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ISOLATION AND PREVALENCE OF *ESCHERICHIA COLI* IN WILD ANIMALS AT THE NATIONAL ZOOLOGICAL GARDEN JOS, NIGERIA

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ABSTRACT

There had been reports of infectious diarrhea leading to death of wild animals at the National Zoological Garden Jos, Nigeria which could possibly be due to *E. coli*. The presence of the zoonotic infection puts the animals, staff, visitors and the general public at risk of contracting the bacteria which could lead to death of wildlife, economic losses, wildlife conservation issues, human sickness, expenditure on treatments or death of man or animals. The record of surveillance of *E. coli* in Nigeria is limited. The study sought to determine the presence and prevalence of *E. coli* in the zoo. 160 faecal samples were collected over a period of three months and analysed for *E. coli* using the conventional biochemical tests and confirmation was done using the Microbact GNB 12E. Seventy seven (48.1%) isolates showed reactions presumptive of *E. coli* after the conventional biochemical tests while 58 (36.3%) were confirmed after testing with Microbact GNB 12E. The confirmed isolates were from primates 18, carnivores 5, herbivores 5, birds 28, and reptiles 2. There was a statistically significant association ($p<0.05$) between the different classes of animals and the isolation of *E. coli*. There was also a statistically significant association ($p<0.05$) between the occurrence of *E. coli* and months of sample collection. The occurrence and high prevalence of *E. coli* implies the organism could be the cause of infectious diarrhoea and death in the zoo, while staff and as many as visit the zoo are at risk of contracting the organisms from the animals or other contaminated objects which could lead to human death and epidemics.

Key words; *E. coli*, Prevalence, Wild animals, Zoonosis, Death

INTRODUCTION

E. coli comprises a diverse group of microorganisms responsible for gastrointestinal diseases in humans and animals, enterotoxigenic and enteropathogenic *E. coli* are important causes of diarrhoea and are associated with significant mortality rates in young children, the elderly and animals (Casswall *et al*., 2000; Asrat, 2001; Rappelli *et al*., 2001; Litchfield, 1980; Adetosoye *et al*., 1976). *Escherichia coli* is an abundant and usually harmless commensal member of the human digestive flora (Selander *et al*., 1986; Kasper *et al*., 1987). Nevertheless, pathogenic strains are an important cause of sickness and mortality throughout the world, particularly for children in developing countries (Cravioto *et al*., 1991). Infections can also result when the immunity of the host is suppressed. *E. coli* is also a common member of the microbial flora of wild animals and birds. Surprisingly, little is known about *E. coli* in populations of wild animals (Selander *et al*., 1987; Whittam, 1996).

Souza *et al*. (1999) screened samples from wild animals from different continents for *E. coli*. Samples were taken majorly from captive wild animals and birds at the end of which 202 *E. coli* strains were obtained from 81 mammalian species, representing 39 families and 14 orders in Australia and the Americas, a strain was obtained from a reptile and 10 from different families of birds collected in Mexico.

A longitudinal study to isolate *E. coli* from wild animals at the Emperor valley zoo in Trinidad and Tobago reported the frequency of *E. coli* isolation being significantly higher in mammals compared with birds and reptiles and the overall frequencies of isolation of *E. coli* from omnivores, herbivores, and carnivores, were 87.2%, 70.0%, and 57.3%, respectively (Gopee *et al*., 2000).

From a total of 103 swab samples from wild animals at the Asa Zoological Park, Hiroshima in Japan, a total of 122 *E. coli* isolates were obtained among other Gram negative bacteria isolated. *E. coli* isolates were the highest of the gram negative bacteria isolated (Ahmed *et al.*, 2007).

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There is paucity of information on the occurrence of *E. coli* in wildlife in Africa, Nigeria and the National Zoological Garden, Jos, Nigeria.

MATERIALS AND METHODS

One gram of faecal sample was inoculated into 9 ml buffered peptone water and incubated for 24 hours at 37^{o}C. A loop full was picked and inoculated onto EMB agar and incubated for 24 hours at 37^{o}C. Green metallic sheen - like colonies were picked and tested in MRVP broth, Simmons citrate, SIM agar, TSI agar and urea agar. Isolates that were positive to methyl red test, negative to Voges-Prokeur test, negative to Simmon citrate test, negative to H_2S production, positive to indole production, motility positive, negative to urea production and that produced acid over acid reaction in TSI were picked for Microbact GNB 12E identification test.

