

Concurrent Neosporosis and Hepatozoonosis in a Litter of Pups

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ABSTRACT

This case report describes a concomitant infection in a litter of puppies with two apicomplexan protozoa *Neospora caninum* and *Hepatozoon canis*. The different potential routes of infection are discussed along with a description of the course of the disease. A private practitioner in the South of Israel (Beer Sheva) submitted blood samples for serological testing for toxoplasmosis and neosporosis from a pup of 6 weeks of age (from a litter of 6 pups) showing neurological symptoms. The results were seropositive for *N. caninum* and seronegative for toxoplasmosis. After 3 weeks another pup also started to develop neurological signs. Samples including blood smears, serum samples for serology for *Neospora* and *Toxoplasma* and fecal samples from all the pups. Out of the 6 pups tested four were found seropositive for *Neospora* while all pups were seronegative for *Toxoplasma*. Of the six pups, four were found to be infected with *Hepatozoon canis*, three of which were co-infected with *N. caninum*. One pup was infected with only *H. canis* and another pup with only *N. caninum* and a third pup was found to be negative for both *H. canis* and *N. caninum*. Treatment consisted of trimethoprim sulfadiazine and clindamycin which were administered for six weeks. The pups recovered completely, with the exception of paresis of the left hind limb in the first diagnosed puppy. An important conclusion from this case report is the need to test all pups in a litter for *N. caninum* where even only one pup shows clinical signs. Furthermore treatment at an early age and for a prolonged period of time appeared to be successful in preventing the progression of the clinical signs. To the best knowledge of the authors this is the first natural concomitant infection case of *N. caninum* and *H. canis* in a litter of puppies.

Keywords: *Neospora caninum*; *Hepatozoon canis*; Concurrent Infection; Dog; Pups.

INTRODUCTION

Neospora caninum, an apicomplexa protozoan from the family Sarcocystidae is the causative agent of abortion in cattle and neuromuscular diseases in dogs (1). The definitive hosts of *N. caninum* are domestic dogs and wild canids. Dogs may be infected by *N. caninum* horizontally by consumption or vertically by *in utero* transmission of tachyzoites during pregnancy (1, 2). In naturally infected dogs and cattle the predominant route of infection is considered to be transplacental (1, 2).

Hepatozoon canis is an arthropod-borne infectious agent of the apicomplexan protozoa from the family *Hepatozoidae* in the suborder *Adeleorin*. *H. canis* transmission takes place by ingestion by the intermediate host, the dog, of the brown dog tick *Rhipicephalus sanguineus*, the definitive host that contains mature oocysts (3). Most domestic dogs probably become infected with *H. canis* through grooming ticks from their hair coat. Also like other apicomplexan parasites horizontal transmission through the uterus from dam to its offspring has been demonstrated to take place (3).

Concurrent infections in dogs are reported to occur with a variety of pathogens (3). Often they may be transmitted by a common vector such as the tick. However co-infecting pathogens seemingly unrelated may be involved, resulting in clinical manifestations which may make the clinical diagnosis more complex. The relationship between the concurrent infections in this case involves two diseases of puppies, neosporosis and hepatozoonosis, both of which can be transmitted *in utero*. On the other hand, infection by one agent may influence the susceptibility to a new infection which may influence the progression of the existing condition.

This case report describes a concomitant infection in a litter of puppies with two apicomplexan protozoa *N. caninum* and *H. canis*. The different potential routes of infection are discussed along with a description of the course of the disease. To the best knowledge of the authors this is the first natural concomitant infection case of *N. caninum* and *H. canis* in a litter of puppies.

CASE REPORT

A sample of serum was received from a private practitioner from a pup named "Fistuk" of 6 weeks of age from a litter of 6 pups born in the city of Beer Sheva in the South of Israel. The pup showed neurological symptoms which in the opinion of the practitioner resembled either that of toxoplasmosis or neosporosis. The clinical signs in the pup were that of paresis of the left hind limb which appeared to be progressing to the right hind limb. In addition the pup appeared to be both fecally and urinarily incontinent.

