

## CONCURRENT CRYPTOSPORIDIOSIS AND EHRlichIOSIS IN A PUP

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DOI 10.29005/IJCP.2024.16.2.107-109}

[Received: 03.10.2024; Accepted: 26.11.2024]

**How to cite this article:** Tresamol, P.V., Saranya, M.G. and Ajithkumar, S. (2024)., Concurrent Cryptosporidiosis and Ehrlichiosis in a Pup. Ind. J. Canine Pract., 16(2): 107-109..

*Cryptosporidiosis* is a parasitic gastrointestinal disease caused by enteric protozoa, *Cryptosporidia* spp., affecting a wide variety of animals as well as humans. This report describes a case of mixed infection of cryptosporidiosis and ehrlichiosis in a pup and its successful therapeutic management. A four-month-old female Siberian Husky pup was presented with a complaint of vomiting and blackish diarrhea with foul smell. Clinical examination revealed fever, accelerated respiration and pulse rates and pale mucous membranes. On faecal examination free oocysts and budding cells suggestive of *Cryptosporidium* could be detected, which were confirmed as *Cryptosporidium parvum* based on the morphology by Modified Ziehl-Neelson staining and micrometry. Blood smear examination revealed morula of *Ehrlichia canis* in the monocytes. Haematological examination showed significant anaemia with neutrophilia, and thrombocytopenia. The animal was successfully treated with azithromycin and oxytetracycline along with supportive treatments including fluids, haematinics, aminoacids and hepatoprotectants. Considering the close association between companion animals and their owners, the role of dogs as a potential risk factor for the zoonotic infection with *C. parvum* should be investigated.

**Keywords:** Cryptosporidia, Ehrlichia, Pup, Treatment.

*Cryptosporidium* is a protozoan parasite that causes severe gastrointestinal disease in animals and humans and is distributed worldwide. It is mainly transmitted by faecal-oral route through contaminated water or food (Ryan *et al.*, 2014). Cryptosporidiosis has been identified as the cause of numerous waterborne and food-borne outbreaks of diarrhea worldwide. Cryptosporidiosis is reported to be one of the major public health concerns. Young animals are mostly affected with symptomatic infections and shed infective oocysts. But infection in adult animals is mostly asymptomatic with fewer oocysts shed in the faeces. Clinical manifestation of cryptosporidiosis includes acute to severe diarrhea along with abdominal pain. Infection is normally self-limiting in immunocompetent animals (Murnik *et al.*, 2022). Infection in dogs is mostly associated with *Cryptosporidium canis* (Itoh *et al.*, 2019; Julien *et al.*, 2019). But *Cryptosporidium parvum* has also been detected in dogs (Murnik *et al.*, 2022). Cryptosporidiosis can be diagnosed by detection of oocysts by

examination of modified Ziehl-Neelson stained faecal smears, faecal floatation techniques, ELISA, Fluorescent antibody tests and polymerase chain reaction. Canine monocytic ehrlichiosis is an important canine tick-borne disease, caused by *Ehrlichia canis* and transmitted by the brown dog tick, *Rhipicephalus sanguineus*. The disease is characterized by fever, depression, lethargy, anorexia, lymphadenomegaly, splenomegaly and bleeding abnormalities. Thrombocytopenia occurs in most dogs in all phases of the disease. Persistent infection in dogs leads a more lethal form of chronic disease where bone marrow and immune system are severely affected. As a result, other opportunistic pathogens will establish infection and aggravate the situation (Aziz *et al.*, 2022). Even though the indirect immunofluorescence antibody (IFA) test is considered as the serological 'gold standard' test for diagnosis of ehrlichiosis, several enzyme-linked immunosorbent assays and molecular techniques are also useful for the diagnosis of ehrlichiosis.

The present study describes mixed infection of cryptosporidiosis and Ehrlichiosis in a pup with successful therapeutic management.

