

Nicholas Kiulia

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- Molecular characterization and epidemiology of enteric viruses in sewage and waste water in Kenya
- Molecular epidemiology of enteric pathogens especially Cryptosporidium, Cyclospora and Microsporidia in Kenya.
- To detect and quantify rotaviruses (RVs), enteroviruses and noroviruses (NoVs) in surface water samples from different geographical regions in Kenya
- Development of a non human primate model for preclinical testing and efficacy of rotavirus and other enteric virus vaccine

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ISID Fellowship Report

Molecular Characterization of Cryptosporidium Isolates in Captive Olive Baboon in Kenya

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Background

Cryptosporidiosis is one of the commonest human enteric infections in developed and developing countries and in most cases is among the four major pathogens causing diarrhoeal diseases especially among children and immunocompromised patients (Antonios et al 2010, Xiao L 2010). Its causative agents the *Cryptosporidium parvum* and *C.hominis* are now considered emerging infectious pathogens (Tzipori and Ward, 2002) and they have major public health implications (Xiao *et al*, 2000). In developing countries cryptosporidium is responsible for 4-19% of cases of diarrhoeal disease with a significant effect on mortality (Gatei *et al*, 2006). Cryptosporidiosis in humans is transmitted by either anthroponotic or anthropozoonotic routes (Sulaiman *et al*, 1998) and many domestic animals, but only a few wild ones, have been implicated as potential reservoirs for human infections (Fayer *et al*, 2000). In Kenya Cryptosporidium is one of the most common protozoan parasites associated with diarrhoea in young children (Gatei *et al*, 2006). Nonhuman primates are genetically related to humans and thus may be susceptible to infection with human parasites and serve as zoonotic reservoirs (10). Cryptosporidium infection has been recorded from many species of captive non-human primates (Li et al, 2011, Muriuki *et al*, 1997, de Graff 1999).

Thus far, very few studies have examined the genetic characteristics of Cryptosporidium in nonhuman primates (Li et al, 2011). Thus, the role of nonhuman primates in zoonotic transmission of Cryptosporidium is not clear. In this study, we investigated the presence of Cryptosporidium infection in captive baboons in Kenya based on the polymerase chain reaction (PCR).

Methods

Specimen collection and molecular characterization

Faecal samples were collected from 50 newly caught and quarantined olive baboons within four weeks of acclimatization (one month). Samples were collected in properly labeled, clean, wide mouthed containers with tightly fitting lids and preserved in 2.5% potassium dichromate at 4°C until further characterization.

Genomic DNA extraction and PCR amplification

Prior to DNA extraction, fecal specimens were washed twice in distilled water. Genomic DNA was then extracted from 0.5 ml of specimens using a FastDNA spin kit for soil (BIO 101, Carlsbad, CA), and eluted in 100 μ l of reagent-grade water as previously described (Jiang et al., 2005). Cryptosporidium in specimens were detected by nested PCR amplification of an approximately 830-bp fragment of the SSU rRNA gene of Cryptosporidium. The primary PCR had 35 cycles of 94°C for 45s, 53°C for 45s, and 72°C for 60s, with an initial denaturation (94°C for 5 min) and a final extension (72°C for 10 min). The condition for the secondary PCR was identical to the primary PCR, except that the annealing temperature was increased to 55°C. Each specimen was analyzed using 1 μ l of the DNA extract per PCR, using DNA of Cryptosporidium baileyi as the positive control. Non-acetylated bovine serum albumin (Sigma-Aldrich, St. Louis, MO) at the concentration of 400 ng/ μ l was used in all primary PCRs to neutralize residual PCR inhibitors in the



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extracted DNA. The PCR was performed in a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA). PCR products were then visualized by electrophoresis in 1.5% agarose containing ethidium bromide.

Results

Of the 50 fecal specimens analyzed, 16% (8/50) were positive for Cryptosporidium species by PCR amplification of the SSU rRNA gene.

