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Cryptosporidium in pet snakes from Italy: Molecular characterization and zoonotic implications

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ABSTRACT

To provide information on the occurrence of *Cryptosporidium* species and genotypes in captive snakes from Italy, faecal specimens from 120 snakes belonging to 13 different genera of the families Boidae, Colubridae and Pythonidae were collected. Faecal samples were taken from the ground of the terrarium when available; otherwise cloacal cotton swabs were used. No clinical signs of cryptosporidiosis were observed in any animal at the time of sampling. Samples were examined for the presence of *Cryptosporidium* by using a direct immunofluorescence antibody test (IFAT) and two-step nested PCR at the small subunit (SSU) rRNA locus. PCR-positive samples were genotyped by restriction fragment length polymorphism (RFLP) analysis with the endonucleases *SspI* and *VspI*.

By IFAT, 42 out of 120 snakes (35.0%) were found to be shedding *Cryptosporidium* oocysts. A significant higher percentage of positive ophidians were detected by using faecal specimens obtained from the terrarium (55.5%) than by cloacal cotton swabs (29.0%).

SSU rRNA gene products were obtained from 25 isolates. Twenty samples tested positive to both microscopy and molecular techniques. Our data reveal a wide extent of cryptosporidial infections in snake-food animals since most of the identified isolates belonged to *Cryptosporidium* species, some of them with zoonotic potential, considered specific for rodents and resulting from ingestion of infected preys. The reptilian-specific species *Cryptosporidium serpentis* was identified in only one isolate.

The common presence of reptile non-specific and, in some cases, zoonotic *Cryptosporidium* oocysts in snake faeces should to be taken into consideration in order to avoid the misidentification of the protozoan as well as the possible public health implications.

1. Introduction

Reptiles (mainly turtles, lizards and snakes) have become popular pets in the last decades (Hassl and Benyr, 2003; Pantchev and Tappe, 2011). In this regard, Wise et al. (2002) reported that the estimated number of U.S. households with reptiles doubled from approximately 850,000 in 1991 to 1.7 million in 2001. Consequently, breeding of reptiles in captivity and capture of wild species have increased recently to meet the demand of pet reptiles. However, captive reptiles are frequently subjected to stressful conditions derived mainly from inadequate management practices, leading to a high risk of acquisition of infectious and parasitic diseases (Ippen and Zwart, 1996).

Cryptosporidium is an apicomplexan protozoan mainly responsible for gastroenteritis in humans and other

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vertebrates (Fayer, 2010). It is also a common parasite of reptiles since it has been reported in more than 80 different reptilian species (Fayer, 2010); adult and captive animals seem to be more susceptible to cryptosporidiosis (O'Donoghue, 1995; Ramirez et al., 2004).

In snakes, two species have been described: *Cryptosporidium varanii* (syn. *Cryptosporidium saurophilum*, Pavlasek and Ryan, 2008) and *Cryptosporidium serpentis*. The former preferentially infects lizards and has been associated with acute enteritis and diarrhoea (Pavlasek et al., 1995; Koudela and Modry, 1998; Plutzer and Karanis, 2007). On the contrary, *C. serpentis* is the main species parasitizing snakes (Xiao et al., 2004; Pedraza-Díaz et al., 2009), causing a chronic gastritis with clinical signs that include anorexia, lethargy, post-prandial regurgitation and weight loss (Brownstein et al., 1977; Cranfield and Graczyk, 1994). Recent molecular studies have identified other non-specific species and genotypes from ophidian faeces, including *Cryptosporidium muris*, *Cryptosporidium tyzzeri* (formerly known as the *Cryptosporidium* mouse genotype I; Ren et al., 2011) and *Cryptosporidium parvum* (Xiao et al., 2004; Pedraza-Díaz et al., 2009; Richter et al., 2011), probably acquired by ingestion of infected preys.

