Molecular Characterization of Cryptosporidium Isolates in Captive Olive Baboon in Kenya

Nicholas M. Kiulia,1 Adamu B. Haileyesus,2 Maureen A. Obara,3 Atunga Nyachieo,1 Lihua Xiao2

1 Enteric Viruses Research group, Institute of Primate Research, P.O. Box 24481, 00502-Karen • Nairobi, Kenya
2 Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Mail Stop D66 Bldg 23, Km 9-168, 1600 Clifton Road • Atlanta, GA, USA 30329-4018
3 Department of Applied Science, Medical Microbiology Program, Jomo Kenyatta University of Agriculture and Technology Karen Campus • Nairobi, Kenya

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 anthropootic 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, faecal 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 μl 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 μl 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/μl was used in all primary PCRs to neutralize residual PCR inhibitors in the

This research was supported with a grant from the International Society for Infectious Diseases (ISID).
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.

<table>
<thead>
<tr>
<th>Specimen Number</th>
<th>18S nested PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>34267</td>
<td>Positive</td>
</tr>
<tr>
<td>34282</td>
<td>Positive</td>
</tr>
<tr>
<td>34287</td>
<td>Positive</td>
</tr>
<tr>
<td>34288</td>
<td>Positive</td>
</tr>
<tr>
<td>34293</td>
<td>Positive</td>
</tr>
<tr>
<td>34294</td>
<td>Positive</td>
</tr>
<tr>
<td>34301</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**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.

---

This research was supported with a grant from the International Society for Infectious Diseases (ISID).
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.

This study was supported by a grant from the International Society for Infectious Diseases (ISID) under the ISID Scientific Exchange Fellowship Program.

References