DOI: 10.5433/1679-0359.2019v40n6Supl3p3797

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Canine isosporosis: a neonatal clinical approach

Isosporose canina: uma abordagem clínica neonatal

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Abstract

Acute diarrhea in young dogs is among the main complaints described by tutors in veterinary services. Complications related to water and electrolyte imbalances due to intestinal alterations can compromise homeostasis in these pups, mainly due to dehydration. Consequently, they do not present a satisfactory clinical response and are exposed to the risk of early death. Among the different types of diarrhea, the infectious diarrhea due to viruses, bacteria, and parasites are particularly significant. Coccidia of the genus *Isospora* (*Cystoisospora* spp.) is an important intestinal pathogen that causes severe clinical manifestations in puppies. Since high parasitic loads render these puppies highly susceptible to opportunistic infections, coccidia infection may cause impaired corporal and cognitive development and even lead to death. Studies regarding this issue, particularly concerning clinical knowledge in neonatology, are scarce in veterinary medicine. Thus, in this study, we intend to discuss the enteric alterations and the most commonly used current diagnostic techniques, as well as to analyze effective therapeutic and prophylactic proposals.
Key words: Diarrhea. *Cystoisospora* spp. Enteritis. Pups.

Resumo

As diarreias agudas em cães jovens estão entre as principais queixas descritas pelos tutores nos serviços veterinários. As complicações relacionadas aos desequilíbrios hídrico e eletrolítico decorrentes das alterações intestinais comprometem a homeostase destes filhotes, principalmente devido a desidratação, não apresentam resposta clínica satisfatória e ficam expostos ao risco de morte precoce. Dentre os diferentes tipos de diarreia, sobressaem as de origem infecciosa. Os agentes podem ser vírus, bactérias, fungos e parasitos. Entre estes últimos agentes, os coccídios do Gênero *Isospora (Cystoisospora* spp.) se destacam como patógenos intestinais que ocasionam manifestações clínicas graves em filhotes, pois elevadas cargas parasitárias propiciam condição de maior susceptibilidade à infecções oportunistas, podendo também prejudicar o desenvolvimento corporal e cognitivo e/ou até mesmo levar a morte do

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Received: Nov. 22, 2018 - Approved: Sept. 30, 2019

cão. Diante de tal temática, na medicina veterinária os estudos são escassos, principalmente quanto ao conhecimento clínico em neonatologia de tal modo tencionamos discutir a respeito das alterações entéricas, métodos de diagnóstico mais utilizados e atuais, bem como analisar as propostas terapêuticas e profiláticas efetivas.
Palavra-chave: Diarreia. *Cystoisospora* spp. Enterite. Filhote.

Introduction

Isosporosis is a disease caused by a protozoan of the genus *Isospora*, which is responsible for causing diarrhea in dogs of several age groups and mainly in puppies (SANTOS, 2013; SCHÄR et al., 2014; HOPPE; MORAES, 2018). Infections of clinical importance are associated with the spreading capacity of *Isospora* spp. oocysts, observed in neonates or young animals; the main transmission route is fecal-oral (JOACHIM et al., 2018).

Mortality is approximately 30% to 40% in neonates. Among the causes of death are illnesses that involve dehydration, diarrhea, inadequate nutrition of the mother, and lack of hygienic-sanitary measures at the breeding place. The same factors are involved in the pathogenesis of isosporosis in dogs (RAMOS et al., 2016). However, different pathogenic agents, such as viral, bacterial, and parasitic (helminths and protozoa), are associated with acute canine gastroenteritis in puppies (DUIJVESTIJN et al., 2016). Thus, diarrhea in neonatal dogs and puppies comprises several causes that should be better investigated with differential diagnostic methods, including important tools, such as coproparasitological techniques (RAMOS et al., 2016).

The increase in the number of household pets, mainly dogs and cats, is associated with a higher risk of contact with zoonotic potential agents, including parasites (FERREIRA et al., 2016). Considering these dynamics, the type of breeding management certainly modulates the dissemination of various diseases, including isosporosis (RAMOS et al., 2016). Research on parasitic diseases in cats and dogs incites increasing interest in the context of public health, since pets and their tutors are constant companions (AL-JASSIM et al., 2017). Thus, hygienic-sanitary measures seem to be the basis for successful prevention and control of various parasitic diseases, especially against isosporosis (RAMOS et al., 2016)

Despite the long-term recognition of the importance of small animal clinical practice in veterinary parasitology, studies specifically concerning the coccidians of the genus *Isospora* spp. are still rare in literature. Therefore, in this study, we intend to elucidate the clinical, parasitological, and therapeutic approaches of coccidia.

