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).
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. caninum (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 titer (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>
<thead>
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<th>Date</th>
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<th>5-Nov-12</th>
<th>11-June-13</th>
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<tbody>
<tr>
<td>Name</td>
<td>Ab titers</td>
<td>Ab titers</td>
<td>Blood smear</td>
</tr>
<tr>
<td></td>
<td>N. caninum</td>
<td>N. caninum</td>
<td>Blood smear</td>
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<tr>
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<td>n.d.</td>
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<td>Hepatozoon</td>
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<td>1:12800</td>
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<td>n.d.</td>
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<td>Hepatozoon</td>
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<tr>
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<td>n.d.</td>
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<td>1:12800</td>
<td>1:12800</td>
<td>Hepatozoon</td>
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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 tend 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 in 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 affects 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 symptomology 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 \textit{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 hepatozoononosis 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 \textit{N. caninum} although the role of \textit{H. canis} in the development of the symptomology could not be established.

**ACKNOWLEDGMENT:**

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**REFERENCES**