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Serological survey of salmonellosis in grey duiker (*Sylvicapra grimmia*) in Asejire, Irewole Local Government Area, Osun State, Nigeria

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Summary

A serological survey of salmonellosis in grey duiker was carried out in Asejire, Irewole Local Government Area (LGA), Osun State, between August and September, 1996. The sera of 50 animals were screened for antibodies to salmonella flagella (H) and/or somatic (O) antigens. Ten "bushmeat" hawkers/processors were interviewed on their state of health. Twenty-two (44%) and 3 (6%) had antibodies to the H and O antigens, respectively. Nine (18%) had antibodies to both the H and O antigens. Antibody titres of 1/320 were detected against *Salmonella paratyphi* serotypes B (12%) and C (2%) and *S. typhi* serotype D (8%). Four (40%) of the interviewed hawkers/processors had had "fever" in the recent past, and 80% of them usually take traditional herbal medicines for most fever-related ailments. A human typhoid epidemiological surveillance carried out within the LGA between 15th March and 4th April, 1995 revealed 64 cases of tentatively diagnosed salmonellosis. Eleven (17.2%) cases were conclusively diagnosed as typhoid fever by laboratory examination. High antibody titres against H and O antigens of *S. paratyphi* serotype B and *S. typhi* serotype were detected. Two (3.1%) of the patients died. The public health importance of these findings is discussed.

Keywords: Grey duiker, salmonellosis, zoonosis, public health, food poisoning.

Résumé

Une étude sérologique de salmonellose dans un "duiker" gris avait été faite à Asejire, à Irewole Gouvernement Local (LGA) dans l'état d'Osun, d'AOUE à Septembre 1996; les sérums de 50 animaux avaient été examinés pour les anticorps de salmonella flagella (H) et pour des antigènes somatiques (O). Dix (10) marchand ambulants ou fumeurs de viande de brousse avaient été interrogés sur leur état de santé. Vingt deux (44%) et trois (6%) avaient des anticorps de H et O antigènes respectivement. Neuf (9) soit 18% avaient des anticorps de 1/320 avaient été détectés contre les sérotypes B(12%) et C(2%) de *salmonella paratyphi* et serotype D (8%) de *s. typhi*. Quatre (40%) des marchands ambulant ou chasseur interrogés ont eu la fièvre dans un passé récent et 80% entre eux prennent des médicaments traditionnels pour la majorité de maladie qui ont pour manifestation la fièvre.

Une étude épidémiologique de typhoïde humaine a été faite dans LGA entre du 15 mars au 4 Avril 1995 montraient 64 cas de diagnostics avec tentative de salmonellose onze (17.20%)

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de les cas avaient été diagnostiqués et confirmés comme fièvre typhoïde par des analyses laboratoires. Un titre élevé d'anticorps contre H et O antigènes de serotype B de *S. paratyphi* et des sérotypes de *S. typhi* avaient été détectés. Deux (3.1%) des patients sont morts. L'importance de ces résultats dans le domaine de la santé publique a été discutée.

Introduction

Salmonellae are enterobacteriae which are gram-negative, fermentative, facultative, anaerobic, oxidase-negative that generally are motile with peritricous flagella, non-spore and non-capsulate [1]. There are many serotypes of the very many species of the organism, but the most common ones are the A, B, C, and D whose identification depends on the detection of the O and H antigens by means of agglutination tests with specific antisera [2]. The O antigens are side chains of repeating sugar units projecting from the outer polysaccharide layer of the bacterial cell wall. These antigens are heat-stable, being unaffected by heating at 100°C for 2½ hours, and alcohol-stable withstanding treatment with 96% ethanol at 37°C for 4 hours. The H antigens, on the other hand, are determinant groups on the flagellar protein, heat- and alcohol-labile [1].