Epidemiology

Dogs may be infected by *Isospor*a or *Cystoisospora canis* (most frequently diagnosed), or by the *Cystoisospora ohioensi*s complex, which is composed of morphologically undifferentiated structures*, C. ohioensis* and *Cystoisopora burrowsi*. (JOACHIM, et al., 2018). Although both may cause clinical disease, *C. canis* is the most pathogenic species, while the *C. ohioensis* complex is the most commonly detected (COELHO, et al., 2012; RAMOS et al., 2016; JOACHIM, et al., 2018).

Isosporosis can be found in several breeding systems and types of sanitation. Conditions of poor hygiene and overcrowding of animals can lead to outbreaks in commercial kennels or veterinary clinic wards, due to the increase in the number of births or introduction of new dogs and cats that are already infected (BRESCIANI et al., 2015).

The small size and weight of oocysts allow them to be easily dispersed by wind, water, and contaminated fomites, or by clothing, shoes, and hands of people, as well as by vehicles for transportation of dogs. Thus, these oocysts are easily carried from one place to another, disseminating the infection (BRESCIANI et al., 2015).

It is important to note that the main form of transmission is the fecal-oral route—the ingestion of non-sporulated oocysts eliminated with feces, which then contaminate the environment and food (RAMOS et al., 2016). Puppies are definitive hosts that also become infected by the ingestion of tissue cysts present in paratenic hosts, such as rats and mice (BRESCIANI et al., 2015). Stressed or immunocompromised animals are more susceptible to this infection, as well as those animals that live in an overcrowded environment with poor sanitation (RAMOS et al., 2016).

Neonates become infected within the first three weeks of life, i.e., before weaning. However, at this stage of neonatal development, puppies are not able to eliminate feces by themselves and depend on the maternal stimulus of licking the perianal region. In most cases, the mother ingests the fecal contents, making it difficult to obtain these stool samples (JOACHIM et al., 2018).

Release of oocysts of *Isospora* spp. into the environment has been observed before 3 weeks of age, although a delayed excretion has been described, and this is directly related to the environmental pressures imposed on the litters (JOACHIM et al., 2018). Several studies have confirmed the occurrence of *Isospora* spp. in samples of dogs worldwide. A prospective, 15-month study of 61 dogs in western Uganda rural area showed a 97.1% rate of intestinal parasitism. *Isospora* spp. was diagnosed in 13.3% by microscopy through the flotation-sedimentation technique (HYEROBA et al., 2017). In East Japan, only 1.2% of *Isospora* spp. of a total of 573 animals was detected using direct microscopy through the sedimentation of fomalin-ethyl acetate (ITOH et al., 2015). In Africa, a study in the Republic of Ghana identified 8.6% positivity of the parasite cited in 380 dogs by the modified McMaster technique (JOHNSON et al., 2015).

In Brazil, 3099 fecal samples of dogs were collected at the Veterinary Hospital of the Faculty of Veterinary Medicine of São Paulo, located in the metropolitan region of São Paulo, SP. This material was processed by flotation and centrifugation-sedimentation techniques, and 46 (1.5%) *Isospora* spp. positive samples were detected (FERREIRA et al., 2016).

In a study performed in northern Poland, 339 fecal samples from dogs were collected from lawn and green areas with direct sunlight for three consecutive years. All samples were examined according to the modified Baermann technique. *Cystoisospora* spp. was found in 10.9% of the total samples. The authors suggest that environmental persistence can be induced by the resistance of the animals to the active principles used by their tutors or due to the low assiduity in the process of deworming during the years (FELSMANN et al., 2017).

In the Small Animal Hospital of the Faculty of Veterinary Medicine at the University of Lisbon, an interview was conducted with 243 tutors of dogs. Approximately 89.7% of participants stated that they used drugs against endoparasites; however, only 11.8% stated that they correctly followed the manufacturer's instructions. Thus, a majority of the tutors administered products indiscriminately and consequently and at ineffective intervals (MATOS et al., 2015).

