CRYPTOSPORIDIOSIS IN SNAKES

Cryptosporidiose bij slangen

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ABSTRACT

Characteristically, cryptosporidiosis in snakes is caused by *Cryptosporidium serpents*. This parasite affects the stomach and may cause considerable morbidity and mortality. This report describes an overview of the current literature. After the importance and prevalence of this parasite are demonstrated, the pathogenesis and the related pathology and clinical signs are described. Subsequently, the different diagnostic methods and therapeutic approaches are mentioned, as well as their use in the prevention and control of cryptosporidiosis in snake collections.

SAMENVATTING

Doorgaans wordt cryptosporidiose bij slangen veroorzaakt door *Cryptosporidium serpents*. Deze maagparasiet kan voor aanzienlijke ziekte en sterfte zorgen. In dit artikel wordt een overzicht van de huidige literatuur gegeven. Na het aantonen van het belang en voorkomen van deze parasiet worden de pathogenese en de ervolgend voortvloeiende pathologie en klinische symptomen beschreven. Vervolgens worden de verschillende diagnostische methoden en therapeutische mogelijkheden aangehaald, evenals hun nut bij de preventie en de bestrijding van cryptosporidiose binnen slangencollecties toegelicht.

HISTORY AND ETIOLOGY

Brownstein *et al.* (1977) wrote the first complete report on snake cryptosporidiosis. In the mean time, the parasite has been found in over 40 snake species (O’Donoghue, 1995). The species most frequently isolated in snakes was named *Cryptosporidium serpents* (Levine, 1980), although there have been occasional reports of *C. saurophylum, C. parvum* and *C. muris* (Xiao *et al.*, 2004). These two latter species could be pseudoparasites originating from prey items. In contrast with most other *Cryptosporidium* species, *C. serpents* mainly infects the stomach, not the gut. *C. serpents* is a monoxenous (asexual and sexual multiplication in one host) parasite, with endogenous sporulation of oocysts and fecal-oral transmission.

PREVALENCE AND GEOGRAPHICAL DISTRIBUTION

Cryptosporidiosis in snakes is widely distributed (Morgan *et al.*, 1999), with prevalences of 4% to 73% being found in the USA (Graczyk *et al.*, 1996b; Lee *et al.*, 2002; Xiao *et al.*, 2004) and Brazil (Kararsawa *et al.*, 2005), with an average higher prevalence in captive snakes than in wild ones. Although cases of snake cryptosporidiosis have been reported in Europe (Valentin *et al.*, 1998), no prevalence data is yet available.

PATHOGENESIS AND PATHOLOGY

*C. serpents* is a facultative pathogen. Several factors such as infectious dose, immunity, co-occurrence of other potential pathogens and virulence differences between...
cryptosporidia strains may play a role in infection (O’Donoghue, 1995). *C. serpen­tis* occurs in wild as well as in captive snakes and is responsible for a high morbidity and mortality, especially in this latter group (Lee et al., 2002).

The most apparent pathological changes in a *C. serpen­tis* infected snake can be found in the stomach. Lesions can vary from mucosal petechiae to multiple focal necroses of the gastric mucosa and increased slime production (Fayer, 1990). The most typical abnormality, however, is an enormous thickening of the gastric wall, which prevents the normal passage of prey items. This provides an explanation for the regurgitation of undigested prey one to three days after feeding (O’Donoghue, 1995).

Microscopic lesions consist of hyperplastic and hypertrophic gastric glands and submucosal edema (Fayer, 1990). Cranfield and Graczyk (1994) mentioned damaging of the brush border of the gastric epithelial cells. Infiltration of lymphocytes, heterophils and macrophages in the lamina propria was also described. Except for the gastric pathology, there have also been reports of catarhal, fibrinous to diphtheroid-necrotic enteritis in *Cryptosporidium* infected snakes. In these cases, the gastric mucosa was hyperemic, but never clearly affected (Valentin et al., 1998). Cimon et al. (1996) described a case of cryptosporidiosis in two *Elaphe guttata guttata* (corn snakes) in which only the bile ducts and the biliary blad­der were affected.

CLINICAL SIGNS

Subclinical infections

Cryptosporidiosis is a disease with high morbidity and variable mortality. The immune status of the animal and the infection pressure seem to play a determining role (Cranfield et al., 1992; O’Donoghue, 1995). This is illustrated by the fact that the disease often can be associated with recent import and captivity (O’Donoghue, 1995; Karasawa et al., 2005). The various rattlesnake species, which are easily stressed, as well as amelanistic snakes, are all more vulnerable to developing the disease (Harr et al., 2000). Infected animals can shift from years of high oocyst shedding to periods of low excretion without clinical signs. In wild populations, only subclinical infections have been noted, which is probably due to the low infection pressure in nature (Upton et al., 1989; Fayer, 1990).

