square kilometres. Nighttime temperatures can drop as low as 11°C from mid-November to late January, making for frigid nights. Bauchi, Kaduna, Benue, and Nasarawa states all have shared borders. There are three agricultural zones in the state: North, central, and south. There are seventeen Local Government Areas (LGAs) in the city. The vegetation is predominantly montane, making it ideal for livestock (including exotic breeds), as well as food production (including maize, guinea corn, millet, fonio grains, Irish potatoes, and yams) (10).

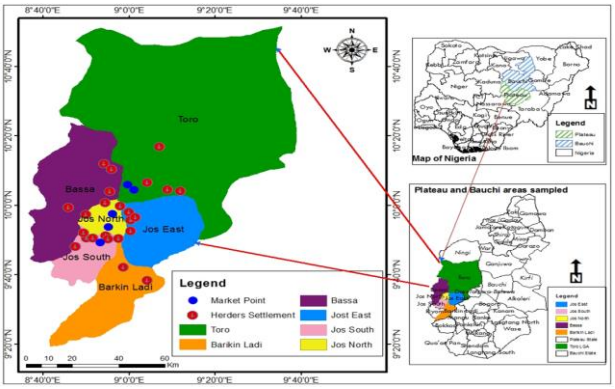

Figure 1: Map of sampling area in Jos-Plateau and environs, Nigeria (Left)
Adapted from the administrative map of Nigeria (Top right).

Study Design and Sampling

A cross-sectional study was conducted in different nomadic settlements from 2017 to 2018, where water samples were collected from various sources (pipe-borne, borehole, well, and stream) twice a month for fifteen months in the early hours of the morning (6:00-7:00 am). A total of 150 water samples were collected, twenty-five (25) samples from each LGA, namely: Jos North, Jos South, Jos East, Barkin Ladi, Bassa, and Toro. Thrusfield's formula (11) was used to compute the sample size. Two hundred (200) mL each of the water samples were collected, placed in Ice-man Cold boxes, and immediately sent to the Bacterial Division (Salmonella Laboratory) of the National Veterinary Research Institute (NVRI),

Headquarters. Vom, Plateau State, for analysis.

Isolation of *Salmonella* Isolates Using ISO 6579:2002 method

The collected water samples were pre-enriched in buffered peptone water (BPW) (OXOID, England) in a 1:9 sample to broth ratio at 37°C for 24hrs. Enrichment for *Salmonella* spp. was carried out on Rappaport Vassiliadis broth (RVB) (SC, Difco USA). Following incubation, 10 mL of each sample was mixed with 90 ml of RVB using a pipette and then incubated at 37°C for 24hr. Following enrichment in RVB, a loop full of broth was smeared and streaked onto Xylose Lysine Deoxycholate Agar (XLD) and Bismuth Sulfite (Oxoid, UK); the plates were incubated at 37°C for 24hr (12).

Biochemical Characterisation of Isolates

Suspected *Salmonella* isolates were subjected to biochemical tests based on indole production, hydrogen sulphide (H_2S) production, motility with Sulfide Indole Motility (SIM) medium (Oxoid Basingstoke, England), Citrate utilisation with Simmons citrate (Oxoid Basingstoke, England), Methyl Red (MR), and Voges-Proskauer (VP) using MR-VP medium (Oxoid Basingstoke, England) and Urease production (Oxoid Basingstoke, England). Further sugar fermentation tests include (glucose, lactose, sucrose, maltose, dulcitol, mannitol, inositol, rhamnose, sorbitol, mannose, and arabinose). According to the manufacturer's instructions, all biochemically identified isolates were confirmed serologically by slide agglutination test using Salmonella polyvalent 'O' group A-S antiserum (Oxoid Basingstoke, England). These tests were undertaken at *Salmonella* Laboratory, NVRI, Vom, Nigeria.

Serotyping of *Salmonella* Isolates

Biochemically confirmed isolates were freeze-dried and shipped to Office International des Epizooties (OIE)/Italian Reference Laboratory for *Salmonella*, *Istituto Zooprofilattico Sperimentale delle Venezie* (IZSVe). Padova, Italy for serotyping. The serotyping of the *Salmonella* isolates was carried out by identification of surface (O-antigens), flagella (proteins, H-antigens), and Vi capsular antigens (13, 14). Pure culture of *Salmonella* species previously confirmed as specified by ISO 6579 was inoculated into a non-selective Tryptose Agar (TA) tube and incubated at 37°C for 18-24 hours. Detection of the O-antigen was performed by slide agglutination. A drop of Poly O antisera was placed on a slide and a small quantity of growth from the Tryptose Agar (TA) tube was mixed with the antisera using the wire loop. The mixture on the slide was then rocked gently for one (1) minute to detect clumping (positive reaction), while a

homogenous suspension indicated a negative reaction. First, the strains were tested in the Poly O-sera-pools. Afterward, they were then tested with individual O-serum represented in the positive O-pool. Those isolates with a positive reaction (agglutination) were tested for flagella antigens.

