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Spectrum of Bacterial Isolates from Captive Non-Human Primates in Jos Wild Life Park

*[1]Okwori, A.E.J., [1]Ofili A. I., [1]Uzoechina. A.R., [2]Nwankiti., O., [2]Chollom, S.C., [1]Echeonwu, G.O.N., [3]Adikwu T.I

[1]Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology, [2]National Veterinary Research Institute, Vom – Jos, [3]Royal Infirmary Hospital, Edinburgh, UK.

Abstract: *Primates refer to a zoological order of placental mammals, consisting of lemurs, bushbabies, tarsiers, marmosets, monkeys, apes, and humans. In an effort to evaluate the occurrence of bacterial organisms with zoonotic and biohazard potential in captive non-human primates (NHP) in the park, we performed a prevalence study examining 18 aparently healthy young adult monkeys and apes. We sampled them by faecal culture on three separate occasions using appropriate media and specific selective culture methods. The survey lasted for a period of three months with sampling at 2 weeks intervals. Enteric organisms potentially transmissible to humans were subcultured and identified based on their morphological and biochemical characteristics. Prevalence results at start and finish points obtained revealed six (6) bacterial organisms as follows: Escherichia coli (100.0%), Salmonella paratyphi A (72.8.0-77.8%), Proteus mirabilis (61.1-83.3%), Campylobacter species (5.6%), Citrobacter ferundii 13(16.7-33.3%), and Yersinia enterocolitica (22.4%). The presence of these pathogenic bacteria has public health significance because of the nature and use of their location (being a park) and its proximity to human dwellings. We recommend proper hygienic husbandry and vigilant sampling of NHP populations anywhere they are kept; also the appropriate use of antibiotics to eliminate shedding of bacteria and spread of the disease should be undertaken.*

Key Words: *Non-human primates, zoonotic bacteria, Jos- Nigeria.*

I. Introduction

A host of bacteria are commonly seen in apparently healthy non-human primates (NHPs). Some include *Mycobacterium tuberculosis*, *Shigella* spp, *Salmonella* spp, *Escherichia coli, Campylobacter* spp and *Klebseilla* spp (Nizeyi *et al.,* 2001); *Helicobacter* spp has also been recently reported in monkeys (Bronsdon and Schoenknecht, 1988; Fox *et al*., 2001).

Some of these pathogens endemic in NHP populations remain clinically inapparent in immunocompetent animals and are in general mild and self limiting; as such they do not adversely affect their lives. The exceptions are those infections associated with the gastrointestinal tract that result in chronic enterocolitis, a condition that, albeit of low incidence, is a persistent and wide spread colony problem in NHPs and is most likely multifactorial in origin (Sestak *et al*., 2003). In addition, immunosuppression, introduced either naturally (e.g., by immunosuppressive retroviruses) or experimentally (e.g., by irradiation, immunomodulatory agents, or chemotherapeutics), can make a previously unexposed animal or group more susceptible to primary outbreaks or induce recrudescence of these pathogens. Bacterial infections commonly associated with immunosuppression include *Mycobacterium avium complex, Rhodococcus equi,* and enteropathogenic *Escherechia coli* (EPEC) (Mansfield *et al*., 2001).

Almost all primates harbour *E. coli, Shigella spp* and *Salmonella spp* in the alimentary tract. Fortunately the most serious human pathogens of these two groups *Shigella dysenterriae* type I and *Salmonella typhi,* (Srivastava *et al.,* 1980) have only rarely been isolated from NHPs. However, several other species including *Shigella flexneri, S. Sonnii, Salmonella typhimirium* and *S. indiana* which are also infectious to man, have been recovered from monkeys (Basu *et al*., 1975; Boro *et al.,* 1980).

