Research Paper

PROFILE OF ENTERIC BACTERIA ASSOCIATED WITH CAPTIVE NON-HUMAN PRIMATES IN JOS ZOO, NIGERIA

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEJ designed the study, wrote the first draft of the manuscript and co-ordinated the research; Author NO,MD helped with sample collection and laboratory analysis; Author OO,AA performed statistical analysis; Author AD,AN,YB helped with literature search. All authors read and approved the final manuscript

ABSTRACT

Non-human primates (NHPs) are distributed worldwide and have several unique features that may account for opportunistic and pathogenic zoonotic bacteria.

Aim: To evaluate the incidence of enteric organisms with zoonotic and biohazard potential in captive NHPs in a zoo setting.

Study Design: Descriptive study.

Place and Duration of Study: *This study was conducted in Jos, Plateau State,* Nigeria between June -September, 2012

Methodology: We examined 33 clinically healthy young adult monkeys and apes over a three months interval. The animals were sampled at six weeks intervals by faecal culture. Samples were inoculated on appropriate media using specific selective culture methods. Suspect isolates potentially transmissible to humans were purified and identified based on their cultural and biochemical characteristics.

Results: The survey revealed six (6) bacterial pathogens using API 20E, *Escherichia coli* (100.0%), *Salmonella paratyphi* A 31(93.9%), *Proteus mirabilis* 14(42.4%), *Campylobacter* species 6(18.2%), *Citrobacter ferundii* 7(21.2%), and *Yersinia enterocolitica* 3(9.1%).

Conclusion: The incidence of infections during the period of study (first week and the twelfth week)indicated increased patterns of transmission between species of primates. Research among primate populations has the potential to predict which pathogens might enter human populations as human contact with these animals both in captivity and in the wild is on the increase.

Key words: Non-human primates, enteric bacterial spectrum, Jos, Nigeria.

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INTRODUCTION

Non human primates (NHPs) and wildlife are susceptible to a wide variety of bacterial agents and infectious disease from environmental contaminants in the air, soil, water and food. Bacterial infections commonly associated with immunosuppression include *Mycobacterium avium complex*, *Rhodococcus equi*, and enteropathogenic *Escherichia coli* (EPEC) [1].

A host of bacteria are commonly seen in apparently healthy NHPs. Some important bacterial species that deserve mention are *Mycobacterium tuberculosis*, *Shigella* species, *Salmonella* species, *Escherichia coli*, *Campylobacter* species and *Klebseilla* species [1], *Helicobacter* species has also been recently reported in monkeys [3, 4].

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*, [5] have only rarely been isolated from NHPs. However, several other species including *Shigella flexneri*, *Shigella sonnii*, *Salmonella typhimirium* and *S. indiana* which are also infectious to man, have been recovered from monkeys [6,7]. The presence of *E. coli* especially the human pathogenic serogroups are indicators of potential hazardous infections of human communities. Toxin producing *Escherichia coli* such as shiga toxigenic *Escherechia coli* (STEC) causes a wide spectrum of ailments from mild diarrhoea to severe disease, in man and animals [8].

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 [9]. The presence of these pathogenic bacteria has public health significance because of proximity of NHPs to human dwellings [10].
A growing literature suggests that cross-species transmission of infectious agents occurs between humans and several primate species in a variety of contexts and in diverse areas [11,12,13,14,15,16]. Most pathogens that affect humans are thought to have originated in animals and subsequently evolved to parasitize human populations [17]. By virtue of their genetic, physiologic and behavioural similarity to humans, NHPs are likely sources of emerging infectious agents with the capacity to infect other primates especially man.

Recent studies in India and the African continent has revealed a large presence of enteric pathogens from non-human primates to humans. These include major pathogens like *Salmonella* species, *Shigella* species and *Campylobacter jejuni*. This report has thus necessitated the need to examine our immediate environment for the presence of these zoonotic pathogens in these animals and their possible role as reservoirs/transmitters.
This study was aimed at identifying enteric bacteria isolated from captive conventionally raised non-human primates and the public health implications.

MATERIALS AND METHODS

Study Area:

This study was conducted at Jos zoo situated in Jos metropolis, Jos North Local Government Area, Plateau State, Nigeria for the determination of bacterial spectrum in apparently healthy, captive non-human primates.

