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Prevalence of *Salmonella* spp. in captive wildlife at the National Zoological Garden Jos, Nigeria

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ABSTRACT

Published reports on *salmonella* in wildlife are few and to the best of our knowledge, no published report exists on the prevalence of *Salmonella* at the National Zoological Garden Jos, Nigeria. There had been reports of death of wildlife among the zoo inmates due to infectious diarrhoea possibly due to *Salmonella* which is of economic importance and of great concern in terms of conservation of wildlife. *Salmonella* is also of great zoonotic importance especially in an animal contact setting like the zoo where many come to visit and interact with the animals. The study therefore sought to screen the zoo animals for *Salmonella* and to determine the prevalence of the organism. 160 faecal samples were collected from wildlife in the zoo over a period of three months and screened for *Salmonella* using the conventional biochemical tests and confirmation was done using the Microbact GNB 12E. Eight isolates were confirmed out of the 160 samples, giving a prevalence of 5%. There was a statistically significant association ($p \leq 0.05$) between the occurrence of *Salmonella* and months of sample collection. The finding of this study is a contribution to the surveillance of *Salmonella* in wildlife; it may also be the reason for the deaths recorded among the inmates due to infectious diarrhea. Considering its zoonotic nature, the staff, visitors to the zoo and the general public is at risk of contracting the bacteria from the animals, infected faecal materials, cages, railings etc. Captive and free range wildlife could be reservoirs and sources of *Salmonella* infection to other wildlife, domestic animals and man.

Keywords: *Salmonella*, Prevalence, Captive wildlife, Zoonosis, Death

INTRODUCTION

Salmonella is the cause of salmonellosis both in animals and humans causing acute and chronic diarrhea and death [1]. They cause heavy economic losses to the animal industry due to high mortality, enteric infections caused by species of *Salmonella* have assumed great zoonotic importance chiefly due to their ubiquitous distribution, the growing number of serotypes, wide host spectrum, complex pathogenesis and the complicated epidemiological chain involving animals, man and the environment [2,3].

Death of wild animals due to infectious diarrhea arising from *Salmonella* have been reported [3,4] with the animals expressing both symptomatic and asymptomatic infections [5]. This poses serious risk to staff, visitors to the zoo and the general public due to the possibility of zoonotic infection from contaminated faecal materials, environment and other objects for example cages railings etc. [6].

MATERIALS AND METHODS

The National Zoological Garden is located in Jos the capital of Plateau State, North Central Nigeria. The State is on latitude 9.8º and longitude 8.9º, the altitude is 1295 above sea level with average daily temperature of 17-29ºC [7]. It occupies a land of about 200 acres and was opened in1956.

A total of 160 faecal samples were collected from captive wildlife at the zoo once every month between February and April 2011. A total of 113 animals comprising 14 carnivores, 30 primates, 15 herbivores, 42 birds and 12 reptiles were housed in 63 cages. Samples were collected by convenience. Collection of samples was done very early in the morning from as many cages as have faecal materials and could be accessed. Individual feacal samples were collected from cages housing one to four animals while two samples were collected from those housing five animals and above. With the distribution of animals in cages the

maximum number of samples that could be collected per round of collection is 65. Fifty two, 53 and 55 samples were, however collected in February, March and April respectively. Freshly voided faeces were given priority as much as possible in the choice of sample to be taken. They were collected using clean polythene bags and transported to the Bacterial zoonosis laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria within three to four hours of collection in an insulated container on ice packs.

One gram of each faecal sample was inoculated into 9ml Selenite F broth as enrichment medium and incubated for 24 hours at 37ºC [8]. After which one loopfull was plated unto xylose lysine desoxycholate (XLD) agar and incubated for 24 hours at 37ºC. Discrete colonies which appear pink or red with or without black centers presumptive of *Salmonella* were inoculated into triple sugar iron (TSI) agar and incubated for 24 hours at 37ºC. Isolates which showed alkaline slant over acid butt reaction with or without hydrogen sulphide and gas respectively were further inoculated into urea agar and incubated for 18-24 hours at 37ºC. Those that produced no reaction in urea were inoculated into nutrient agar slant for further biochemical tests [9, 10].

The ability of the isolates to ferment 12 sugars was tested. The sugars were sorbotol, raffionse, sucrose, xylose, rhamnose, lactose, glucose, maltose, inositol, manitol, dulcitol and arabinose, manufactured by Oxoid (U.K.). They were prepared and used according to manufacturer's instruction [11].

