PREVALENCE OF CRYPTOSPORIDIOSIS IN BELGIAN SNAKE COLLECTIONS

Prevalentiestudie van cryptosporidiose bij Belgische slangencollecties

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ABSTRACT

This report describes a prevalence study of cryptosporidiosis in 105 snakes from 7 Belgian snake collections. Several non-European studies have shown high prevalences in snake collections of zoos and in snake populations in the wild. In spite of correct sampling and proper detection procedures, not a single positive sample was found in the present study. Therefore one can conclude that the importance of this parasite in the collections examined is negligible.

SAMENVATTING

In dit artikel wordt de prevalentie van cryptosporidiose beschreven bij 105 slangen afkomstig van 7 Belgische slangencollecties. Verschillende niet-Europese studies toonden niet te verwaarlozen prevalenties aan bij slangencollecties van dierentuinen en bij slangenpopulaties in het wild. In onze studie echter kon geen enkel positief staal worden aangetoond, ondanks correcte staalnames en geschikte detectiemethoden. Daarom kan besloten worden dat het belang van deze parasiet bij de onderzochte slangen verwaarloosbaar is.

INTRODUCTION

In Belgium, data concerning the occurrence of *Cryptosporidium serpentis* in captive snakes is lacking. In order to obtain an estimate of the prevalence of this pathogen in 7 Belgian snake collections, gastric lavages were performed in 105 snakes belonging to 38 snake species and five snake families (Table 1). These snakes were part of the collections of private owners, reptile shops and a zoo. Snakes with a very diverse background concerning management and predisposing factors for cryptosporidiosis were included, and samples were obtained both from imported animals caught wild and from animals bred in captivity. The samples were analyzed with a direct immunofluorescence test (IFT) and with carbol fuchsin staining. As there is no effective treatment, the control and prevention of the condition deserve special attention.

MATERIALS AND METHODS

Snakes examined

Sampling was performed in snakes from four private collections, one zoo and two reptile shops. A file was created for every snake containing the following data: species, origin, age, sex, morphological characteristics, condition score and clinical status. For each sampling group the management conditions applied, hygiene and prey items were described. This was done to enable the identification of predisposing factors for cryptosporidiosis.

Sampling

Sampling consisted of a gastric lavage according to the following protocol:

Table 1. The number of sampled snakes for each species and subspecies included in the present.

Snake species	No. of snakes sampled for this study
1. Family Boidae	
a) Subfamily *Boinae* (boas)	
Acrantophis dumerili	3
Boa constrictor constrictor	3
Candoia aspera	2
Corallus caninus	2
Corallus hortulanus enydris	2
Epicrates cenchria cenchria	6
Epicrates cenchria maurus	3
Sanzinia madagascariensis	1
b) Subfamily *Erycinae* (sandboas)	
Eryx johnii	1
c) Subfamily *Pythoninae* (pythons)	
Antaresia childreni	1
Liasis mackloti savuensis	1
Morelia amethistina	1
Morelia spilota macrospila	1
Morelia spilota variegata	2
Python curtus	3
Python molurus bivittatus	1
Python regius	11
Python reticulates	2
2. Family Colubridae (typical snakes)	
a) Subfamily *Colubrinae*	
Boiga dendrophila melanota	4
Boiga dendrophila gemmicincta	1
Boiga drapiezii	1
Boiga tanahjampeana	3
Drymarchon melanurus	2
Elaphe guttata guttata	7
Elaphe obsoleta rossalleni	1
Elaphe situla	1
Lampropeltis getula californiae	8
Lampropeltis getula nigrita	4
Lampropeltis triangulum annulata	1
Lampropeltis triangulum campbelli	2
Lampropeltis triangulum sinaloae	1
Ortriophis taeniurus friesi	2
Ortriophis taeniurus ridleyi	1
Pantherophis obsoletus lindheimeri	1
Pituophis melanoleucus	2
Spilotes pullatus pullatus	1
b) Subfamily *Natricinae*	
Thamnophis sirtalis parietalis	2
c) Subfamily *Xenodontinae*	
Heterodon nasicus nasicus	1
Philodryas baroni	3
3. Family Cylindrophiidae (pipesnakes)	1
Cylindrophis ruffus ruffus	
4. Family Viperidae	
a) Subfamily *Crotalinae* (pitvipers)	2
Bothriechis schlegelii	1
Bothops asper	1
Trimeresurus albolabris	
b) Subfamily *Viperinae* (pitless vipers)	1
Cerastes cerastes	
5. Family Elapidae	
Subfamily *Elapinae* (cobras)	2
Naja haje legionis	2
Naja kaouthia	

The volume of PBS that had to be administered (2% of the body weight) was prepared in a syringe and the gastric tube was marked to prevent it from being inserted further than halfway the length of the snake's body, which is the position of the stomach (in the family of Boidae, the stomach is situated a little more caudally). In most snakes examined, tubes 0.52 m long and $5.3x10^{-3}$ m in diameter (Medicoplast, Jillingen, Germany) were used. In the smaller specimens, tubes 0.50 m long and $2.7x10^{-3}$ m in diameter (Sherwood Medical, Tullamont, Ireland) were used. Due to the size of the tubes, sampling was restricted to snakes with a body length of between 0.90 and 1.00 m (without the tail). After the syringe containing PBS was fixed to the tube, the mouth of the snake was opened, with an extra person assisting to stretch the snake's body, and the tube was inserted. In some animals a considerable resistance could be felt, which was due to contractions of the somatic muscles. This could be overcome by applying gentle pressure, by rotating the tube a little bit or by lubricating the tube. When the tube had reached the stomach, the PBS was injected, followed by some air to clear all fluid out of the tube. Then, the tube was withdrawn a few mm and the PBS was aspirated. The animal was held head-down to facilitate this procedure. The aspirated sample was collected in a sterile poly-ethylene recipient and stored at 4°C for a maximum of five days. For each snake, the volume of PBS which could be aspirated was noted. After extubation, the mouth mucosa was inspected for lesions.