### Case History and Diagnosis

A four-month old female Siberian Husky pup weighing 5.6 kg was presented to the University Veterinary Hospital, Mannuthy, with a history of vomiting and blackish diarrhea with foul smell since two weeks, with poor response to prior treatment with antibiotics and vitamin supplements. Clinical examination revealed fever (103.8°F), accelerated respiration (46/minute) and pulse rate (90/minute) and mucous membrane was pale. The pup was anorectic and dehydrated. A lateral flow technique using faecal swab was negative for canine parvoviral antigen. Microscopic examination of wet blood film was also negative for any moving parasites. The faecal sample was collected and subjected to microscopical

examination. Direct microscopical examination revealed smooth, colorless and spherical or slightly ovoid bodies suggestive of protozoan oocysts. Faecal smears were stained with modified Ziehl-Neelson stain and microscopical examination revealed acid fast oocysts suggestive of *Cryptosporidium parvum* based on morphology and micrometry. Blood smear examination revealed morula of *Ehrlichia canis* in monocytes. Based on history, clinical signs, blood smear and faecal smear examination, a diagnosis of canine cryptosporidiosis and ehrlichiosis was made. Haematological examination revealed significant anaemia with low values of haemoglobin (4.3g/dL), volume of packed red cells (12%) and erythrocyte count ( $3.20 \times 10^6/\mu\text{L}$ ). Total leukocyte count was normal with neutrophilia, and lymphopaenia and thrombocytopenia was also observed (Table 1).

**Table 1. HAEMATOLOGICAL PROFILE OF AFFECTED PUP**

Sl No.	Parameter	Prior to treatment	Two weeks after treatment
1	Haemoglobin	4.3g/dL	9.7g/dL
2	VPRC	12%	30%
3	Erythrocyte count	$3.20 \times 10^6/\mu\text{L}$	$4.84 \times 10^6/\mu\text{L}$
3	TLC	9600/cmm	9500/cmm
4	Neutrophils	89%	67%
5	Lymphocytes	11%	31%
6	Platelet	41,000/ $\mu\text{L}$	4,66000/cmm

### Treatment, Results and Discussion

The animal was treated with azithromycin orally @25mg/kg BW for one week and oxytetracycline (10mg/kgBW) intravenously with normal saline for two weeks with supportive treatments including fluids, haematinics, aminoacids and hepatoprotectants. Protein supplementation was also given. Animal was cured after 2 weeks of treatment. Faecal smear examination and blood smear examination after 2 weeks revealed absence of cryptosporidium oocysts and morulae of *Ehrlichia canis* respectively. Post-treatment,

the animal showed no signs of diarrhea or vomiting and began eating again. As in this case, young dogs are reported to be more susceptible to cryptosporidiosis as also reported by Itoh *et al.* 2019, attributing to the stress associated with weaning and resulting negative impact on the immune system as also mentioned by Murnik *et al.*, 2022. The concurrent infection with *E. canis* may occur leading to severe disease as also reported by Aziz *et al.*, 2022. Although there is no effective treatment for cryptosporidiosis, various chemical compounds such as metronidazole, paromomycin, nitazoxanide,

azithromycin and spiramycin have been used for the disease.

Canine Ehrlichiosis affects multiple organs and systems and has three clinical manifestations, including acute, subclinical, and chronic. Definitive diagnosis involves visualization of morulae on cytology, detection of antibodies through an indirect immunofluorescence test (IFAT) and DNA amplification by polymerase chain reaction as also elicited by Aziz *et al.*, 2022. Canine monocytic ehrlichiosis is successfully treated with antibiotics that belong to the tetracycline family as also highlighted by Sainz *et al.*, 2015. Since, dogs do not develop life-long immunity prevention of re-infection should be attempted by vector control using acaricides at regular intervals and monitoring of environmental factors to reduce tick growth.

### Summary

Occurrence of mixed infection of cryptosporidiosis and ehrlichiosis in a pup is described and therapeutic management using azithromycin and oxytetracycline was attempted successfully. Given the close contact between pets and their owners, dogs can serve as a source of zoonosis for humans.

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