The serum sample was tested by the immunofluorescence antibody test (IFAT) for both *Toxoplasma gondii* and *Neospora caninum*. A pronounced high titer for were found for *N. cani-*

num (1:12,800) while no reaction to *T. gondii* were observed. The pup was treated orally with trimethoprim sulfadiazine (Resprim, Teva Medical, Petah Tikva, Israel) and clindamycin (Dalacin C, Pfizer Pharmaceutical, Israel). Two weeks later the pup showed an improvement in the right hind limb but not in the left hind limb.

When the pups reached 9 weeks of age, another pup, named Pufi, also started to develop neurological signs of paresis in the left hind limb and furthermore had difficulty in rising. Samples including blood smears, serum samples for serology for *Neospora* and *Toxoplasma* and fecal samples were collected from all the pups. Out of the 6 pups tested four were found seropositive for *Neospora* with high antibody titers (Table 1) while all pups were seronegative for *Toxoplasma*. Of the six pups, four were found to be infected with *Hepatozoon canis*, diagnosed from examination of their blood smears and three of which were co-infected with *N. caninum*. One pup was infected with only *H. canis* and another pup with only *N. caninum* and a third pup was found to be negative for both *H. canis* and *N. caninum*. No oocysts, or other parasites were found in fecal samples.

Clinical signs resembling those of neosporosis were only seen in the two pups described above. The other two pups with hepatozoonosis and/or neosporosis did not appear to exhibit any detectable clinical signs of disease.

Positive pups for either *N. caninum* or *H. canis* parasites were treated *per os* with trimethoprim sulfadiazine (15mg/kg q12h) (Resprim, Teva, Petach Tikva, Israel) and clindamycin (10mg/kg q12h) (Dalacin, Pfizer) for six weeks (2). All pups were found to be healthy after 6 weeks after treatment. The incontinent pup Fistuk improved although the left hind limb remained paralytic. The pup named Pufi recovered completely.

No *H. canis* parasites were observed in the blood smears

Table 1: Antibody titer for *Neospora caninum* and the detection of *Hepatozoon canis* gamonts in a litter of 6 pups

Date	10-Oct-12		5-Nov-12		11-June-13	
	Name	Ab titers <i>N. caninum</i>	Ab titers <i>N. caninum</i>	Blood smear	Ab titers <i>N. caninum</i>	Blood smear
Fanta		n.d.	1:12800	Hepatozoon	1:12800	Negative
Pupa		n.d.	1:12800	Negative	1:12800	Negative
Lipstick		n.d.	Negative	Hepatozoon	Not done	Negative
Popcorn		n.d.	Negative	Negative	Not done	Negative
Pufi		n.d.	1:12800	Hepatozoon	1:12800	Negative
Fistuk		1:12800	1:12800	Hepatozoon	1:12800	Negative

n.d. = Not done

examined at the end of the six week treatment period. The four *Neospora* seropositive puppies continued with high antibody titers.

DISCUSSION

The predominant route of natural infection in dogs by *Neospora caninum* is considered to be transplacental (1, 2). The bitch from this report from the south of Israel was adopted by the current owner as a stray dog. It is suggested that free roaming dogs in urban areas have a greater seroprevalence for *N. caninum* than companion pets (4).

It has been reported that a variable number, but not all pups in a litter from a dam infected with *N. caninum* may have clinical manifestations (4, 5, 11). Indeed, in this litter four of the six pups presented as infected, judged by serological investigation (IFAT) and of them only two showed clinical signs at different intervals after birth. An important conclusion from this study is the need to test all puppies in a litter for *N. caninum* where even only one pup shows clinical signs. From the frequency of the disease among the pups in this litter this conclusion appears to be applicable to both *N. caninum* and *H. canis*.