The environmental survival of the sporulated oocyst of *Isospora* spp. can last for months, due to resistance capacity to routinely used disinfectants (DAUGSCHIES et al., 2013). The largest concentration of research and industry to produce parasiticides for pet animals (52% of the total) is in the United States of America; only 1% is in the African continent, further contributing to the greater occurrence of neglected parasitic diseases in these countries, due to the country's local political issues or lack or failure of public health programs (OTRANTO, 2015).

Dogs from rural communities, especially in economically developing countries, are susceptible and participate effectively in the maintenance and

spread of infectious diseases among themselves or even present as zoonotic threats to public health. However, in economically developed countries, urban expansion has reduced free spaces for tutors to walk their dogs, thus, forcing them to use small areas, such as parks or beaches; sharing this space with other animals and humans maximizes the risk of environmental contamination (FELSMANN et al., 2017).

Pathogenesis and Cycle

Coccidia show similar biological characteristics among themselves; however, four distinct endogenous stages are common to all species of *Isospora*—excystation, schizogony, gametogony, and sporogony. An extra-intestinal phase can also occur in definitive and paratenic hosts in which monozoic tissue cysts are formed, containing a sporozoite in the interior portion (called a hypnozoite) (DUBEY et al., 2009).

Infection with *Isospora* spp. causes inflammation of the intestinal wall, with epithelial destruction. The transmission of this parasite occurs by the elimination of oocysts in the feces of infected animals that can contaminate the environment. In this manner, oocysts that are ingested with food or water settle in the gut and penetrate the epithelial cells. In combination with inadequate food, adverse climatic conditions, or poor conditions of management, a greater or lower severity of the disease can occur (VASCONCELOS et al., 2008). While *C. canis* is located in the proximal portion of the small intestine, inducing enteritis due to direct lesion of the mucosa (due to schizogony and gametes), the *C. ohioensis* complex infects the enterocytes in the lamina propria of the small intestine, cecum, and colon, causing atrophy of intestinal villi, necrosis of enterocytes, and inflammation of intestinal crypts (BRESCIANI et al., 2015).

During the acute phase of infection, which occurs in the intestine, the occurrence of intense cellular necrosis can be observed. In the chronic phase, tissue cysts and mucosal atrophy are seen with inflammation of the lamina propria, loss of intestinal villi, and hyperplasia of Peyer's plaques (JONES et al., 2000). After the complete endogenous development of *Isospora* spp., the oocysts are excreted through the feces and sporulate in the environment in a few days (RAUSCHER et al., 2013).

Immunity develops after the first infection and is more effective with advancing age of the exposed animals. Immunosuppression in dogs and cats facilitates reactivation of tissue cysts with release of sporozoites contained within these structures. Therefore, the enteric cycle restarts and causes recurrence of intestinal coccidiosis (BRESCIANI et al., 2015).

Clinical Manifestations

The clinical manifestations of isosporosis vary according to the species involved (BRESCIANI et al., 2015). In general, frequent symptoms in dogs are aqueous diarrhea with mucus or blood, abdominal pain, selective appetite, anorexia, dehydration, anemia, weakness, vomiting, fever, emaciation, decreased growth, mental depression; death can occur in severely infected animals (MITCHELL et al., 2009).

Diagnosis

Diagnosis is made by the detection of oocysts of *Isospora* spp. in fecal samples associated or not with the onset of diarrhea. Parasitological tests, such as centrifugal-flotation in zinc sulphate (Faust technique) or a sugar saturated solution (Sheater's technique), fluctuation in saturated sodium chloride solution (Willis-Mollay's technique), and in vitro sporulation followed by oocysts morphometry can be performed (BRESCIANI et al., 2015). Due to the cycle of *Isospora* spp., diarrheal samples analysis does not result in visualization of oocysts (BRESCIANI et al., 2015).

Coprophagia is a common behavior in dogs, resulting in ingestion of their own, other dogs', and even other species' feces. This behavior could make diagnosis more difficult, since the ingestion of non-canine feces changes the way different parasite structures can be observed and identified morphologically, thus resulting in an inconclusive coproparasitological diagnosis (NIJSSE et al., 2014).