Study Population: Non-human primates (NHPs):

The study population cut across 33 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 wild in various parts of the country. The species sampled were: *Cercopithecus mona* (Three), *Chlorocebus tantalus* (Ten), *Erythrocebus patas* (Eight), *Papio Anubis* (Seven) *and Pantroglodytes* (Five).

Samples, Sampling and Processing:

Samples were fresh excreta from the animals. Stool samples were collected at 3 different moments using sterile swab sticks on freshly passed faeces. (at the start of the

study - 6th week - 12th weeks). 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, National Veterinary Research Institute Vom.

Bacteriological Procedures:

Improved Preston blood-free Agar plates, MacConkey Agar (MCA) plates, Selenite F broth and PBS were directly inoculated with samples. After 24 hours of incubation, Selenite F broth cultures were streaked onto Deoxycholate Citrate Agar (DCA) plates. All results obtained were compared against a standard biochemical chart for *Enterobacteriaceae* (API 20E) [18, 19, 20].

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 [21]) and Okwori *et al* [19]. (Mycobacterium species were not searched for in this study).

RESULTS

All 33(100%) of the animals sampled were positive for *Escherichia coli.* This figure remained constant throughout the entire survey period. *Salmonella paratyphi* A was isolated from 29(87.9%) samples which later increased to 31(93.9%) by the end of the study. The prevalence of *Proteus mirabilis* showed 12(36.4%), 13(39.4%) and 14(42.4%) progressively. Prevalence of *Campylobacter* species showed that 6(18.2%) animals were infected. That of *Citrobacter ferundii* progressively increased from 1(3.0%) to 5(15.2%) and finally 7(21.2%) by the end of the study. The prevalence of *Yersinia enterocolitica* was 1(3.0%) which increased to 3(9.1%). The incidence of bacterial infections during the period of study (first week and the twelfth week) indicated increased patterns of transmission between primate species. These results are as presented in details in Tables 1 to 3.

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

Primate species	**Nos of primates examined**	**Nos of NHPs infected with particular isolates (%)**					
		Escherichia coli	***Salmonella paratyphi***	***Proteus mirabilis***	***Campylobacter* species**	***Citrobacter ferundii***	***Yersinia* enterocolitica**
Cercopithecus mona	3	3(100.00)	3(100.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Chlorocebus tantalus	10	10(100.00)	8(80.00)	2(20.00)	0(0.00)	0(0.00)	0(0.00)
Erythrocebus patas	8	8(100.00)	6(75.00)	3(37.50)	0(0.00)	1(12.50)	1(12.50)
Papio Anubis	7	7(100.00)	5(71.00)	3(42.90)	4(57.10)	0(0.00)	0(0.00)
Pantroglodytes	5	5(100.00)	5(100.00)	4(80.00)	2(40.00)	0(0.00)	0(0.00)
Total	**33**	**33(100.00)**	**29(87.90)**	**12(36.40)**	**6(18.20)**	**1(3.00)**	**1(3.00)**

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

Primate species	**Nos of primates examined**	**Nosof NHPs infected with particular isolates (%)**					
		Escherichia coli	***Salmonella paratyphi***	***Proteus mirabilis***	***Campylobacter* species**	***Citrobacter ferundii***	***Yersinia* enterocolitica**
Cercopithecus mona	3	3(100.00)	3(100.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)

Chlorocebus tantalus	10	10(100.00)	8(80.00)	2(20.00)	0(0.00)	4(40.00)	1(10.00)
Erythrocebus patas	8	8(100.00)	6(75.00)	3(37.50)	0(0.00)	1(12.50)	0(0.00)
Papio Anubis	7	7(100.00)	7(100.00)	4(57.10)	4(57.10)	0(0.00)	2(28.00)
Pantroglodytes	5	5(100.00)	5(100.00)	4(80.00)	2(40.00)	0(0.00)	0(0.00)
Total	**33**	**33(100.00)**	**31(93.90)**	**13(39.40)**	**6(18.20)**	**5(15.20)**	**3(9.10)**