Detection of *Cryptosporidium* oocysts

All samples were examined for the presence of *Cryptosporidium* oocysts with the direct immunofluorescence test (IFT) (Merifluor® Meridian Diagnostics Inc., Cincinatti, Ohio, USA) as described by Graczyk *et al*. (1996) and Harr *et al.* (2000). After centrifugation (3000 rpm, 5 minutes), 20µl of the sediment were transferred to a well on a pre-treated microscopic slide, and one drop both of detection reagent and of counterstain was added. After rinsing, the samples were screened for fluorescent green positive, round to oval oocysts. The first twenty samples were additionally examined using a carbol fuchsin stain (Graczyk and Cranfield, 1996a; 1996b) in order to compare the two methods. To do this, a variable amount of sediment was applied on a microscopic slide with a transfer loop, three drops of carbol fuchsin stain were added and a smear was made. Light microscopy was used for the detection of oocysts with correct dimensions and morphology.

RESULTS

Samples were collected from 105 snakes, belonging to 5 families, 25 genera and 38 species. Twenty-five samples originated from reptile shops, 42 from private keepers and 24 from a zoo. The average sampled snake weighed 493 grams and was 0.82 m long. The majority of snakes were sub-adults and adults. The study included 51 males, 42 females and 12 snakes of unknown sex. Seventy-one animals were captive bred, 18 were wildcaught and 16 snakes were of unknown origin. Most of this data was provided by the owners. About 90% of the animals were in good condition, a few snakes showed mild to severe clinical signs, such as chronic stomatitis and cachexia, but none of these signs were indicative of cryptosporidiosis. The average volume of PBS that could be aspirated was 56.6% of the administered volume.

The samples analyzed with IFT and carbol fuchsin were all negative for cryptosporidia. The positive and negative IFT controls yielded the results described by the manufacturer. The samples only contained background material, which was of a dull orange or red color. Sometimes green fluorescent particles could be seen, but these did not show the characteristic dimensions or morphology of *Cryptosporidium* oocysts.

DISCUSSION

The objective of the present study was to gain insight into the prevalence of *C. serpentis* in snakes kept in Belgium. To this end, gastric lavages were performed in private collections and in a zoo, as well as in reptile shops and in recently imported animals. Thus, results were obtained from animals living in more or less closed collections with suitable management, but also from snakes kept in less favorable conditions. For example, snakes in shops often live in small enclosures and come into contact with newly acquired animals quite regularly. Furthermore, shops do not always offer the perfect environmental conditions for every species and sometimes hygiene is a problem as well. All these disadvantageous conditions were also applicable to one private collection, which almost exclusively consisted of recently imported animals. These animals were facing the stress of recent transport and were more susceptible to infection with opportunistic pathogens. However, it must be stated that most snakes in this collection were in good health.

It has already been demonstrated that immune suppression underlying disease, stress and bad hygiene promote cryptosporidiosis in several animal species and in man (Tzipori, 1983; Fayer and Ungar, 1986; O'Donoghue, 1995; Tzipori and Griffiths, 1998). However, due to the absence of

C. serpentis in the snake collections studied, no predisposing factors for cryptosporidiosis could be identified.

The average length of the snakes in this study made it possible to use tubes of 50 cm. The volume of PBS administered was 2% of the body weight. The average aspirated volume was 56.6% of the original volume. This is in agreement with the results of the gastric lavages performed in snakes by Graczyk *et al.* (1996). Therefore we can assume that the tubes and the PBS actually entered the stomach and that the sampling was well performed.

Snakes have a low feeding frequency and can fast for weeks or even months. Therefore, the physiology of their gastro-intestinal system is totally different from that of other animal species and man (Secor 2003). The stomach of a snake has an impressive capacity to upregulate its mechanical and metabolic activity following a meal. As *C. serpentis* infects the stomach, most of the parasitic properties are related to this intermittent gastric activity. A low gastric activity results in low multiplication and excretion of the parasite (Harr *et al.*, 2000). In this respect, Graczyk *et al.* (1996) and Harr *et al.* (2000) found that the number of positive *C. serpentis* results was negatively correlated with the interval between the last meal and sampling. Although most of the snakes in the present study were clinically healthy and had a good appetite, sampling mostly was performed several days or even weeks after their last meal. This may thus have caused false negative results.

Although there have been individual reports of snake cryptosporidiosis in Europe (Valentin *et al.*, 1998), prevalence data is not yet available. Based on studies in the USA and papers demonstrating the wide geographical distribution of cryptosporidia (Kilani and Wenman, 1994; Morgan *et al.*, 1999), there is no reason to believe that cryptosporidiosis is not present in Belgian snake collections. However, in the present study none of the snakes tested positive for *C. serpentis*, which makes us conclude that its importance in Belgium is rather low.

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