Polyvalent antiserum agglutination test was done by suspending a loopfull of the growth from XLD agar in one or two drops of normal saline and mixing with one drop of *Salmonella* polyvalent antiserum on a glass slide. Agglutinating serum *Salmonella* polyvalent O groups A – S (Remel Europe Ltd. Dartford England DAZ-6PT, Ref- ZCO2) was used according to manufacturer's instruction. The slide was then tilted back and forth several times. Positive tests were indicated by rapid, complete agglutination of the bacterial cells on the glass slide [12, 13].

All isolates were subcultured on XLD agar at 37^{o}C for 24 hours. One to two colonies were picked using a sterile loop and thoroughly suspended into 5ml of sterilized normal saline in test tubes.

The Microbact GNB 12E wells were labeled with the corresponding isolate identification codes. The adhesive seal of the wells were opened. Using sterile pipette, each labeled Microbact GNB12E well was filled to ¾ level with the corresponding isolate suspension, after which they were resealed. The filled plates were then incubated for 24 hours at 37^{o}C. Two drops of Kovac's reagent, one drop each of VPI and VPII and one drop of TDA were added to wells eight, 10 and 12 respectively before taking the readings. Colour changes were compared with the standard colour chart provided by the manufacturer and number grades assigned to each well. The grades were summed up to three numbers and inputed to the Microbact 2000 identification software provided by the manufacturer which then gave the identification of test organisms as percent probabilities.

RESULTS AND DISCUSSION

Out of total of 160 faecal samples screened for *Salmonella*, nine samples had characteristic biochemical reaction similar to *Salmonella* after the conventional biochemical tests while eight showed typical *Salmonella* reactions after Microbact GNB 12E testing having % probability score of 75 and above. Eight *Salmonella* isolates were therefore confirmed by Microbact test kit. The Kappa statistics analysis between Microbact test kit and the conventional biochemical test was 0.938 which is an almost perfect agreement (Table 1).

The samples collected each month and the corresponding *Salmonella* isolates obtained were subjected to fisher's exact test. There was a statically significant association ($p \leq 0.05$) between the occurrence of *Salmonella* and months of sample collection (Table 2).

The rate of recovery of *Salmonella* within the carnivores is 12.5%. While the class specific prevalence for primates, herbivores, birds and reptiles are 1.96%, 0%, 7.02% and 0% respectively. The overall prevalence of confirmed *Salmonella* isolates by Microbact GNB 12E is 5%. This could be obtained also by adding together the overall prevalence for all the classes of animals; (1.87, 0.63, 0, 2.5 and 0) (Table 3).

The prevalence for the isolated *Salmonella* in this study is 5%. This has established the occurrence and prevalence of *Salmonella* at the National Zoological Garden Jos, Nigeria. The sources of these infections could be food fed to the animals by the owners of the facility. Since most of the animals that yielded the organisms were carnivores, birds that eat flesh and primates that the visitors often give food whenever they enter the zoo. *Salmonella* have been reported in meat, milk, fruits and other food items [14]. These are part of what visitors give the animals to eat when they come into the zoo especially the primates. Water could also be a source of the infection in the zoo, contaminated water fed to the animals could lead to infection. Migratory bats and other birds periodically migrate to the zoo and hang on the trees over the cages, defeacating inside the cages, water and food. These birds could be sources of transmission of *Salmonella*. Rodents, grasscutters, lizards, flies etc. enter the cages and could play important roles in the transmission of the organisms. Staff of the facility could also be sources of *Salmonella* to the zoo animals [13].

Table 1. Codes and description of wild animals from which *Salmonella* were confirmed, number of positive isolates after conventional biochemical tests and Microbact test result.

Description of type of wild animals	No of suspected isolate after conventional biochemical tests	No of positives after Microbact Test	Percentage probability by Microbact
Peafowl	1	1	87.45%
African hawk eagle	1	1	87.45%
2nd cage, Spotted hyena	1	1	87.47%
1st cage, Lion 4	1	1	87.45%
3rd cage, Chimpanzee	1	1	98.87%
African hawk eagle	1	1	98.44%
Bateleur eagle	1	1	98.44%
1st cage, Stripped hyena	1	0	-
2nd cage, Stripped hyena	1	1	87.45%
Total	9	8	

Kappa statistic (Microbact and conventional biochemical test) = 0.938

Table 2. Months of sample collection, total number of samples collected per month and the total number of sample positive for *Salmonella* in the zoo.