E. coli Identification by Microbact GNB 12E.

All isolates were sub cultured on EMB agar at 37^{o}C for 24 hours. One to two pure culture colonies were picked using a sterile loop and suspended into already prepared, sterilized 5 ml normal saline in test tubes. The adhesive seals of the Microbact GNB 12E kit wells were opened. Sterile pipettes were used to dispense the prepared isolate suspension into the labeled Microbact GNB 12E kit wells after which the seals were returned. The inoculated plates were incubated for 24 hours at 37^{o}C, following which two drops of Kovac reagent, one drop each of VPI and VPII and one drop of TDA respectively were added to wells 8, 10 and 12 respectively before taking the readings. Colour changes were compared with the standard colour chart provided by the manufacturers and number grades assigned to each well, these were summed up to give a set of three numbers and inputted to the Microbact 2000 identification software provided by the manufacturer which gives the identity and probability of the organism tested.

RESULTS AND DISCUSSION

Out of the 160 samples collected, 77 (48.1%) were positive after the initial biochemical tests while 58 (36.3%) of the samples were confirmed *E. coli* following testing with Microbact GNB 12E. Out of the 58 confirmed *E. coli*, 18 were from primates, five from carnivores, five were also from herbivores, 28 from birds while two were from reptiles. Using Fisher's exact test there was a statistically significant association ($p<0.05$) between the different classes of animals and the isolation of *E. coli.* The samples collected each month and the corresponding *E. coli* isolates obtained were subjected to Chi square test. There was a statistically significant association ($p<0.05$) between the occurrence of *E. coli* and months of sample collection.

Table 1. Confirmed *E. coli* isolates from the zoo and percentages by class of animal

Class of Animal	Number Positive	Percentages
Primates	18	31.03
Carnivores	5	8.62
Herbivores	5	8.62
Birds	28	48.28
Reptiles	2	3.45
Total	58	100

Table 2. Class of wildlife animal in the zoo, total number of samples collected per class for the three months period, number of *E. coli* confirmed positive, class specific and overall prevalence.

Class of Animal	No of Samples Collected	*E. Coli* Positive From Microbact	Class Specific Prevalence	Overall Prevalence
Carnivores	24	5	20.83	3.12
Primates	51	18	35.29	11.3
Herbivores	16	5	31.25	3.13
Birds	57	28	49.12	17.5
Reptiles	12	2	16.7	1.25
Total	160	58		36.3

Fisher's exact test p=0.03.

Table 3. Months of sample collection, total number of samples collected per month and the total number of sample positive for *E. coli*. and their percentages

Month of sampling	Total no of samples collected/month	Total no of Sample positive for *E. coli* / (%)
Feb. 2011	52	8(15.38)
Mar. 2011	53	26(59.06)
Apr. 2011	55	24(43.64)
Total		58(36.25)

Chi square test value=14.86, p value= 0.0006

The prevalence of 36.3% for *E. coli* confirms the presence and abundance of the bacteria in the Zoological Garden. The isolated *E. coli* which could either be enteropathogenic, enterohaemorhagic or commensal are possible causes of infection to both wildlife and humans considering the zoonotic nature of the organism. They could be the cause of the reported infectious diarhoea and death of wildlife in the zoo while staff, visitors and the general public are at risk of contacting the bacteria (Ekwonu, 2010, personal communication).

The sources of infection could not be ascertained. Food, water, visitors, rodents, lizards, bats and other migratory birds found in and around the garden could however, be possible sources. There were distinct differences between the isolation of *E. coli* and the classes of wildlife. The birds and the primates had the highest yield, this may be due to the fact that visitors have more access to these set of animals, with food and drinks given uncontrollably as compared to carnivores, herbivores and reptiles. This is in agreement with the findings of Gopee *et al*. (2000) who reported isolation of 87.2% in omnivores compared to 57.3% and 70% in carnivores and herbivores respectively.

It is not understood why the yield of *E. coli* in February was significantly lower than that of March and April. Further research work could be done to understand the effect of the climate on the isolation of the organism, determine the strain of *E. coli* isolated and investigate the sources of infection to the animals.

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