Table 1: The positivity of the Cryptosporidium identified in the baboon.

Specimen Number	18S nested PCR
34267	Positive
34282	Positive
34287	Positive
34288	Positive
34293	Positive
34294	Positive
34301	Positive

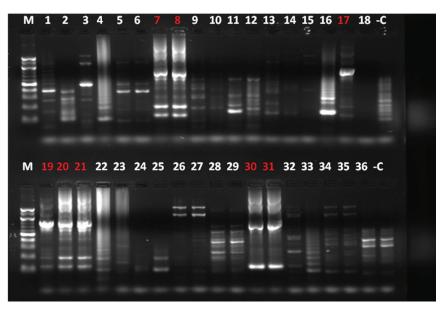


Figure 1: Amplification products corresponding to the 18S rRNA gene of Cryptosporidium species identified from baboon (Papio anubis) in Kenya.

Discussion and Conclusion

The prevalence of Cryptosporidium in wild caught baboon in this study was 16%. The present of Cryptosporidium in baboon as seen in this study clearly indicate that newly captive baboons in Kenya harbor Cryptosporidium species and they may be a source of infection in human.

With the ever-increasing expansion of human populations into uninhabited areas, anthropozoonotic disease assumes an important role in the health of humans as well as wildlife and its conservation. This is particularly so for non-human primates that shares a close phylogenetic relationship with humans and where common intestinal metazoan and protozoans parasites have been found. Baboons in Kenya have also been shown to harbor some human-pathogenic Cryptosporidium genotypes.

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More studies are needed to fully elucidate the importance of non-human primate especially the baboons and understanding the epidemiology of cryptosporidiosis in human population. Surface water in the area where baboons live in close contact with human should also be tested to monitor the spread of Cryptosporidium due to contamination of drinking source water by these reservoir hosts.

Public health measures should also be put in place to reduce contact between wild non human primates and susceptible human populations especially people living with HIV/ AIDs in areas where baboon lives.

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Calendar of Events 2012

14–16 November

4th Conference of Virology Sharm El-Shaikh, EGYPT

http://www.merc-cerealviruses.com

Activities include food virology, vaccinology and vaccine production, virus identification, detection and typing, bio-informatics for virology. The conference will cover plant, animal and human virology.

14–18 November

XVI Congreso Latinoamericana de Pediatria, ALAPE 2012

Cartagena de Indias, COLOMBIA

http://www.congresosalape.com

This Congress is being held in participation with the Colombian Society of Pediatrics and is aimed towards pediatricians, nurses, therapists, nutritionists, psychologists and family physicians. Topics range from neonatology, infectious diseases and vaccines, adolescent and sport medicine, nutrition, interventions in primary care and more.

21–23 November

II International Conference on Antimicrobial Research (ICAR2012) Lisbon, PORTUGAL

http://www.formatex.org/icar2012/

Conference will cover topics on antimicrobial resistance, (early) microbial and resistance detection, enhancement of innate defences against pathogens, as well as methods & techniques.

3–5 December

ISDS Conference: Expanding Collaborations to Chart a New Course in Public Health Surveillance,

San Diego, California USA

http://www.syndromic.org/annual-conference/2012

The International Society for Disease Surveillance Annual Conference will highlight the importance of working together across agencies, sectors, and disciplines to improve surveillance methods and population health outcomes.

7–8 December

1st Conference of the Ethiopian Society of Tropical and Infectious Diseases, Addis Ababa, ETHIOPIA

www.estaids.org

The inaugural conference of ESTAID will be centered on the theme of *Environmental Change* and Threats of Tropical and Infectious Diseases: Public Health and Economic Implications. The Sub-themes are: Environmental Change and HIV/ STIs, Environmental Change, Malaria and Vector-Borne Diseases, Environmental Change and Diarrheal Diseases, Environmental Change, Tuberculosis and Other Respiratory Diseases, and Environmental Change and Zoonotic Diseases.