CRYPTOSPORIDIOSIS IN SNAKES

Cryptosporidiose bij slangen

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ABSTRACT

Characteristically, cryptosporidiosis in snakes is caused by *Cryptosporidium serpentis*. 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 serpentis*. 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 eruit 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 serpentis* (Levine, 1980), although there have been occasional reports of *C. saurophilum*, *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. serpentis* mainly infects the stomach, not the gut. *C. serpentis* 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 (Karasawa *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. serpentis 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 catarrhal, 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., Cincinatti, Ohio, USA). Merifluor® is a direct immunofluorescence test (IFT) designed for the simultaneous detection of *Cryptosporidium* and *Giardia* 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 defecation 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 gastrotomy 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 (Vancraeynest *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 nephrotoxic 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.

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