Clinical infections

The incubation period is unknown and although there have been reports of young snakes with overt disease (Godshalk et al., 1986), others suggested that cryptosporidiosis mainly affects adult animals (Brownstein et al., 1977; Carmel and Groves, 1993).

Clinical signs in snakes with gastric infection include persistent or intermittent postprandial regurgitation one to three days after feeding, diarrhea, an externally visible bulge in the gastric region, weight loss and lethargy. The disease slowly progresses and results in death (Brownstein et al., 1977; Frey, 1991). According to Frye (1991), gastric enlargement and chronic postprandial regurgitation are pathognomonic for gastric cryptosporidiosis, although the externally visible gastric bulge was not noted in other cases (Graczyk et al., 1998).

Some cases of *Cryptosporidium* infections in snakes only resulted in enteric symptoms of maldigestion, progressive cachexia, occasional vomiting and diarrhea. Bacterial and viral co-infections could often be demonstrated in these snakes (Valentin et al., 1998).

DIAGNOSIS

Gastric lavage

This technique is very suitable for the diagnosis of subclinical *C. serpen­tis* infection (Harr et al., 2000). It is performed by intubating the animals intra-gastrically to administer 2% of their body-weight of phosphate-buff­ered saline (PBS), which is subsequently aspirated. After centrifugation (7500g, 15 min), the sediment of the samples is examined for the presence of *Cryptosporidium* oo­cysts by means of the Merifluor® test (Meridian Diagnostics Inc., Cincinnati, Ohio, USA). Merifluor® is a direct immunofluorescence test (IFT) designed for the simultaneous detection of *Cryptosporidium* oocysts in feces. *Cryptosporidium* oocysts can also be detected by acid fast staining of smears made from the sediment of gastric lavage samples (Graczyk et al., 1996b). Gastric lavage of infected snakes produces significantly more positive test results three days after feeding than seven days after feeding, regardless of whether IFT or staining is used (Graczyk et al., 1996b; Harr et al., 2000).

Fecal examination

Clinically affected snakes shed large amounts of oo­cysts via their feces (Carmel and Groves, 1993). However, this fecal elimination is often intermittent, which can result in false negative results on fecal examination (Graczyk and Cranfield, 1995; 1996a; Karasawa et al., 2005). An additional difficulty is the low feeding and de­fication frequency in snakes, which makes sampling more difficult than in other animal species. Other ham­pering factors are the low numbers of oocysts shed by sub-
clinically infected snakes and the unsatisfying detection limits. Therefore, Graczyk and Cranfield (1995) postulated that fecal examination does not suffice to make managerial decisions in preventing cryptosporidiosis in snakes.

Several methods have been described to detect fecal oocysts. For example, samples can be stained with an acid-fast stain or with carbol fuchsin after sedimentation. When using stains, one has to take into account the fact that oocysts are more easily masked by the substrate when feces are being stained than when gastric lavages are being stained (Harr et al., 2000; Karasawa et al., 2005). A second possibility is to detect fecal oocysts through immunofluorescence. This technique has a better detection limit than staining (Graczyk and Cranfield, 1995; Graczyk et al., 1996b). A technique that is less frequently used is the analysis of fecal samples using PCR to detect the presence of the 18S rRNA gene (Kimbell et al., 1999), the COWP gene (Xiao et al., 2000a) or the SSU rRNA gene (Xiao et al., 2004) of C. serpentis.

**Mucus sample**

Postprandial regurgitation is a frequent clinical sign of cryptosporidiosis. Therefore, the mucus covering the regurgitated prey can be sampled and examined by means of an acid-fast stain (Graczyk et al., 1996b).

**Post-mortem histology, gastric biopsy and cytology**

These are the most reliable methods to detect Cryptosporidium. Examination of several histological sections of gastric tissue upon necropsy is the most definitive technique for diagnosis (Cranfield and Graczyk, 1994). Gastric biopsy is possible through either gastroscopy or endoscopy. However, Graczyk and Cranfield (1996a; b) stated that this technique might lead to confusing, false negative results due to the random distribution of cryptosporidia in the gastric mucosa.

**DIFFERENTIAL DIAGNOSIS**

Other infectious pathogens, wrong management, neoplasia, and acute stress due to manipulation and liver- or kidney failure can also cause vomiting (Cranfield and Graczyk, 1996). In the family of Boidae (boas and pythons), chronic regurgitation is often due to a viral disease known as Inclusion Body Disease (Van Craeynest et al., 2006).

