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Cryptosporidium ubiquitum n. sp. in animals and humans

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ABSTRACT

A new species, *Cryptosporidium ubiquitum*, previously identified as the *Cryptosporidium* cervine genotype and infrequently as the cervid, W4 or genotype 3 genotype, is described. In published studies this genotype was reported in wild and domesticated ruminants, rodents, carnivores and primates including humans. In the present study oocysts were found in feces from a captive prehensile-tailed porcupine and her infant. Oocysts from the porcupine were transmitted to 4 boer goats. Oocysts from the goats were transmitted to a calf (calf 1) and oocysts from calf 1 were transmitted to gerbils and BALB/c mouse pups. Calf 2 housed near calf 1 became contaminated and excreted oocysts of *C. ubiquitum*. Oocysts collected from calf 2 were transmitted to a calf 3. When calf 2 stopped excreting *C. ubiquitum* oocysts it was challenged with oocysts of *C. parvum* and became infected, indicating a lack of cross-species immunity. Oocysts of *C. ubiquitum* from calf 1 measured 4.71–5.32 μm × 4.33–4.98 μm (mean = 5.04 μm × 4.66 μm) with a length/width shape index of 1.08 (n = 50). Purified PCR products of the SSU rRNA, actin and COWP genes were sequenced and analysis of the 3 unlinked loci demonstrated the new species to be distinct from all other species and also demonstrated a lack of recombination, providing further evidence of species status. Based on morphological, molecular and biological data, this geographically widespread parasite infectious for a wide range of mammalian hosts is recognized as a new species and is named *C. ubiquitum*.

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1. Introduction

Cryptosporidiosis is a diarrheal disease of humans and animals caused by parasites in the genus *Cryptosporidium*, a genus comprising 20 valid species and nearly 2-fold as many genotypes (Fayer, 2010; Fayer et al., 2008; Ryan et al., 2008; Power and Ryan, 2008). Molecular characterization of these species and genotypes has revolutionized our understanding of the biological diversity of the organisms in this genus. Most human infections are caused by *C. hominis* and *C. parvum* (Cacciò et al., 2005). However, *C. meleagridis*, *C. felis*, *C. canis*, *C. suis*, *C. muris*, *C. andersoni* and the *Cryptosporidium* cervine, horse, skunk and monkey genotypes also have been detected in feces of immunocompetent and immunocomprised humans (Pieniazek et al., 1999; McLauchlin et al., 2000; Ong et al., 2002; Cama et al., 2003; Mallon et al., 2003; Learmonth et al., 2004; Leoni et al., 2006; Chalmers et al., 2009). Of these four genotypes, the cervine genotype has been detected in feces from more animals and over a greater geographic range than most species of *Cryptosporidium*. It has been reported in feces from humans in Canada, New Zealand, England, Slovenia, the United Kingdom and the USA (Ong et al., 2002; Learmonth et al., 2004; Blackburn et al., 2006; Feltus et al., 2006; Leoni et al., 2006; Soba et al., 2006; Chalmers et al., 2009; Davies et al., 2009) and in a colony of non-human primates (lemurs) in NC (da Silva et al., 2003) (Table 1). It has been found in feces from wild and domesticated ruminants including among others white-tailed deer in NY (Perz and LeBlancq, 2001), a blesbok, a mouflon and a nyala in a zoo in the Czech Republic (Ryan et al., 2003), an ibex in China (Karanis et al., 2007), sika deer in China (Wang et al.,

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Table 1
Reported primate hosts of the *Cryptosporidium* cervine genotype, their geographic location and the genes used to characterize the genotype. When available GenBank accession numbers were included.

Host	Number	Location	Gene(s) (GenBank accession number)	Author
Human	3	British Columbia, Canada	SSU rRNA (AY030086–AY030088), COWP (AF411632–AF411633)	Ong et al. (2002)
Human	2	New Zealand	SSU rRNA (AY458613–AY458614)	Learmonth et al. (2004)
Human	7	British Columbia, Canada	CP23 (DQ389174), HSP70 (DQ389176), actin (DQ389175), β-tubulin (DQ389177)	Wong and Ong (2006)
Human	1	Ontario, Canada	SSU rRNA (DQ192500)	Trotz-Williams et al. (2006)
Human	1	WI, USA	SSU rRNA (DQ640639–DQ640640), COWP	Feltus et al. (2006)
Human	1	OH, USA	SSU rRNA	Blackburn et al. (2006)
Human	1	England	SSU rRNA (DQ116570)	Leoni et al. (2006)
Human	1	Slovenia	SSU rRNA (AJ849465), COWP (AJ849460)	Soba et al. (2006)
Human	1	United Kingdom	SSU rRNA (FJ031238)	Davies et al. (2009)
Human	4	England and Wales	SSU rRNA, COWP	Chalmers et al. (2009)
Human	1	Lima, Peru	SSU rRNA	Xiao and Cama (unpublished)
Coquerel's sifaka (lemurs)	4	Duke University, NC, USA	SSU rRNA (AF442484), COWP (AF436074 and AY057839)	da Silva et al. (2003)