Detection of the H-antigens for the first phase was performed by slide agglutination. The first phase identification was performed using the same procedure described for somatic antigens but using Poly H antisera. Flagella agglutinations are less "floccular" in appearance than somatic agglutinations and sometimes form only around the edge of the drop. The strains were first tested in the Poly H-antisera-pools. Afterward, they were tested in the individual H-antisera represented in the positive H-pool (15). After identifying the first phase, the detection of the second phase was then carried out by "phase inversion", using specific antisera inhibiting the identified first phase and allowing the expression of the second phase. About 5 ml of Swen Gard medium was poured into a small sterile Petri dish and allowed to solidify. Two (2) drops of antiserum against the detected H-antigen were added onto the already solidified Swen Gard medium and allowed to spread over the surface of the medium to block the first phase and enable the expression of the second phase. The strain was then inoculated in one spot at the centre of the Swarm Gard plate and incubated overnight at 37°C. The following day, a drop of Poly H-antigen was then placed on a glass slide and mixed with a loop full of bacterial growth from the edge of the motility zone on Swarm Gard medium. The slide was then rocked gently for a maximum of one (1) min for the appearance of agglutination. The strains were first tested in the Poly H-antisera-pools. Afterward, they were tested in the individual H-antisera represented in the positive H-pool (15). A positive reaction was seen as a visible agglutination, while physiological saline was used as a negative control (homogeneous milky turbidity). A late or weak agglutination should be considered negative. Negative reactions may be due to a strain expressing the Vi antigen, a strain not covered by the antisera used, or a strain not being *Salmonella*. Reactions from the O, H, and Vi antigens were combined to identify the specific *Salmonella* strain (serovar), and the Kauffmann-White Scheme was consulted for the identification.

Data Analyses

The prevalence of *Salmonella* serovars was determined using statistics and depicted as a multiple bar chart, with the results summarised as mean values and

standard error. Using Minitab version 17, a principal component analysis (PCA) biplot was utilised to connect prevalence and *Salmonella* serovar distributions in water sources and locales. P 0.05 values were considered statistically significant.

RESULTS

In the study areas, a total of 18 Salmonella serovars were isolated, with an overall prevalence of 12.0% (Table 1). Salmonella Kentucky (2/18: 1.33 %), S. Lille (5/18:3.33 %), S. Lagos (10/18: 6.67 %), and S. Gatineau (1/18: 0.67 %) were among the Salmonella serovars detected (Table 1). The presence of Salmonellae was more common in stream water (9/15: 52%) and well water (8/80: 10%); and no borehole water was positive for Salmonella (Table 2). According to location, the highest frequency of *Salmonella* was found in Bassa LGA (28.0%), while no *Salmonella* was found in Jos East LGA (0.00%) (Table 3).

Table 1: Prevalence of *Salmonella* serovars isolated from water samples in Jos-Plateau and environs, Nigeria

Serovars	Total	No. positive	Prevalence (%)
Salmonella Kentucky	150	2	1.33
Salmonella Lille	150	5	3.33
Salmonella Lagos	150	10	6.67
Salmonella Gatineau	150	1	0.67
Total	**150**	**18**	**12.00**

Table 2: Distribution of *Salmonella* serovars isolated from water sources in Jos-Plateau and environs, Nigeria

Source of water	Total	No. positive	Prevalence (%)
Pipe-borne	8	1	12.50
Bore-borne	4	0	0.00
Well water	80	8	10.00
Stream water	58	9	15.52
Total	**150**	**18**	**12.00**

Table 3: Prevalence of *Salmonella* serovars isolated from water samples in different Locations of Jos-Plateau and environs

Location	No. examined	No. positive	Prevalence (%)
Jos North	25	2	8.00
Jos South	25	4	16.00
Jos East	25	0	0.00
Barkin Ladi	25	2	8.00
Bassa	25	7	28.00
Toro	25	3	12.00
Total	**150**	**18**	**12.00**

The *Salmonella* serovars' highest prevalence and distribution were found in Bassa and Jos South LGAs, while none was found in Jos East (Figure 2).

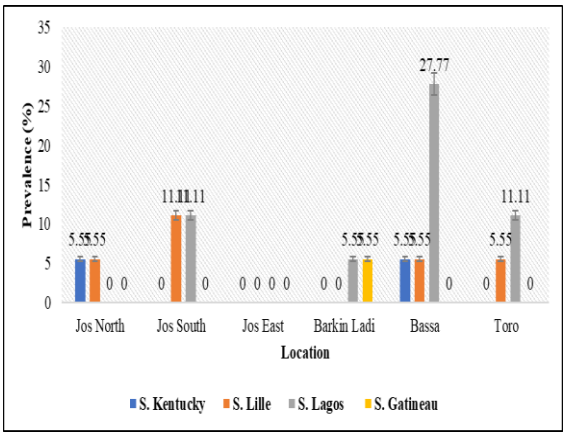

Figure 2: Distribution of *Salmonella* serovars isolated from water samples across sampling locations in the study area

The serovars S. Lagos and S. Lille were substantially linked with stream and well waters in Bassa, Toro, and Jos South LGAs, as shown in Figure 3. Well, stream water had a weak and positive connection with S. Kentucky and S. Gatineau. There was no correlation between serovars and borehole water (Figure 3).