The clinical signs presented by the pups were typical of neosporosis for their age: Congenitally infected pups tends to exhibit a more severe form of the disease (2). Pups of less than 6 months of age exhibit a severe disseminated form showing ascending paralysis of the limbs with the pelvic limbs affected more severely than the thoracic limbs (1, 6). Muscle atrophy, contracture and fibrosis occur in many pups (2). Gradual, progressive, ascending paralysis with hyperextension of the hind limbs in congenitally infected puppies is the most common clinical manifestation of the disease in dogs less than 6 months of age (2).

H. canis infection causes disease ranging in severity from an incidental hematologic finding in an apparently healthy dog to a debilitating and life threatening illness (3). Even so, when high parasitemia is present *H. canis* may also induce severe clinical manifestations (e.g. lethargy, fever, anorexia, weight loss, lymphadenomegaly and anemia) associated with a high parasite load (3, 7). The possibility that the clinical signs were exacerbated due to the concurrent infection with *H. canis* cannot be completely discounted, however *H. canis* is found primarily in the hemolymphatic tissues and not directly related to the neurological system (3).

Concurrent infections with *H. canis* in dogs have been described with *Ehrlichia canis* and *Babesia canis* where all three infections are transmitted by the same tick vector, *R. sanguineus*. Other pathogens reported to be involved in concurrent infections include parvovirus, canine distemper, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Toxoplasma gondii* (8) and *Leishmania infantum*. To the best knowledge of the authors' coinfection of *Heptozoon canis* with *Neospora caninum* has not been previously described.

The seroprevalence of *H. canis* in dogs in Israel has been reported to be 33% (9). The incidence of clinical disease for *H. canis* is considered to be much lower than the exposure rate with most dogs probably undergoing a subclinical form of the disease. In fact in the litter described in this study one pup was infected with *H. canis* only and was not reported to show clinical signs. Although the route of infection cannot be definitely ascertained, it seems likely that the pups were infected *in utero*. Horizontal transmission through the uterus from the dam to its offspring has been demonstrated in *H. canis*. In a study of naturally infected pregnant bitches meronts were found in the spleen of a pup that died 16 days after birth and blood gamonts were detectable as early as 21 days in other pups. The possibility of these pups being naturally infected by ingestion of the *Rhipicephalus sanguineus* ticks cannot be completely discounted, however taking into account the young age of the pups and the relatively high rate of infection within the litter this seems unlikely. Furthermore it would have been necessary for the young pups to ingest the ticks and this makes the possibility of infection after birth as even more unlikely.

Symptoms seen in the two pups were strongly suggestive of infection with *N. caninum* (1). Treatment with trimethoprim sulfadiazine and clindamycin for six weeks was successful in treating the two affected pups, one pup had recovered completely, and the other remained with paresis of the left hind limb whereas the other signs including urinary and fecal incontinence were alleviated. Although the current treatment protocol for *H. canis* is imidocarb dipropionate alone or in combination with doxycycline, a similar treatment used in this study has been recommended for several years in the treatment of *H. canis* (10). This treatment regime was not applied in the treatment of two puppies in this study indicating that *H. canis* may not have been involved in the symptomatology of this concurrent infectious condition.

The exact interaction of *N. caninum* and *H. canis* in the

pathogenesis of the symptoms is unknown but from the treatment outcome it does appear that *N. caninum* did play a prominent or maybe even a singular role.

In conclusion, this case study is to the best knowledge of the authors the first report of a concomitant infection of neosporosis and hepatozoonosis in a litter of puppies. Treatment at an early age and for a prolonged period of time appeared to be successful in preventing the progression of the clinical signs and possibly preventing clinical signs in other litter mates which were infected. Our study is in agreement with others who shown that in naturally infected dogs, treatment can improve clinical signs but may not eliminate the infection, as the puppies remained seropositive (11).

The clinical signs were strongly indicative of those caused by *N. caninum* although the role of *H. canis* in the development of the symptomology could not be established.

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