2008), sheep at an abattoir in Western Australia (Ryan et al., 2005a) and sheep on a farm in MD, USA (Santin et al., 2007) and on farms in Scotland (Elwin and Chalmers, 2008), China (Wang et al., 2010), Belgium (Geurden et al., 2008) and Spain (Diaz et al., 2010) (Table 2). It has been found in feces from a variety of wild rodents including squirrels, chipmunks, and a woodchuck, beaver, deer mouse and raccoon (Feng et al., 2007; Ziegler et al., 2007) (Table 2). It has been identified in water samples in various geographic locations (e.g. Xiao et al., 2000; Jiang et al., 2005; Ryan et al., 2005b) (Table 3).

Different names have been used for the cervine genotype in publications and in GenBank leading to confusion of its identification (Santín and Fayer, 2007). There are 131 available sequences in GenBank for 7 genes (112, 8, 4, 4, 1, 1 and 1 for the SSU rRNA, COWP, actin, HSP-70, HSP-90, Cp23 and β-tubulin, respectively). To improve identification and thereby the detection, reporting and understanding of the epidemiology of this genotype the present study was undertaken to obtain morphometric and biological data to add to the molecular data so the cervine genotype could be identified as a separate species using parameters proposed by Xiao et al. (2004). Data obtained in the present study indicate that the *Cryptosporidium* cervine genotype qualifies as a new species based on its biological and molecular uniqueness and as such is named *C. ubiquitum* because of its great geographic range and diverse host species.

2. Materials and methods

2.1. Experimental design

Fecal specimens were also collected from prehensile-tailed porcupines (*Coendou prehensilis*) at the Smithsonian National Zoo in Washington, DC. All oocysts were less than 1-month-old when used for infectivity studies. Animals that received oocysts were housed and cared for in animal buildings approved by the Beltsville Area Animal Care and Use Committee at the BARC. Initially, oocysts from a naturally infected mother and infant porcupine were used to infect boer goats (goats 1–4). Oocysts from goat 2 were used to infect a neonatal calf (calf 1). Oocysts from calf 1 were used to infect, gerbils (gerbils 1 and 2) and BALB/c mouse pups (mouse 1–9). A second neonatal calf (calf 2) was housed in a stall adjacent to that of experimentally infected calf 2 but was not experimentally infected with oocysts of *C. ubiquitum*. A third calf (calf 3) was experimentally infected using oocysts from calf 2.

All feces from infected animals were processed to obtain oocysts at the USDA, ARS laboratory in Beltsville, MD as previously described by Fayer et al. (2000). *C. ubiquitum* oocysts from naturally infected porcupines were pooled and used to infect 4 goats. All experimental infections in calves, mice and gerbils were administered per os with 100,000 oocysts per mouse, gerbil and calf cleaned of fecal debris and suspended in dH_2O. Mice and gerbils received a 0.2 or 1.0 ml suspension via a 26 ga gavage needle fitted to a syringe, respectively. Goats and calves received a 50 ml suspension in nippled bottles. To test for cross-species immunity calf 2 received a challenge dose of 100,000 oocysts of *C. parvum* within 2 weeks after excretion of *C. ubiquitum* oocysts ceased.

2.2. Oocyst collection and processing

Feces from the goats, calves and gerbils were collected daily for 21–24 days beginning on the day of inoculation and placed in individual specimen cups with lids, labeled and held at 4 °C until processed. Five to fifteen grams of feces from each goat and calf specimen were transferred from each specimen cup to a 50 ml centrifuge tube containing approximately 35 ml dH_2O. Likewise, 3–6 pellets from each gerbil specimen were transferred from each specimen cup to a 15 ml centrifuge tube containing approximately 10 ml dH_2O. Contents were thoroughly mixed using a Vortex-Genie (Scientific Industries, Bohemia, NY). The fecal suspension was sieved through a 45 μm pore size wire screen, placed in another centrifuge tube, and the volume adjusted to 50 or 15 ml, with dH_2O. After centrifugation at 1800 × *g* for 15 min supernatant was dis-