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

Primate species	**Nos of primates examined**	**Nos of NHPs infected with particular isolates (%)**					
		Escherichia coli	***Salmonella paratyphi***	***Proteus mirabilis***	***Campylobacter* species**	***Citrobacter ferundii***	***Yersinia enterocolitica***
Cercopithecus mona	3	3(100.00)	3(100.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Chlorocebus tantalus	10	10(100.00)	8(80.00)	2(20.00)	0(0.00)	6(60.00)	1(10.00)
Erythrocebus patas	8	8(100.00)	6(75.00)	3(37.50)	0(0.00)	1(12.50)	0(0.00)
Papio Anubis	7	7(100.00)	7(100.00)	4(57.10)	4(57.10)	0(0.00)	2(28.00)
Pantroglodytes	5	5(100.00)	5(100.00)	5(80.00)	2(40.00)	0(0.00)	0(0.00)
Total	**33**	**33(100.00)**	**31(93.90)**	**14(42.40)**	**6(18.20)**	**7(21.20)**	**3(9.10)**

DISCUSSION

The isolation of *E. coli* in all apparently healthy subjects is quite interesting. With the high prevalence rate of this organism in these animals, there is no doubt that a majority of these organisms are normal residents of the gut microflora though some may be enteric pathogens. The presence of *E. coli* (especially if of the human pathogenic serogroups) are indicators of potential hazardous infections of sorrounding human communities. Enteropathogenic *Escherichia coli* (EPEC) infection has been observed in approximately 20% of normal healthy neonatal and adult monkeys (rhesus macaques) [1, 22, 23]. Toxin producing *Escherichia coli* such as shiga toxigenic *Escherechia coli* (STEC) has been reported to cause a wide spectrum of ailments from mild diarrhoea to severe disease, in animals [8].

Salmonella paratyphi A was the second most encountered organism in this research work. Isolation of *Salmonella* species has also been previously reported from NHPs [2, 10, 24]. The high occurrence of *Salmonella paratyphi* A in NHPs may be attributed to its ability to survive in cool moist conditions for weeks outside the living body, as they have also been reported to have been found in dried excrement after over 2.5 years. Its main mode of transmission is contaminated water, flies or infected dust; fruits and vegetables especially those grown beside river banks, which possibly may have been contaminated by human

waste, have also been implicated in its transmission and these constitute a vast majority of the animals' diet. Its occurrence in these NHPs may thus indicate an enhanced anthropozoonotic transmission of these organisms. The prevalence of *Salmonella paratyphi, Campylobacter* and *Yersinia,* organisms is similar to findings of [25] where these organisms were documented as being of a low prevalence in NHP.

The incidence of *Citrobacter ferundii* and *Proteus mirabilis* has although not yet been widely publicized. The increasing incidence of *Salmonella, Proteus, Citrobacter and Yersinia* infections during the period of study displays the continuing transmission and re-infection amongst captive NHPs. The source and mode of *Yersinia enterocolitica* infection in captive NHP is still unclear, although this may be attributed to the exhilarating feeding phenomenon displayed by most zoo visitors over the years with a host of materials not minding their sources and unsanitary conditions.

Campylobacter jejuni has previously been reported to account for a large percentage of diarrhoeal illness in colony raised monkeys [22, 26, 27]. Both the duration and clinical presentation being similar to those seen in humans [28, 29, 30]. Epidemiological studies have further revealed that infection and reinfection with multiple strains of *C. jejuni* are common and recovered monkeys are often asymptomatic [30]. Isolation of *Salmonella paratyphi*, and *Campylobacter* species are of particular interest. Studies in the African continent have also revealed presence of similar enteric pathogens from non-human primates, *Salmonella* spp and *Campylobacter jejuni* [26].

Chronic gastritis and enterocolitis, either asymptomatic or associated with chronic diarrhoea, are however reported to be relatively common in NHPs [31, 32, 33]. Due to the inadvertent unavailability of gastric biopsy specimens these organisms could not be sought for in this study. Although *H. pylori* is generally asymptomatic, both *H. pylori* and *H. heilmanni*-type bacteria have been associated with gastritis, peptic ulcers, gastric carcinomas, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas in humans [34].

Contamination of the environment by faecal matter is usually when animal wastes are not properly disposed. Direct human contamination may probably involve zoo visitors and zoo keepers. This preliminary investigation on enteric bacterial pathogens in non-human primates though yielding information, barely scratches the surface on possible zoonotic pathogens capable of emanating from non-human primates in close proximity to humans.

Shigella flexneri, Shigella sonnii, Salmonella typhimirium and *S. indiana* were not isolated in this study as documented by Basu[6] and Boro[7]. Some of the NHPs were not colonized with bacterial pathogens probably because they not susceptible to these spectrum of bacterial pathogens.