The primate carrying any of these organisms can have a fulminating fatal infection at anytime, with excretion of large numbers of organisms during the course of the disease or as silent shedders, which can then be transmitted to humans (Mohan *et al.,* 1973). The presence of these pathogenic bacteria has public health significance because of proximity of NHPs to human dwellings (Mohan *et al.,* 1973) and cross species transmission of infection (Lerche *et al*., 2001; Engel *et al*., 2002; Wolfe *et al*., 2004; Engel *et al*., 2002., 2006) though this is dependent on several factors: the prevalence of infectious agents in primate reservoirs, the context of interspecies contact and the frequency with which contact occurs (Engel *et al*., 2006). By virtue of their genetic, physiologic and behavioural similarity to humans, NHPs are particularly likely sources of emerging infectious agents with the capacity to infect humans, and primate-to-human cross-species transmission of infectious agents has become a focus of scientific enquiry. Moreover, despite significant advances in the

diagnosis of infectious diseases, unrecognized or adventitious agents are common in NHPs and have the potential to confound experimental work (Barley and Mansfield, 2010).
Recent studies in India and Africa have revealed a large presence of enteric pathogens from non-human primates including major pathogens like *Salmonella* spp, *Shigella* spp and *Campylobacter jejuni*. These reports has necessitated the need for us to examine such animals in close contact with humans in our environment for the presence of these zoonotic pathogens.

II. Methodology

STUDY AREA

This study was conducted at Jos zoo situated in Jos metropolis, Plateau State, Nigeria for the determination of enteric bacterial microflora in apparently healthy, captive non-human primates.

STUDY POPULATION: NON-HUMAN PRIMATES (NHPs)

The study population cut across 18 non-human primates (monkeys and apes) held in captivity. The majority of animals studied in the 90 days period were initially received by the zoo as donations from private individuals. Most other primates were obtained from the wild from various parts of the country. The species present were: *Cercopithecus mona* (1), *Chlorocebus tantalus* (1), *Erythrocebus patas* (5), *Papio Anubis* (8) *and Pantroglodytes* (3).

SAMPLES, SAMPLING AND PROCESSING

Samples were fresh excreta from the animals. They were taken in duplicates by deeply inserting and rotating a sterile cotton swab 15 cm long into freshly passed faeces. They were then transported with minimal delay to the laboratory. Samples were analyzed at the Bacteriology laboratory of Federal College of Veterinary and Medical Laboratory Technology, N.V.R.I, Vom.

BACTERIOLOGICAL PROCEDURES

The procedures for the culture and isolation of *Campylobacter* species were similar to the methods of Bolton *et al.* (1988), while those for *Yersinia* species and other enteric bacteria were a modification of the methods of FDA/CFSAN (2001) and Okwori *et al* (2005) respectively.
Swabs were used to directly inoculate Improved Preston blood-free Agar plates and MacConkey Agar (MCA) plates, and were then placed in Selenite F broth. After 24 hours of incubation, Selenite F broth cultures were streaked onto Deoxycholate Citrate Agar (DCA) plates.

About 1gram of faecal sample was aseptically inoculated into 10ml of phosphate buffered saline (PBS) pH 7.2, homogenized for about 30 seconds and incubated at 4ºC for 21 days (FDA/CFSAN, 2001). The inoculated PBS was subcultured onto solid culture plates of BA and MCA, and incubated at room temperature (24ºC) for 24 and 48 hours respectively.

Plates of Improved Preston blood-free medium were examined for Campylobacter organisms after 48 hours microaerophilic incubation at 37ºC; grey, moist, flat-spreading colonies; creamy-grey, moist slightly raised greenish colonies were selected. Preferences were given to isolates that gave the following results:
(i) Gram reaction - Negative 'S' shaped bacilli
(ii) Oxidase - Positive
(iii) Catalase - Positive
(iii) Motility - Positive (actively darting)

Culture plates of BA and MCA incubated at 24ºC for 24 and 48 hours respectively were examined for *Yersinia* species. On BA, smooth, translucent, non-haemolytic colonies measuring 2-3 mm in diameter were selected. On MCA pinpointed non-lactose fermenting colonies were selected. The selected colonies were further inoculated into Christensen's urea agar by stabbing with a straight wire and incubated at room temperature for 18 hours.

