Rapid Communication

Complex epidemiology and zoonotic potential for Cryptosporidium suis in rural Madagascar

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Cryptosporidium spp. is the most important parasitic diarrheal agent in the world, is among the top four causes of moderate-to-severe diarrheal disease in young children in developing nations, and is problematic as an opportunistic co-infection with HIV. In addition, Cryptosporidium is a persistent challenge for livestock production. Despite its zoonotic potential, few studies have examined the ecology and epidemiology of this pathogen in rural systems characterized by high rates of overlap among humans, domesticated animals, and wildlife. To improve our understanding of the zoonotic potential of Cryptosporidium species in the rural tropics, we screened humans, livestock, peridomestic rodents, and wildlife using PCR-RFLP and sequencing-based approaches to distinguish species of Cryptosporidium in rural southeastern Madagascar. Cryptosporidium of multiple species/genotypes were apparent in this study system. Interestingly, C. suis was the dominant species of Cryptosporidium in the region, infecting humans (n = 1), cattle (n = 18), pigs (n = 3), and rodents (n = 1). The broad species range of C. suis and the lack of common cattle Cryptosporidium species (Cryptosporidium parvum and Cryptosporidium andersonii) in this system are unique. This report represents the fifth confirmed case of C. suis infection in humans, and the first case in Africa. Few rural human and livestock populations have been screened for Cryptosporidium using genus-specific genotyping methods. Consequently, C. suis may be more widespread in human and cattle populations than previously believed.

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1. Introduction

Among the top four causes of moderate-to-severe diarrheal disease in young children in developing nations, Cryptosporidium spp. is the most important parasitic diarrheal agent and is problematic as an opportunistic co-infection with HIV (Tzipori and Widmer, 2008; Cabada and White, 2010; Kotloff et al., 2013). In addition, Cryptosporidium is a persistent challenge for livestock production as a
common infection in calves (Fayer et al., 2000). Despite its zoonotic potential, few studies have examined the ecology and epidemiology of this pathogen in rural systems characterized by high rates of overlap among humans, domesticated animals, and wildlife (Appelbee et al., 2005).

There are more than 26 accepted species and approximately 50 Cryptosporidium genotypes described for humans and animals (Xiao and Feng, 2008; Xiao, 2010; Elwin et al., 2012). Although Cryptosporidium parvum and Cryptosporidium hominis account for greater than 90% of investigated human cases, Cryptosporidium meleagridis, Cryptosporidium canis, Cryptosporidium felis, Cryptosporidium viatorum, Cryptosporidium cuniculus, Cryptosporidium ubiquitum, Cryptosporidium muris, Cryptosporidium suis and a few other Cryptosporidium species and genotypes are also known to infect humans (Xiao, 2010). To improve our understanding of the zoonotic potential of Cryptosporidium species in the rural tropics, we screened humans, livestock, peridomestic rodents, and wildlife using PCR-RFLP and sequencing-based approaches to distinguish species of Cryptosporidium in rural southeastern Madagascar.

2. The study

Two communities located on the edge of Ranomafana National Park, Madagascar, were selected as the focus of this study: Ambodiaviavy (GPS Coordinates: 21°15.849 S 047°29.087 E, population = 363) and Ankialo (GPS Coordinates: 21°08.062 S 047°20.638 E, population = 361). In July and August 2011, household and individual surveys were administered in both communities: Ambodiaviavy (n = 65, total households = 10) and Ankialo (n = 70, total households = 10). Informed consent of participants was obtained prior to specimen collection and survey. Participants were anonymously given unique identifiers. Surveys were comprehensive with inquiries of demographic information, health status, hygiene, medication usage, water usage, and exposure to livestock and wildlife (70 questions for individual survey and 40 variables for the household survey). Potential behaviors associated with risk for diarrheal disease and infection with Cryptosporidium were queried in both surveys.

All survey participants were asked to provide a fecal specimen for examination of Cryptosporidium and 89% complied. Concurrently, domesticated animals of participants (bovine, canine, feline, and porcine) were sampled and baited rodent live-traps were set inside participant homes overnight. The following morning, fecal specimens were collected from trapped peridomestic rodents. Wildlife (lemurs, rodents, and fossa) within Ranomafana National Park were opportunistically and non-invasively sampled. All fecal specimens were preserved upon collection in RNAlater® (Qiagen Inc., Valencia, CA).