REFERENCES

1. Mansfield, K.G., Pauley, D., Young H.L. and Lackner, A.A. (1995). *Mycobacterium avium* complex in macaques with AIDS is associated with a specific strain of simian immunodeficiency virus and prolonged survival after primary infection. *J. Infect. Dis.* **172**: 1149 – 1152.
2. Nizeyi, J.B., Innocent, R.B., Erume, J., Kalema, G.R., Cranfield, M.R. and Graczyk, T.K. (2001). Campylobacteriosis, Salmonellosis, and Shigellosis in free ranging human habituated mountain gorillas of Uganda. *Journal of Wildlife Disease*. **37**: 239 – 244
3. Bronsdon, M.A. and Schoenknecht, F.D. (1988). *Campylobacter pylori* isolated from the stomach of the monkey, *Macaca nemestrina*. *Journal of Clinical Microbiology*. **26**(9): 1725-1728.

4. Fox, J.G., Handt, L., Xu, S., Shen, Z., Dewhirst, F.E., Paster, B.J., Dangler, C.A., Charles, A., Lodge, K., Motzel, S. and Klein, H. (2001). Novel *Helicobacter* species isolated from rhesus monkeys with chronic idiopathic colitis. *Journal of Medical Microbiology.* **50**(5): 421-429

5. Srivastava, L., Mathew, T., Suri, J.C., Edward, E.J. and Sood, P.L. (1980). Carriage of Salmonella species by laboratory animals. *Indian Journal of Pathological Microbiology*. 23: 69 – 71.

6. Basu, S., Suri, J.C., Mohan, K. and Madan, M. (1975). Salmonella Indiana (1,4,12:Z: 1,7): a serotype isolated for the first time in India. *Indian Journal of Medical Research*. 63: 170 – 1.

7. Boro, B.R., Sarma, G. and Sarmah, A.K. (1980). Bacteriological investigation of enteric infections in zoo animals. *Indian Journal of Animal Health*. 19: 39 – 41.

8. Hall, G.A., Reynolds, D.J., Chanter, N., Morgan, J.H., Parsons, K.R., Debney, T.G., Bland, A.P. and Bridger, J.C. (1985). Dysentery caused by *Escherichia coli* (S102 – 9) in calves: natural and experimental disease. *Veterinary Pathology*. 22: 156 – 163.

9. Mohan, K., Pal, S.C., Ghosh, J.N., Chakravarty, A.K., Pattanayak, S. and Arya, S.C. (1973). Enteric infections in captive monkeys. *Indian Journal of Medical Research*. 61: 363 – 8.

10. Marshall, J.C. (1991). The ecology and immunology of the gasterointestinal tract in health and critical illness. *J. Hosp. Infect.* 19 Suppl C: 7 – 17.

11. Lerche, N.W., Switzer, W.M., Yee, J.L., Shanmugam, V., Rosenthal, A.N., Chapman, L.E. (2001). Evidence of infection with simian type D retrovirus in persons occupationally exposed to nonhuman primates. *J. Virol*. 75: 17839.

12. Jones-Engel, L., Engel, G.A., Schillaci, M.A., Bobo, R. and Froehlich, J. (2001). Detection of antibodies to selected human pathogens among wild and pet macaques (*Macaca tonkeana*) in Sulawesi, Indonesia. *Am. J. Primatol*. 54: 1718.

13. Jones-Engel, L., Engel, G.A., Schillaci, M., Rompis, A., Putra, A. and Suaryana, K.G. (2005). Primate-to-human retroviral transmission in Asia. *Emerg. Infect. Dis.* 11: 102835.

14. Jones-Engel, L., Engel, G.A., Schillaci, M.A., Lee, B., Heidrich, J. and Chalise, M. (2006). Considering human to primate transmission of measles virus through the prism of risk analysis. *Am. J. Primatol*. In press.

15. Wolfe, N.D., Switzer, W.M., Carr, J.K., Bhullar, V.B., Shanmugam, V. and Tamoufe, U. (2004). Naturally acquired simian retrovirus infections in central African hunters. *Lancet*. 363: 9327.

16. Engel, G.A., Jones-Engel, L., Schillaci, M.A., Suaryana, K.G., Putra, A. and Fuentes, A. (2002). Human exposure to herpesvirus B-seropositive macaques, Bali, Indonesia. *Emerg. Infect. Dis.* 8: 78995.

17. Cleaveland, S., Laurenson, M.K. and Taylor, L.H. (2001). Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philos Trans R Soc Lond B Biol Sci*. 356: 9919.