Plates of MCA and DCA incubated at 37ºC were purified and examined for enterobacteriaceae. Pure isolates were inoculated into TSIA slants and peptone water broth and further examined for hydrolysis of urea, utilization of citrate and indole production, motility and deamination of phenylalanine. All results obtained were compared against a standard biochemical chart for Enterobacteriaceae.

III. Results

All 18(100%) of the NHP sampled were positive for suspected bacterial infection. This figure remained constant throughout the entire survey period. We isolated the following bacteria with the prevalences at the start and end of our investigations as follows: *Escherichia coli* (100.0%), *Salmonella paratyphi* A (72.8.0– 77.8%), *Proteus mirabilis* (61.1-83.3%), *Campylobacter* species (5.6%), *Citrobacter ferundii* 13(16.7-33.3%), and

Yersinia species (22.4%) through out the course of our investigations. Details are as presented in tables 1 to 3.

Table 1: Isolates from sampled NHPs on the first sampling (first week)

Primate species	Nos of primates screened	Nos of NHPs infected with particular isolates (%)					
		Escherichia coli	*Salmonella paratyphi*	*Proteus mirabilis*	*Campylobacter* species	*Citrobacter ferundii*	*Yersinia* enterocolitica
Cercopithecus mona	1	1(100.0)	1(100.0)	1(100.0)	0(00.0)	1(100.0)	0(00.0)
Chlorocebus tantalus	1	1(100.0)	1(100.0)	1(100.0)	0(00.0)	0(00.0)	0(00.0)
Erythrocebus patas	5	5(100.0)	4(80.00)	5(100.0)	1(20.00)	1(20.0)	3(60.0)
Papio Anubis	8	8(100.0)	6(75.0)	1(12.5)	0(00.0)	0(00.0)	0(00.0)
Pantroglodytes	3	3(100.0)	1(33.2)	3(100.0)	0(00.0)	1(33.3)	1(33.3)
Total	**18**	**18(100.0)**	**13(72.2)**	**11(61.1)**	**1(5.6)**	**3(16.7)**	**4(22.4)**

Table 2: Isolates from sampled NHPs on the second sampling (sixth week)

Primate species	Nos of primates screened	Nos of NHPs infected with particular isolates (%)					
		Escherichia coli	*Salmonella paratyphi*	*Proteus mirabilis*	*Campylobacter* species	*Citrobacter ferundii*	*Yersinia* enterocolitica
Cercopithecus mona	1	1(100.0)	1(100.0)	1(100.0)	0(00.0)	1(100.0)	0(00.0)
Chlorocebus tantalus	1	1(100.0)	1(100.0)	1(100.0)	0(00.0)	0(00.0)	0(00.0)
Erythrocebus patas	5	5(100.0)	4(80.00)	5(100.0)	1(20.00)	1(20.0)	3(60.0)
Papio Anubis	8	8(100.0)	6(75.0)	5(62.5)	0(00.0)	0(00.0)	0(00.0)
Pantroglodytes	3	3(100.0)	2(66.7)	3(100.0)	0(00.0)	1(33.3)	1(33.3)
Total	**18**	**18(100.0)**	**14(77.8)**	**15(83.3)**	**1(5.6)**	**3(16.7)**	**4(22.4)**

Table 3: Isolates from sampled NHPs on the third sampling (twelft week)

Primate species	Nos of primates screened	Nos of NHPs infected with particular isolates (%)					
		Escherichia coli	*Salmonella paratyphi*	*Proteus mirabilis*	*Campylobacter* species	*Citrobacter ferundii*	*Yersinia* enterocolitica
Cercopithecus mona	1	1(100.0)	1(100.0)	1(100.0)	0(00.0)	1(100.0)	0(00.0)
Chlorocebus tantalus	1	1(100.0)	1(100.0)	1(100.0)	0(00.0)	0(00.0)	0(00.0)
Erythrocebus patas	5	5(100.0)	4(80.00)	5(100.0)	1(20.00)	1(20.0)	3(60.0)
Papio Anubis	8	8(100.0)	6(75.0)	5(62.5)	0(00.0)	3(37.5)	0(00.0)
Pantroglodytes	3	3(100.0)	2(66.7)	3(100.0)	0(00.0)	1(33.3)	1(33.3)
Total	**18**	**18(100.0)**	**14(77.8)**	**15(83.3)**	**1(5.6)**	**6(33.3)**	**4(22.4)**