Salmonellosis, one of the most serious zoonotic diseases, is caused by some 2,000 or more serotypes of *Salmonella*, including members of the *arizona* group [3]. All known types are pathogenic, or potentially so, for humans or animals or both. However, only about a dozen serotypes account for more than 75% of all isolates from man and animals [4]. These coliforms are among the foremost causes of food- and water-borne enteric infections in the world [5].

Salmonellae are versatile in antigenicity and disease manifestations, and may cause typhoid fever, enteric fevers, which basically resemble typhoid fever but are milder; "salmonella food poisoning", a form of acute gastroenteritis; gram-negative septicaemia and localized abscesses; inflammatory foci in almost any body organ, and a chronic carrier state in man [6]. In animals, however, salmonellosis is regarded as many diseases by reason of the variety of animal species that are susceptible, the variety of *Salmonella* species that are pathogenic and the poorly defined variety of circumstances in which host and pathogen interact to produce the disease [3, 7]. Adult animals are less likely to suffer generalized or septicaemic infections than are the young. They are also likely to cast it off or become symptomless carriers for an indefinite period [8]. In addition to causing septicaemia, leucopenia, fibrino-necrotic and ulcerative enterocolitis and proctitis, salmonella organisms may cause abortion and

reproductive wastage in animals, both domestic and wild [3].

Clinical survey on the epizootics of wild animals in tropical Africa has gone a long way in recent times. The works carried out on wildlife diseases were initiated because of the need to reduce frequent decimation by poaching, diseases and parasites as well as provide information which will enhance ecotourism and bioconservation of wildlife resources [9]. Many of the highly priced species such as elephants, chimpanzee, hippopotamus, leopard and water chevrotin are currently facing the threat of extinction in Nigeria, Benin Republic, Cameroon, Ghana, Ivory Coast and Liberia [10]. Also, over 80% of the population in Nigeria are rural dwellers who depend on wild animal meat, commonly called "bushmeat" as their main source of animal protein. Of these, the grasscutter and grey duiker are the most abundant and most highly patronized by hawkers as well as buyers [11]. Many elite urban dwellers also patronize "bushmeat" centres, usually located at strategic "bank on hand" bushmeat sales point along most major highways in Nigeria.

Most of these hunted wildlife species are reservoirs of many zoonotic diseases [9] which can be transmitted through handling and/or consumption by both hunters, hawkers/processors and buyers [7]. The blood, bile, vomitus, urine and faecal materials from these animals, if not handled and/or processed properly may serve as sources of infection [1]. Salmonella food poisoning is very rampant in our environment, and has led to many deaths especially, within the past two decades, and this problem is more compounded by the appearance of drug-resistant strains of the organisms [8] and the always exorbitant prices of the newer and more efficacious drugs.

The objectives of this study are to determine the prevalence and public health significance of salmonellosis in the grey duiker and its possible role in food poisoning in man.

Materials and methods

The study area

Irewole Local Government Area (LGA) is one of the 23 LGAs in Osun State with a projected population of 238,879 people (1973 National Census). It is made up of 10 wards of which Asejire is one. By its location, Asejire is the gateway to Osun State, being a border village between Oyo and Osun States. Asejire was a hamlet of about 1000 inhabitants in 1970 when the construction of the Asejire Water Dam on the Osun River was started. This brought the migration of workers from urban areas (notably from Ibadan) to the area. The location of Coca-Cola Factory and a cassava and starch processing industry in the 1980s and 1990, respectively, led to the influx of people into the area. Presently the village has a projected population of 8142 people (1993 National Census).

Collection of blood samples

Grey duiker (*Silvicapra grimmia*), a wild small ruminant of the antelope family, is widely distributed throughout the savanna and the rainforest zones of Nigeria (11). In the daytime, duikers lie in shady thickets, often ruminating, while they move out at night grazing on succulent foliage and grasses, and drink water at brooks, ponds or sides or large flowing streams. The individuals usually live singly, but in fairly close proximity to one or two other members of the species. Because of their nocturnal habit, these animals are vulnerable to the hunter's scotching and blinding calcium carbide-powered lamps and shots

from local dane guns. Most killings or maiming usually occur in the early hours of the day, such that the animals are brought to the hawking/processing spots quite fresh and during the pre-rigor state. The consent of the hawker or buyer was sought and approval obtained before bleeding. Blood samples were collected between 07.00 and 08.00 hours. The survey took place between August and September 1996.