Treatment

In diseases with acute gastrointestinal involvement, the first therapeutic measure is correction of water and electrolyte disturbances. Patients are presumed to have lost a sufficient amount of isotonic fluids until they become dehydrated due to diarrhea. Restoration of normal circulating volume is an immediate priority both to prevent functional renal damage, to minimize further damage to the gastrointestinal tract, as well as to maximize the action of drugs used for treatment. Thus, the administration of fluids in these patients is critical (MARTINS; SHIH, 2015).

Considering the pre-patent periods mentioned above, the definition of a treatment should consider two steps; the application of drugs that block the release of oocysts (metaphylaxis) and the improvement of clinical signs. The first step, which is the treatment administered during metaphylaxis, seems to be the most effective in the control of clinical signs (when there is still no lesion in the intestinal epithelium) and in the reduction of oocyst excretion. In order to evaluate a possible influence of other enteric pathogens of importance in puppies, especially *Giardia* spp., other diagnostic tests should be performed (JOACHIM et al., 2018).

The administration of sulfamethoxazole and trimethoprim, along with supportive therapies, such as restoring of physiological water and electrolyte balance, and the administration of metronidazole resulted in clinical recovery and control of spread of *Isospora* spp. oocysts (CARVALHO, 2015).

However, sulfas are known to precipitate crystals in the renal tubules and promote acute interstitial nephritis by a sensitivity reaction, consequently leading to acute renal failure. Therefore, patients need rehydration, mainly for satisfactory drug action and correction of water imbalance (CARVALHO, 2015). Table 1 lists the recommended drugs for canine therapy, regardless of the patient's age.

In conditions of acute diarrhea, the rates of water and electrolyte loss must be considered. In addition, it is necessary to measure serum and electrolyte replacement, especially potassium. The identification and correction of glycemic concentrations is always recommended in animals with clinical signs of weakness, hypothermia, and even stupor. This can be carried out by intermittent bolus or continuous infusion, associated with crystalloid solutions (LEE; COHN, 2017).

Table 1. Main drugs used in the treatment of canine isosporosis (adapted from Bresciani, 2015).

Active principle	Doses (mg/kg)	Interval between doses (hours)	Treatment duration (days)	Route of Administration
Amprolium	300 a 400	24	5	PO
Diclazuril	25	24	1	PO
Sulfadimethoxine	50 a 60	24	5 a 10	PO
Sulfaguanidine	100 a 200	8	5	PO
Toltrazuril	15	24	3	PO
Trimethoprim	50	24	5	PO, SC
Sulfonamide	15 a 30	12 a 24	-	-

- Table caption: SC: subcutaneous; PO: Orally.

To evaluate the effectiveness of the treatment against *Isospora* spp. in the litter, it is recommended that fecal samples be collected and examined weekly, in an individualized manner, thus, allowing for the correct identification of puppies with potential environmental release of oocysts, until weaning (JOACHIM, et al., 2018).

Prophylaxis and Control

Control measures involve the isolation of infected animals from animals with other types of diseases, reduction of overcrowding in kennels to avoid contact of healthy dogs with oocysts spread by infected dogs, and finally, awareness of an adequate hygienic environment and fomites with disinfection using solutions containing quaternary ammonia (HOPPE; MORAES, 2018). Adequate disinfection of incubators, eaters, and drinkers is important. Moreover, personal hygiene measures between veterinarians and nurses need to be strictly followed, such as hand washing before and after handling the patient, as well as the use of gloves. If necessary (as in diarrheal cases), coats could be worn for further protection (MURTAUGH, 2006; RAMOS et al., 2016). The control of insects where animals live also helps to reduce the number of infected animals, since insects can transmit oocysts from one environment to another (RAMOS et al., 2016).

Final Considerations

Canine isosporosis is a disease that affects neonates and pups, resulting in water-electrolyte imbalance due to diarrhea. In addition, coccidiosis favors the installation of opportunistic agents and/or promotes fragility in the health status of the animal with consequent risk of death. Intimate and necessary dependence between the mother and offspring may be problematic in perpetuating the environmental cycle; therefore, prophylactic measures need to be adopted. However, hygienic-sanitary measures are the basis for the success of the prevention and control of this parasitosis.

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