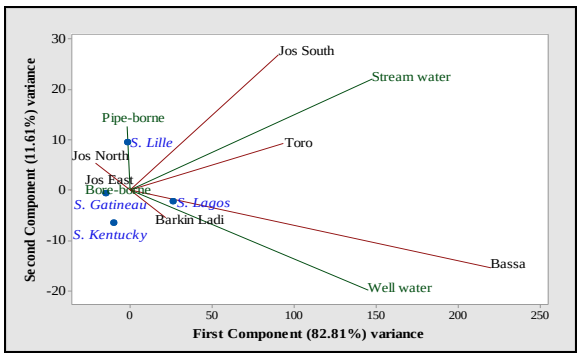

Figure 3: Principal component-based (PCA) biplot of sampling locations, sources of water, and *Salmonella* serovars in Jos-Plateau and environs, Nigeria

DISCUSSION

Salmonella was shown to be more prevalent in stream water than in pipe-borne, borehole, and well water in this investigation. This finding could be due to the dumping of household garbage into streams, and human and animal faeces could encourage the spread of biological contaminants. *Salmonella* spp. is uncommon in borehole water, possibly due to the water's depth and decreased contamination from surface and subsurface sources. In Jos, Nigeria, Karshima *et al*. (16) found a low incidence of *Salmonella* spp. in borehole water (1.3%) compared to stream water (6.8%). Dekker *et al*. (17) made a similar observation in Ghana's Asante Akyem district, where the river water had the highest prevalence (14.5 percent), followed by well water (4.0 percent), and none was detected in the borehole and pipe-borne water. These results contrast those of Ekelozie *et al*. (8) in Anambra State, Nigeria, who found *Salmonella* prevalence of 8.03 percent, 5.4 percent, and 1.8 percent in well, borehole, and stream water, respectively. The disparity in *Salmonella* prevalence in different water sources could be due to the organism's relative contamination of the sources. Nwaiwu *et al*. (18) have linked anthropogenic sources such as home sewage, livestock manure, septic tank leaks, and pit latrines to water contamination from boreholes, wells, and streams.

On-the-spot inspection of wells in the studied environment showed that they lacked covers, allowing contamination of the wells. *Salmonella* Lagos was the serovar with the highest prevalence, while *Salmonella* Gatineau had the lowest prevalence. Fashae and Hendriksen (19) found a reduced frequency of Salmonella Lagos in pig farms in Ibadan, Nigeria.

This study is the first to report the occurrence of *S*. Lagos, *S*. Gatineau, and *S*. Lille in water sources in the study area. *Salmonella typhi* was recovered from

Nasarawa State wells (20), Kano State tap water (21), and Ogun State wells (22). As a result, *Salmonella* serovars can survive in water from various sources, regardless of depth from the surface. The Principal Component Analysis (PCA) biplot used in this study further confirms the interlink between *Salmonella* serovars contamination of stream and well waters.

The overall prevalence (12.00 %) of *Salmonella* found in this study is of public health significance. The presence of Salmonella in food goods can result in their recall from the market by the FAO/WHO Codex Alimentarius Commission regulation (23). The high incidence is often attributed to poor sanitary conditions, which may be related to poor water quality, resulting in a massive health burden posed by dirty water and its sources (1). Furthermore, due to the rising human population without corresponding improvements in sanitation and hygiene, the potential of pathogen contamination of these drinking water sources is significant. This scenario could also explain the high prevalence of *Salmonella* spp. in water from the Bassa LGA. Because all *Salmonella* serovars are harmful to humans, the occurrence of diverse *Salmonella* serovars in Jos-Plateau and its environs is of severe public health concern. The *S.* enterica subspecies enterica causes a broad spectrum of food and water-borne illnesses in humans and animals, constituting a severe public health problem in Jos-Plateau, Nigeria, with worldwide implications (24, 25).

CONCLUSION

This investigation found four different *Salmonella* serovars and an overall prevalence of *Salmonella* species to be 12.0% in water samples from various sources. Serovars of *Salmonella* present in cattle rearing environment in Jos and environs are *S.* Lagos (10:(6.67%), *S.* Lille (5: 3.33%), *S.* Kentucky (2:1.33%), and *S.* Gatineau (1: 0.67%). Bassa LGA had the highest prevalence (28.0 %), while none was identified from water samples from Jos East LGA. The presence of Salmonella serovars detected in water sources in the present study poses serious zoonotic concerns to humans, and this highlights the need for immediate public health intervention in Jos-Plateau and its environs to avert salmonellosis outbreaks.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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