Fecal specimens (n = 278) were screened for Cryptosporidium including 120 human (n = 59 from Ambodiaviavy and n = 61 from Ankialo), 82 domesticated animal (n = 62 bovine, n = 17 porcine, n = 1 canine, and n = 2 feline), 48 peridomestic rodent, and 28 wildlife (n = 25 lemur, n = 2 wild rodents and n = 1 fossa) specimens. DNA was extracted from fecal specimens (n = 278) preserved in RNAlater® using the FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH), following the manufacturer-recommended procedures. All DNA samples were then examined by a PCR-restriction fragment length polymorphism (RFLP) approach capable of detecting and differentiating Cryptosporidium species as described by Xiao et al. (2001). Briefly, an 834-bp segment of the Cryptosporidium small subunit (SSU) rRNA gene was amplified by nested PCR. Primers and amplification conditions used in this study are described elsewhere (Xiao et al., 1999), except that the reverse primer used in the primary PCR was 5’-CCCATTCCTTGCAGAGA-3’. Genotype identification was made by restriction digestion of the secondary PCR product with SspI and VspI. Each sample was analyzed at least twice in 2 independent PCR-RFLP analyses. Fragment sizes for each enzyme were then cross referenced to the expected fragment sizes for species of Cryptosporidium. PCR products of the 41 positive samples were sequenced in both directions on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) to confirm species/genotype. There was complete agreement in identification of Cryptosporidium species between RFLP and DNA sequence analyses.

All protocols, including obtaining oral consent from participants, were reviewed and approved by the Ministry of Health of the government of Madagascar, the Stony Brook University Internal Review Board and Institutional Animal Care and Use Committee. As approved by the Stony Brook Internal Review Board, oral informed consent of participants was obtained prior to specimen collection and survey. In the case of minors, a parent or guardian provided informed consent. Given the low literacy rate of the population being studied, we opted for oral consent administered and recorded on the survey sheets by the native interpreter conducting the interview. All participants were anonymously given unique identifiers. Permits were not required for sample collection from the animals in this study. All cows and pigs sampled were handled according to the guidelines of the National Veterinary Services Laboratories (publication N231597), USDA, Fort Collins, CO. Rodents were handled following protocols outlined by the CDC (Millis et al., 1995). The Centers for Disease Control and Prevention did not participate in sample collection and did not receive personal identifiers.

Cryptosporidium of multiple species/genotypes were apparent in this study system C. suis was recovered from one human (a 43-year-old male) in the rural village of Ankialo, Madagascar, where 25% of pigs sampled were also positive for Cryptosporidium. Three of the four Cryptosporidium-positive pigs were infected with C. suis (Table 1). However, the infected person and his household did not own pigs. The infected person reported having no diarrheal symptoms but did report a fever and nausea/vomiting and indicated the use of three different medications: acetaminophen (analgesic), levamisole (antihelmintic), and calcium lactate (anti-acid). This infected person also reported behaviors that increase risk for Cryptosporidium infection and/or diarrheal symptoms such as: eating food contaminated with rodent fecal matter, eating uncooked meat, and eating animals with visible sores. Further survey results can be found in Bublitz et al., 2014. Interestingly, the infected person reported recently
trapping, cooking, and eating a wild pig, which may account for his exposure to C. suis. This novel pathway of infection is supported by the fact that the C. suis-infected subject was the only Cryptosporidium-infected human in either of the villages sampled, despite high prevalence of Cryptosporidium in resident cattle (29%), pigs (24%), and peridomestic rodents (33%). Further studies examining the frequency of hunting in the area would allow the significance of this potential novel pathway of transmission to be assessed.

3. Conclusion

The broad species range of C. suis and the lack of common cattle Cryptosporidium species (C. parvum and C. andersoni) in this study system are unique (Fayer et al., 2000; Tzipori and Widmer, 2008). This report represents the fifth confirmed case of C. suis infection in humans. Most commonly found in pigs, C. suis has been identified previously in an HIV+ patient in Lima, Peru, an HIV+ patient in Henan, China, and two patients in London (Xiao et al., 2002; Leoni et al., 2006; Wang et al., 2013). Our finding expands the geographic range of known human C. suis infections. Few rural human and livestock populations have been screened for Cryptosporidium using genus-specific genotyping methods. Consequently, C. suis may be more widespread in human and cattle populations than previously believed.

Further studies are needed to better understand the complex epidemiology of Cryptosporidium in the Ranomafana region of Madagascar and in other rural sites where similar transmission dynamics may be at play.

Conflict of interest statement

None of the authors of this manuscript had financial or personal relationships with other people or organizations that could inappropriately influence the work presented. None of the funding sources for this study played any role in study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

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References