In a recent investigation, the presence of gastrointestinal parasites infecting reptiles in Italy was assessed using microscopic techniques (Papini et al., 2011); no *Cryptosporidium* positive snakes were detected, and only a small percentage of lizards and turtles shed oocysts of the protozoan. Nevertheless, no information regarding the implicated species was available. The main objective of this research is to provide up-to-date information on the occurrence of *Cryptosporidium* species in captive snakes from Italy using a molecular approach and to unravel their public health significance.

2. Materials and methods

Faecal specimens from 120 snakes belonging to 13 different genera of the families Boidae, Colubridae and Pythonidae (Table 1) were collected in snake breeders, pet shops and private collections in Italia. All animals were kept in individual cages and were sampled once. No other animals were introduced into the terrarium, excepting the feeding mice. Cleaning of the terrarium was carried out once a week, removing all the faecal material; once a month, all the substrate was also removed. Forty-one ophidians were males and 79 females, and the age ranged from 2 months to 8 years. Most of the animals ($n = 94$) were born in captivity, whereas 26 were collected directly from the wild. No clinical symptoms of cryptosporidiosis were observed in any animal when sampled. Fresh faecal samples were collected from the ground of the terrarium when available ($n = 27$); otherwise cloacal cotton swabs were used ($n = 93$). Individual samples were maintained at 4 °C until microscopical examination, never later than 72 h from collection.

Faecal specimens (3 g) were homogenized in 30 ml of phosphate buffered saline (PBS) pH 7.2, filtered through a 40 μm mesh size sieve and centrifuged at 1000 × *g* for 10 min. The supernatant was removed and 20 μl of the pellet was examined for the presence of *Cryptosporidium* oocysts using a direct immunofluorescence antibody test (IFAT) (Crypto Cel, Cellabs Pty Ltd., Brookvale, Australia). Oocysts were removed from cloacal swabs by immersing and rubbing the head of the swab against the wall of a 2-ml microcentrifuge tube containing 1 ml of PBS. The oocyst suspensions were concentrated to a final volume of 220 μl by centrifugation at 6000 × *g* during 3 min. Twenty μl of each suspension was used for IFAT examination. The number of oocysts in each sample was expressed as oocysts

Table 1
Cryptosporidium in snakes sampled in Italy. Microscopic and molecular results.

Scientific name	Number of samples	Origin[a]	IFAT +	PCR +	*Cryptosporidium* species
Acrantophis dumerili	1	1 CB	0	–	
Acrantophis madagascareiensis	1	1 CB	1	0	
Aspidites ramsayi	2	2 CB	0	–	
Boa constrictor constrictor	11	8 WC + 3 CB	3	1	1 *C. parvum*
Boa constrictor imperator	52	2 WC + 50 CB	16	4	3 *C. parvum*, 1 ND
Boiga irregularis	5	5 WC	1	0	
Boiga nigriceps	1	1 WC	0	–	
Epicrates cenchria cenchria	1	1 CB	1	1	1 *C. serpentis*
Lampropeltis floridiana	1	1 CB	1	1	1 *C. tyzzeri*
Leioheterodon modestus	1	1 WC	0	–	
Liopholidophis lateralis	1	1 WC	0	–	
Mimophis mahfalensis	1	1 WC	0	–	
Morelia amethistina	1	1 CB	1	1	1 *C. tyzzeri*
Morelia spilota	1	1 CB	1	1	1 *C. muris*
Morelia viridis	3	1 WB + 2 CB	1	1	1 ND
Pantherophis guttata	1	1 CB	0	–	
Python molurus bivittatus	3	3 CB	0	–	
Python molurus molurus	3	3 CB	0	2	1 *C. parvum*, 1 ND
Python regius	28	6 WC + 22 CB	14	11	7 *C. tyzzeri*, 2 *C. muris*, 2 ND
Python sebae	1	1 CB	1	1	1 *C. tyzzeri*
Sanzinia madagascariensis	1	1 CB	1	1	1 *C. tyzzeri*
Total	120		42	25	

–, no PCR was performed; ND, no data.
[a] CB, captive-born; WC, wild-caught.