**TREATMENT**

**Hyperimmune bovine colostrum (HBC) therapy**

This treatment has resulted in positive effects in AIDS patients with cryptosporidiosis (Greenberg and Cello, 1996). It has also proved to be beneficial in several animal species (Tzipori et al., 1994). Therefore, it was also evaluated for the treatment of snakes (Graczyk et al., 1998). Colostrum was obtained from a C. parvum immunised cow and administered to C. serpentis infected snakes. An increasing number of HBC treatments resulted in a decrease in oocyst shedding. Animals that were clinically ill were not cured, but the number of oocysts that could be demonstrated was much lower than expected on the basis of the lesions. In subclinically infected snakes, no Cryptosporidium stadia could be found in histological sections upon necropsy. Therefore Graczyk et al. (1998) considered them cured and suggested that HBC treatment can play an important role in the prevention and control of cryptosporidiosis in snake collections.

**Halofuginone, spiramycin, trimethoprim-sulfonamides and paromomycin**

Due to their beneficial effects in the treatment of mammals with cryptosporidiosis, these drugs were also suggested for the therapy of snakes. Although spiramycin (80-160 mg/kg, p.o.; three times a day), halofuginone (0.5-1 mg/kg/day or alternate day therapy, p.o.) (Cranfield and Graczyk, 1994; Graczyk et al., 1996a) and trimethoprim-sulfonamides (30mg/kg/day, p.o.) (Mirtschin and Ormerod, 1990; Valentin et al., 1998) resulted in a decrease in oocyst shedding and environmental contamination, the treatments were not able to eliminate the pathogen. Paromomycin (100mg/kg/day, p.o.) induced no changes in the shedding pattern (Valentin et al., 1998). Clinical signs, such as postprandial regurgitation, were not influenced by any of the treatments, and hepatotoxic and nephrototoxic changes were obvious after halofuginone administration (Graczyk et al., 1996a).

**PREVENTION AND CONTROL**

Due to the excretion of high numbers of highly resistant oocysts, high infection pressures may arise within the occasionally limited enclosures of captive snakes, especially when hygiene and proper management are lacking. Wild living snakes face lower infection pressure and less predisposing factors. The endogenous sporulated oocysts released in the environment have a long survival time and are resistant against a high number of dis-
infectants (Holton et al., 1994). For example, oocysts can survive up to three months at 25-30°C, the temperature which is preferred by most snakes (Fayer et al. 2000). Oocysts also remain viable after routine chlorination of water (Zu et al., 1992). Some possible strategies to destroy the infectivity of Cryptosporidium oocysts are the application of hypochlorite, ammonia, formaldehyde, lyophilization and exposure to temperatures above 65°C for 30 minutes (Valentin et al., 1998).

To avoid the intake of Cryptosporidium in collections, a good screening procedure is important. It is of utmost importance to detect subclinically infected animals and intermittent oocyst shedders. The best way to do this is to examine gastric lavages (Graczyk et al., 1996b). Wild-caught animals and sensitive species should be observed very closely when they are purchased and should be checked several times during a quarantine period of minimum 30, but ideally 90 days. A routine control of all animals in zoo collections, followed by isolation or euthanasia of affected animals, is advised.

The distribution of C. serpentis via water is very efficient, which may have important implications for wild as well as for captive populations (Cranfield and Graczyk, 1994). Methods have been described to examine water samples for the presence of Cryptosporidium oocysts and diagnostic tools have been developed to identify several cryptosporidial species in these samples (Xiao et al., 2002). The application of this knowledge to prevent and control outbreaks of cryptosporidiosis in snake collections has not yet been reported.

The role of prey items in the transmission is still a point of debate. Carmel and Groves (1993) stated that transmission via mice is possible. However, others have shown that prey animals are refractory hosts (Koudela and Modry, 1998). Other authors postulate that cryptosporidia of mice can cause false positive results in the screening of snakes. By submitting prey items to a freeze-thaw cycle, the oocysts, which may be present in their intestines, will be digested in the stomach of the snake (Graczyk et al., 1996b). Serological tests may be an additional tool in the screening of collections (Graczyk et al., 1996b).

**Zoonotic Aspect**

Up to now, there have been no reports of C. serpentis infections in humans. However, the occurrence of such an infection in immunocompromised patients cannot be excluded (Xiao et al., 2000b). Moreover, snakes may spread cryptosporidial oocysts of prey items, including C. parvum, which is an important zoonotic pathogen.

**REFERENCES**


Graczyk T.K., Cranfield M.R., Owens R. (1996b). Diagnosis of subclinical cryptosporidiosis in captive snakes based on
stomach lavage and cloacal sampling. *Veterinary Parasitology* 67, 143-151.