Month of sampling	Total no of samples collected/month	Total no of Sample positive for *Salmonella*/ (%)
Feb. 2011	52	1(1.92)
Mar. 2011	53	1(1.89)
Apr. 2011	55	6(10.91)
Total	160	8(5)

Fisher's exact test p value= 0.05

Table 3. Class of wildlife animal in the zoo, total number of samples collected per class for the three months period, number of *Salmonella* confirmed positive, class specific and overall prevalence.

Class of animal	No of samples collected	*Salmonella* positive from Microbact	Class specific prevalence (%)	Overall prevalence (%)
Carnivores	24	3	12.5	1.87
Primates	51	1	1.96	0.63
Herbivores	16	0	0	0
Birds	57	4	7.02	2.5
Reptiles	12	0	0	0
Total	160	8	0	5

Table 4. Results of conducted biochemical tests on *Salmonella* isolates compared to results of standard tests.

TESTS	Pf2A	Ahe1B	2Sh1B	1L4C	3Cm2C	Ahe1C	Be2C	1SrhC	2SrhC	STANDARD
XLD	RED	RED+BLK	RED+BLK	RED	RED+BLK	RED+BLK	RED	RED	RED+BLK	**RED±BLK**
TSI	K/AG	K/A+H2S	K/A+H2S	K/AG	K/AG+H2S	K/A+H2S	K/A	K/AG	K/A+H2S	**K/A(G)+H2S**
Urea	—	—	—	—	—	—	—	—	—	**—**
Sorbitol	†	†	†	†	†	†	†	†	†	**†**
Raffinose	—	—	†	—	†	—	†	†	—	**—**
Sucrose	†	†	†	†	—	†	†	†	†	**—**
Xylose	—	—	—	—	†	—	—	—	—	**†**
Rhamnmose	—	—	†	—	†	—	†	†	—	**†**
Lactose	—	—	†	†	†	—	—	—	—	**—**
Glucose	†	†	†	†	†	†	†	†	†	**†**
Maltose	—	†	†	†	†	†	—	†	—	**†**
Inositol	†	†	†	—	†	†	—		†	**†**
Manitol	†	†	†	—	†	†	†	†	†	**†**
Docitol	—	†	†	—	†	†	—	†	†	**†**
Arabinose	—	—	—	—	—	—	—	—	—	**—**
O Antiserum Aggl	†	†	†	†	†	†	—	†	†	**†**

†; Positive, —; negative

Falade and Durojaiye [4] reported a prevalence of 9.5% from the University of Ibadan zoo, 7% was reported by Gopee *et al*. [13] at the Emperor Valley zoo while Okoh and Onazi [15] reported 1.76% from the Kano zoo for *Salmonella* isolation. There was an outbreak of 'unexplained diarhoea' amoung the inmates of the University of Ibadan when the study was carried out, Gopee *et al*. [13] carried out their research over a period of three years. Outbreak of infection with infectious diarhoea as signs and research work taking

longer period of time giving room for more samples to be collected may be the reasons for the higher prevalence in both cases compared with this study. It is not totally understood why the prevalence in Kano zoo is lower compared with this study, probably the conditions under which samples were processed could be a factor [15].

The implication of this finding is that the wildlife in the zoo are at risk of coming down with salmonellosis and dying. Both humans and animals could also be infected with *Salmonella* when they come in contact with the zoo animals or other contaminated materials from them because of the zoonotic nature of the organism [16].

CONCLUSION

The results of this study have shown that *Salmonella* is present in wildlife animals kept at the National Zoological Garden Jos, Nigeria and they may actually be responsible for morbidity and mortality among the animals. The isolation of these organisms from apparently healthy animals may be evidence of asymptomatic infections and the fact that they may be carriers, shedding the organisms periodically; the public health importance of the findings of this study cannot be over emphasized. *Salmonella* is zoonotic and can be transmitted from animals to man. With thousands of people including children visiting the zoo weekly especially during festive seasons, it is evident that infection could be contracted through touching and climbing of contaminated railings, cages, barriers, etc. staff that clean up the cages daily, feed the animals and enter to treat the animals periodically are also at risk of contracting *Salmonella*.

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