Ten millilitres (ml) of blood was collected from each animal by cardiac puncture into sterile plain tubes and allowed to stand slanting in a cotton wool-padded flask and immediately transported to the laboratory for analysis. Serum was separated from each sample by centrifugation (Gallenkamp, UK) at 2,000rpm for 10 minutes and transferred into sterile storage tubes. The sera were either analyzed immediately or kept at -25°C for use within not more than 72 hours of collection.

Serological analysis

The commercial Widal Cromatest reagents kit (Linear Chemicals, Badalona, Spain) was used for the serological analysis of the sera for the presence of antibodies to the H and O antigens of four serotypes – A, B and C of *Salmonella paratyphi* and serotype D of *S. typhi*. The tube agglutination test [12] was used. Duplicate serial dilutions of each serum sample at 1:20, 1:40, 1:80, 1:160 and 1:320 in 1.0ml 0.85% saline solution were mixed with 2 drops each of the suspensions of positive and negative control reagents in the kit. The contents in the tubes were mixed thoroughly and incubated according to manufacturer's specification (50°C for 4 hours for the O antigens and 50°C for 2 hours for the H antigens). Readings were carried out macroscopically by observation of large floccular agglutinins for H antigens and grandar or small flaky aggregates for the O antigens, noting and recording the end-point titres.

Human typhoid epidemiological surveillance

Based on a report of an increased incidence of typhoid fever from the Disease Surveillance Officer for Irewole LGA, the Epidemiological Preparedness Team of the Osun State Ministry of Health visited the LGA. There had been a report of acute potable water shortage within the LGA and environs for some weeks prior to this report. A total of 23 medical clinics, including 7 private and 16 public health institutions were visited. Case reports of in- and outpatients were examined. Specifically, patients with enteric and fever-like syndromes were noted and further investigated for laboratory examinations, treatments and mortalities, if any. Where laboratory examinations were carried out, such laboratories were visited and recorded diagnoses obtained. A total of 3 such laboratories within the LGA were visited.

Results

The results of the serological survey of grey duiker are as shown on Table 1. Titres of 1:20 and 1:40 were regarded as negative, 1:80 optimum, 1:160 low positive, while 1:320 was high positive. Of the 50 sera samples screened, 25 (50%) consisting of 11(42%) male and 14 (58%) females, were positive, with titres of 1:160 and above, for either or both of serotypes A, B, C, or D. Of these, 22 (44%) were positive for H antigens only, 3 (6%) were positive for O antigens only and 9 (18%) were positive for both H and O antigens. The highest titre of 1:320 was recorded for 6 and 1 H antigens of serotypes B and C,

respectively of *S. paratyphi* and 4 for H antigen of serotype D of *S. typhi*.

Table 1: Serological profiles of grey duiker for the "H" and/or "O" antigens of *Salmonella*