18. Sharma, N. K., Doyle, P. W., Gerbasi, S. A. and Jessop, J. H. (1990). Identification of *Yersinia* species by API 20E. *Journal of Clinical Microbiol.* 28: 1443-1444.

19. Okwori AEJ., Obiora C., Agina SE., Olabode AO., Onwuliri FC (2008) Occurrence of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in locally fermented cow milk (*nono*) in Bauchi metropolis, Nigeria. Nigerian Journal of Biotechnology Vol.19:5-11

20. Okwori , AEJ., Pilar OM., Maria Fredriksson-Ahomaa., Agina. SE, Korkeala H(2009) Pathogenic *Yersinia enterocolitica* 2/o:9 and *Yersinia pseudotuberculosis* 1/o:1 strains isolated from human and non-human sources in the Plateau State of Nigeria. *Journal of food Microbiology (26): 872- 875.*

21. FDA/CFSAN. (2001). *Yersinia enterocolitica* and *Yersinia pseudotuberculosis.* Bacteriological Analytical Manual. Online Chp. Pp.1-7. FDA - Centre For Food Safety and Applied Nutrition.

22. Sestak, K., Merritt, C.K., Borda J., Saylor, E., Schwamberger, S.R., Cogswell, F., Didier, E.S., Didier, P.J., Plauche, G., Bohm, R.P., Aye, P.P., Alexa, P., Ward, R.L. and Lackner,

A.A. (2003). Infectious agent and immune response characteristics of chronic enterocolitis in captive rhesus macaques. *Infec. Immun*. 71: 4079 – 4086.

23. Wachtman, L.M. and Mansfield, K.G., (2008). Opportunistic infections in immunologically compromised nonhuman primate. *ILAR J* 49: 191 – 208.

24. Robert, C.G., Bessie, D.M. and Toshio, K. (1969). Enteric pathogens in monkeys. *Journal of Bacteriology*. 97: 1048-1055.

25. Mikov, M. (1994). The metabolism of drugs by the gut flora. *Eur. J. Drug. Metab. Ph*. 19: 30-39

26. Kalashnikova, V.A., Dzhikidze, E.K., Stasilevich, Z.K. and Chikobava, M.G. (2002). Detection of *Campylobacter jejuni* in healthy monkey and monkeys with enteric infections by PCR. *Bull. Exp. Biol. Med*. 134: 299 – 300.

27. Russell, R.G., Rosenkranz, S.L., Lee, L.A., Howard, H., DiGiacomo, R..F., Bronsdon, M.A., Blakdey, G.A., Tsai, C.C and Moton, W.R. (1987). Epidemiology and etiology of diarrhoea in colony-born *Macaca nemestrina*. *Lab. Anim. Sci*. 37: 309 – 316.

28. Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P. and Blaser, M.J. (1988). Experimental *Campylobacter jejuni* infection in human. *J. Infect. Dis*. 157: 472-479.

29. Fitzgeorge, R.B., Baskerville, A. and Lander, K.P. (1981). Experimental infection in rhesus monkeys with a human strain of *Campylobacter jejuni, J. Hyg. Camb*. 86: 343 – 351.

30. Russell, R.G., Blaser, M.J., Sarmiento, J.I. and Fox. J. (1989). Experimental *Campylobacter jejuni* infection in *Macaca memestrina. Infect. Immun*. 57: 1438 – 1444.

31. Russell, R.G., Sarmiento, J.I., Fox, J. and Panigrahi, P. (1990). Evidence of reinfection with multiple strains of *Campylobacter jejuni* and *Camplobacter coli* in *Macaca nemestrina* housed under hyperendemic conditions. *Infect. Immun*. 58: 2149 – 2155.

32. Bolton, F.J., Hutchison, D.N. and Parker, G. (1988). *European Journal of Clinical Microbiological Infectious Diseases*, 7, Pp. 155-160.

33. Russell, R.G., O'Donnoghue, M., Blake, D.C. Jr., Zulty, J. and DeTolla. L.J. (1993). Earlycolonic damage and invasion of *Campylobacter jejuni* in experimentally challenged infant *Macacamulatta*. *J. Infect. Dis*. 168: 210 – 215

34. Bruorton, M.R., Davis, C.L. and Perrin M.R. (1991). Gut microflora of vervet and samango monkeys in relation to diet. *Appl environ. Microbiol.* 57: 573 – 578.