IV. Discussion

A high number of isolates was recorded in this survey. Isolation of *E. coli* (100%) at a constant rate throughout our study (especially if finally identified asbeing of human pathogenic serogroups) is an indicator of potential hazardous infections of sorrounding human communities. Enteropathogenic *E. coli* infection has been observed in approximately 20% of normal healthy neonatal and adult monkeys (rhesus macaques) (Mansfield *et al*., 2001; Sestak *et al*., 2003; Wachtman and Mansfield, 2008). Toxin producing *Escherichia coli* such as shiga toxigenic *Escherechia coli* (STEC) can cause a wide spectrum of ailments from mild diarrhoea to severe disease, in animals (Hall *et al.*, 1985).

Prevalence of *Salmonella paratyphi* A showed mild increase by the end of or study, like wise *Proteus mirabilis*. Their increasing incidence during the period of study displays the continuing transmission amongst captive NHPs. Rates of occurrence of *Campylobacter* species, *Citrobacter ferundii, Yersinia enterocolitica* remained the same. It may imply that the mecahnisms which supported the thriving of *S. paratyphi* and *P. mirabilis* combined with their modes of spread were not condusive or supportive for these bacteria. Isolation of *Salmonella* species from NHPs and their further transmission to other primates has been reported by Mohan *et al*., (1973), Nizeyi *et al*., (2001) and Robert *et al*., (1969). They asserted that this could be due to its ability to survive in cool moist conditions for weeks outside the living body. Likewise they have been found in dried excreta for over 2.5 years. Its occurrence in these NHPs may thus indicate an enhanced anthropozoonotic transmission of these organisms.

We noted that the cage floors of these primates were quite unhygienic, the animals constantly littered them with faeces and urine, and these floors were not routinely cleaned and sanitized. In addition, the quadrupedal movement of these animals on the ground enhanced their contact with pathogens. These conditions

no doubt provided pointers to some of the high prevalence values we recorded, being avenues for bacterial infection, re-infecton and dissemination of existing infections amongst previously unaffected animals.
Several reports have been presented on the isolation of *Yersinia* species from a variety of animals and human beings (Shepel *et al*., 2001., Skiriken, 2004., Okwori *et al*, 2008., 2009); descriptions of observed clinical manifestations or pathoanotomical changes are sparse (Hurvell, 1981; Schiemann, 1989).
Despite the successes in excluding select pathogens (Barry and Strelow, 2008; Morton and Capuono, 2008; Solnick *et al,* 1999) of NHP colonies, elimination of many other infectious diseases from NHP colonies has proven difficult for several reasons. The standard approach- based on cesearean derivation and isolation used to produce specific pathogen free population of many species of laboratory animals is difficult to reproduce in primates, given that parental input is critical in the normal social development of infants and juveniles. This approach may lead to future poor reproductive performance and behavioral issues (Harris *et al*, 2002; Suomi, 1997). Common primate housing and breeding strategies that are used to enhance socialization and welfare may promote transmission of many agents. The continued use of feral and free ranging source colonies introduces variability in exposure that is difficult to control. In addition, the long span and high value of many NHP species can restrict options available in the event of infection of animals.

The presence of these pathogenic bacteria has public health significance because of the proximity of these non-human primates to human dwellings (Mohan *et al.*, 1973).

V. Conclusion

In this study we have identified bacteria present in NHP populations in Jos wild life park. We therefore caution that many of them may resurface in human habitations in close proximity to these animal dwellings. These organisms are transmitted majorly by the faecal-oral route which occurs mostly when animal wastes are not properly disposed. It is possible to control active bacterial infections by vigilant sampling of NHP populations and the appropriate use of antibiotics to eliminate shedding of bacteria and spread of the disease (Black-Schultz *et al*., 1997; Wolfensohn, 1998).

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