S/No	Sex	Flagella (H) antigen				Somatic (O) antigen D			
		A [®]	B [®]	C [®]	D [#]	A	B	C	D
1	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
2	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
3	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
4	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
5	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
6**	M	1/20	1/320	1/20	1/20	1/20	1/160	1/20	1/160
7*	F	1/20	1/20	1/20	1/20	1/20	1/160	1/20	1/20
8**	F	1/20	1/320	1/20	1/20	1/20	1/160	1/20	1/160
9	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
10	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
11	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
12*	F	1/20	1/160	1/20	1/20	1/20	1/20	1/20	1/20
13*	M	1/20	1/320	1/20	1/160	1/20	1/20	1/20	1/20
14*	M	1/20	1/160	1/20	1/160	1/20	1/20	1/20	1/20
15*	F	1/20	1/160	1/20	1/20	1/20	1/20	1/20	1/20
16*	F	1/20	1/20	1/160	1/20	1/20	1/20	1/20	1/20
17*	F	1/20	1/160	1/20	1/20	1/20	1/20	1/20	1/20
18	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
19**	F	1/160	1/20	1/20	1/320	1/20	1/160	1/20	1/20
20**	F	1/20	1/160	1/20	1/320	1/20	1/160	1/20	1/20
21	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
22	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
23	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
24	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
25**	F	1/20	1/320	1/20	1/20	1/20	1/160	1/20	1/20
26	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
27	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
28	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
29	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
30**	F	1/20	1/320	1/20	1/160	1/20	1/20	1/20	1/160
31**	M	1/20	1/20	1/20	1/320	1/20	1/20	1/20	1/160
32*	M	1/20	1/320	1/320	1/20	1/20	1/20	1/20	1/20
33*	F	1/20	1/20	1/160	1/20	1/20	1/20	1/20	1/20
34	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
35*	F	1/20	1/20	1/20	1/20	1/160	1/20	1/20	1/20
36	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
37	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
38*	M	1/20	1/20	1/20	1/160	1/20	1/20	1/20	1/20
39*	M	1/20	1/320	1/20	1/160	1/20	1/20	1/20	1/20
40	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
41	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
42*	F	1/20	1/20	1/20	1/160	1/20	1/20	1/20	1/20
43	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
44	F	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
45	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
46*	M	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/160
47*	F	1/20	1/160	1/20	1/20	1/20	1/20	1/20	1/20
48**	F	1/20	1/160	1/20	1/20	1/20	1/20	1/20	1/160
49*	M	1/20	1/20	1/20	1/320	1/20	1/20	1/20	1/20
50**	M	1/20	1/160	1/20	1/160	1/20	1/160	1/20	1/20

® - *Salmonella paratyphi*; # - *Salmonella typhi*;

* - Positive for either H or O antigen;

** - Positive for both H or O antigens

Table 2 shows that serotype B of *S. paratyphi* and serotype D of *S. typhi* were more commonly encountered in salmonella-infected grey duiker as 20 (40%) of the animals were positive for serotype B of *S. paratyphi*, while 17 (34%) were positive for serotype D of *S. typhi*. Serotypes A and C of *S. paratyphi* were found in 2 (4%) and 3 (6%) animals, respectively.

Table 2: Summary of grey duiker positive for the various serotypes of *Salmonellae*

Serotypes	A [®]	B [®]	C [®]	D [#]
<u>H antigen</u>				
1/160	1	7	2	7
1/320	-	6	1	4
<u>O antigen</u>				
1/160	1	7	-	6
1/320	-	-	-	-
Total	2	20	3	17

® - *Salmonella paratyphi*; # - *Salmonella typhi*;

1/160 - Low titre positive

1/320 - High titre positive

Of the 10 "bushmeat" hawkers/processors interviewed for malaria-like or fever-related symptoms or illnesses presently or in the past few weeks, 4 (40%) said they have had 'fever' within the past two weeks. The remaining 60% said they had such symptoms some months ago but are presently doing fine. However, some of the hawkers interviewed volunteered information on two other hawkers who were still sick and at home for fever-like illnesses during our survey. Eight (80%) of the hawker interviewed would prefer taking traditional herbal medicines for curing fever-related ailments.

The results of the epidemiological surveillance of human typhoid cases reported in Irewole LGA of Osun State, Nigeria between 15th March and 14th April, 1995, revealed that 64 cases were tentatively diagnosed as having salmonellosis (Epidemic Preparedness Team, Osun State Ministry of Health). Of these, 43 and 21 cases were diagnosed at the seven private and twenty-one public medical clinics, respectively, located within the LGA. Eleven (17.2%) cases were conclusively diagnosed as typhoid fever using both bacteriological and haematological screening techniques in three of the laboratories visited. All the 11 (100%) had a titre of 1/160 to the H and O antigens of *S. paratyphi* serotype B, while 3 (27.3%) had antibody titres of 1/320 and 1/160 to the H and O antigens, respectively, of *S. typhi* serotype D. Titres of 1/20 (negative) were recorded for serotypes A and C of *S. paratyphi*. A mortality of 3.1% (2 out of 64) was recorded.

