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Clinical Case Report on Diarrhea Caused by *Escherichia coli* in Horsfields Tortoise (*Testudo horsfieldi*) at Zoological Garden, University of Ibadan, Nigeria

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Abstract: A clinical case of diarrhea in Horsfields tortoise (*Testudo horsfieldi*) was reported. It was characterized by weakness, anorexia, dullness, dehydration, depression and with profuse, watery and foul smelling feces. Microbiological cultural examination of fresh faecal samples from tortoises revealed that the diarrhea was due to *E. coli* infection, while by parasitological examination of helminthes' eggs were negative. The infection might be due to environmental pollution from human visitors, wild carnivores and other wild captive animals around the enclosures of the tortoises.

Key words: Horsfield Tortoise · *Escherichia coli* · Diarrhea · Zoo

INTRODUCTION

Horsfields tortoise (*Testudo horsfieldi*) is the most widely distributed tortoise in the relatively inhospitable terrain in central Asia. Other species like leopard tortoises (*Geochelone pardalis pardalis*) are found in central and southern Africa [1]. They can be also found throughout the savannas of Africa, from Sudan to the southern Cape. They are commonly kept as pets and adapt well to captivity in most areas barring coastal Natal where the humidity affects them adversely [1]. These large tortoises need a large area if confined in an enclosure, though it is preferable to give them the run of a garden.

Escherichia coli is a normal inhabitant of the gastrointestinal tracts of animals and human beings [2, 3]. The microorganism has been responsible for both intestinal and extra-intestinal infections, thus making chemotherapy important for control [4-6]. Exposure of animals to microorganisms from various sources, especially in foods and the environment, may then facilitate the transmission of resistant bacteria [7]. To date, however, there is very limited information available in the literature on the antibiograms of *E. coli* strains from wildlife, either free-ranging or captive [7, 8]. *E. coli* possesses virulence markers such as O-antigens [9, 10], haemolysin production [11, 12], K (capsule) production [13] and pilus production [14] amongst others, which play significant role in the pathogenesis of human and animal strains of the micro organism. However, it is known that the majority of *E. coli* strains lead a symbiotic existence and are considered harmless [15]. Enteropathogenic *E. coli* (EPEC) strains are responsible for enteritis diseases in humans, principally associated with neonatal and infantile diarrhea [5, 16, 17]. Although EPEC strains have been isolated to date, an animal reservoir has not been well established as is currently known for verocytotoxigenic *E. coli* (VTEC) where cattle serve as the major reservoir [6, 18]. Presently, there is a dearth of information on diarrhea induced by the prevalence of *E. coli* in wildlife especially in Tortoise. The present study investigated a clinical case of diarrhea in Horsfields tortoise (*Testudo horsfieldi*) at the Zoological Garden, University of Ibadan.

MATERIALS AND METHODS

History and Clinical Examination: The zookeeper at the University of Ibadan, Zoological Garden reported to the duty Veterinarian at the Zoological garden that the tortoises were passing watery diarrhea for over two weeks

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and they appeared very dull and inactive. This dullness was evident in the reaction of the tortoise to its food when fed. Observations revealed that the tortoises preferred a secluded corner and when approached by the handler they did not put up any resistance as they would normally do. Physical examination of the tortoises revealed dullness, anorexia, weakness and passage of watery and foul smelling diarrhea. Within the tortoise environment are other captive wild species such as wild carnivores, ducks, feral pigeons and water fowls that are carriers of *E. coli* as reported by Fowler and Miller [19]. Human visitors may also be source of infection.

Bacteriological Investigation: Twelve (12) fresh faecal samples obtained from tortoises were examined for the presence of bacteria and helminthes. The faecal samples were directly streaked out on Columbia agar supplemented with 5 % sheep blood (BioMérieux, Vienna, Austria), Chromocult® Coliform Agar with coliform Selective-Supplement (Merck, Vienna, Austria), SM2 agar (BioMérieux, Vienna, Austria) and Sabouraud's gentamicin chloramphenicol agar (Bio- Mérieux, Vienna, Austria). With the exception of the Sabouraud's gentamicin chloramphenicol agar, the other agar plates were incubated at 37°C aerobically for 24 h. The Sabouraud's gentamicin chloramphenicol agar plates were incubated at 28°C aerobically for 48 h. All colonies with characteristic metallic sheen were selected and subjected to biochemical tests using standard methods [20]. In all, 2-5 colonies from each plate were selected for identification. The disc diffusion method [21] was used to determine the antibiogram of *E. coli* isolates. The following antimicrobial agents and concentrations were used: ofloxacin (30µg), gentamicin (10µg), augmentin (30µg), tetracycline (30µg), amoxicillin (10µg), cotrimoxazole (25µg), nitrofurantoin (25µg), nalidixic acid (30µg) and ciprofloxacin (30µg). The interpretation of sizes of zones of inhibition followed the recommendation of the disk manufacturer (Difco, Detroit, Michigan, U.S.A.).

Parasitological Investigation: Faecal samples were examined parasitologically using the flotation technique as described by Boch and Supperer [22].

RESULTS

The cultures of the faecal samples collected from the enclosure of the tortoises yielded heavy growth of *E. coli* based on the cultural and biochemical tests. The parasitological results indicated that the tortoises were free from helminthes. *E. coli* isolates were susceptible to gentamicin 10µg , ofloxacin 30µg and resistant to augmentin, tetracycline, amoxycillin, cotrimoxazole, nitrofurantoin, nalidixic acid and ciprofloxacin.

DISCUSSION

In this report it was observed that the tortoises were weak, dull, anorexic, dehydrated with profuse, watery and yellowish feces with progressive depression which agrees with [23] who reported that enterotoxigenic *E. coli* caused secretory diarrhoea in domestic animals because of the elaboration of enterotoxins after colonization of the intestinal mucosa. The faecal samples of the tortoises were found to be positive for *E. coli*, this agrees with Adesiyun and Downes [24] who reported that faecal, rectal and cloacal cultures from wild mammals and birds from Trinidad and Tobago were often positive for *E. coli* but were consistently negative for the O157 antigen associated with human disease.

All the tortoises were removed from their enclosure and kept in another enclosure. The food remnants and feces were packed. The floor of the enclosure was washed three times with blue detergent and Izal. This place was allowed to dry and the tortoises were treated with gentamicin intramuscularly for five days.

The observation in this report showed that *E. coli* may be transferred from human visitors, flies, soil manures, feed supplies, domestic and other zoo animal strains themselves. Therefore screening of the human visitors, domestic and other captive wild species should be instituted to minimize transfer by contact. This will serve to prevent avoidable huge financial losses due to *E. coli* infection in rare captive species such as the tortoises.

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