Discussion

The results of this survey have shown that grey duiker may be very important in the epidemiology of salmonellosis in domestic animals and man in the area under study. This resembles the sylvatic cycle of disease transmission observed in rabies, another important zoonosis [13]. The presence of agglutinating

antibodies to various combinations of serotypes A, B and C of *S. paratyphi* and serotype D of *S. typhi* in 50% of the population of grey duiker examined, and especially a very high titre of 1/320 in of 25 (44%) positive sera shows that these animals were carrying the infection [14].

This trend could have far-reaching and significant implications as antelopes are frequently in contact with domestic livestock such as cattle, especially during transhumance. Also, the grey duiker has a feeding range of up to 50km² [11] during which it makes formal and informal contacts with man and his domestic stock like sheep and goats, by the contamination with faeces and urine of farm vegetation and/or crops on which they act as pests. Another seemingly dangerous dimension is the possibility of direct transmission of salmonellosis to man through the handling and consumption of improperly processed killed animals by hunters, hawkers/processors of "bushmeat" as well as the poor rural and elite urban dwellers who depend on this animal as prime source of animal protein and delicacy, respectively. Even though no reliable population figure is available on grey duiker in this area, the abundance and the rate at which the animals are being hunted and sold at "bushmeat" processing centres within and around southwestern Nigeria suggests a reasonably high population [11].

The dualization of the Ife-Ibadan Road coupled with the presence of Asejire Water Dam and the opening up of the massive forest reserves for timber thinning and game poaching activities in this area have made Asejire a commercially important "bushmeat" and fish supply gateway to Ondo, Osun, Oyo, Ogun and Lagos States. It is very common to observe both private and commercial vehicle drivers and passengers make stop-overs and purchase either or smoked fish and "bushmeat" while in transit.

Our observation at the Asejire "bushmeat" processing centre revealed a very high demand for grey duiker, either fresh or smoked. However, very little attention was paid to proper hygienic processing and smoking. In fact, it is not uncommon for the processors to consume part of the undone meat often without washing of hands. The interviews held with some of the hawkers and processors revealed a high incidence of fever-like syndromes, ill-health and loss of man-hours amongst the traders. The fact that most of them would patronize herbal medical practitioners and not the conventional western modes of typhoid therapy may make rational treatment impossible and a high prevalence of healthy carriers of the causative organisms [8]. The mode of preparation of meat (that is, smoking, boiling and/or frying) in our local culture notwithstanding, it is known that some strains of *Salmonella* may withstand very high temperatures, 100°C for 2½ hours, due to the presence of the H antigens [1]. In contrast, some elite urban dwellers may adopt the "Western" barbeque style of "bushmeat" preparation in which the meat will be half-cooked, and hence are exposed to the organisms in blood, tissue and other body fluids of infected animals.

In conclusion, there appears to be no social stratification to the menace of salmonellosis. Very high antibody titres (1:320) to the H antigens of serotype B of *S. paratyphi* and serotype D of *S. typhi* were detected in 13 and 11 grey duiker, respectively in this study. These same serotypes were confirmed in 11 (17.2%) of conclusively laboratory-diagnosed typhoid fever in Irewole LGA and with a record of two deaths from the disease within the one-month survey. The transmission

of salmonella food poisoning and other ailments through the consumption of improperly processed grey duiker and other types of "bushmeat" should not be overlooked. The public health importance and hence, the implication of these findings is such that health education, especially to hunters, hawkers and processors and consumers of "bushmeat", in whatever form, is a must. This will help in no small measure to control the menace of the carriers of salmonellosis in our environment, and to state that infections are not only through drinking water, milk, pork and eggs [5].

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