Prevalence of Hemoparasite in Dogs in Nongchok, Bangkok, Thailand

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Abstract: A study of the prevalence of hemoparasites in dogs attending The Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Nongchok districts, Bangkok, Thailand were conducted. One thousand, one hundred and four blood samples of dogs were collected during January to December 2010. Their blood samples were examined for hemoparasites by Diff-Quick stains and the microscopy. One hundred and fifty samples (13.59%) were found to have at least one kind of the hemoparasites. The overall incidence was 1.27% (14/1,104) for Babesia vogeli, 8.7% (96/1,104) for Ehrlichia canis, 2.45% (27/1,104) for Hepatozoon canis, 0.82% (9/1,104) for Anaplasma platys and 0.36% (4/1.104) for the microfilaria of Dirofilaria immitis. The present study suggested a widespread transmission of tick and mosquito in dogs from this area.

Keywords: hemoparasite, dogs, Bangkok

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บทความ: การศึกษาความสูญหายของพยาธิในเลือดในสุนัขที่เขามาทำการตรวจที่โรงพยาบาลเพื่อการเรียนการสอนด้านสัตว์เลี้ยง คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีมahanok จังหวัดแพร่ จ. 10530

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คำสำคัญ: พยาธิในเลือด สุนัข รางฟัน

# รูปแบบขอความเห็น

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Introduction

In this study the term tick-borne hemoparasites includes all tick-borne organisms which are visible with light microscope and which occur in the circulating blood as part of their normal life-cycle. Tick-borne hemoparasites occur on every continent and affect all domestic animals. Ticks are the most important ectoparasites of domestic animals, because of the direct damage they can inflict (Ullenberg, 1995). In the veterinary field, protozoan and rickettsial tick-borne diseases are by far the most important.

The parasitic diseases caused by hemoparasites such as Babesia vogelli, Ehrlichia canis, Hepatozoon canis, Dirofilaria immitis, Trypanosoma evansi and Dipetalonema reconditum cause severe infection in dogs throughout the sub-continent and are found all over the world (Gadahi et al., 2008).
*Ehrlichia canis* is a major health problem for dogs. Symptoms may vary from mild to those of severe tropical pancytopenia. Other ehrlichial species of dogs are *Ehrlichia platys* and *Ehrlichia ewingii*. *Ehrlichia canis* and *Anaplasma platys* are the two best known pathogens that cause canine ehrlichioses. Both agents have a worldwide distribution and were thought to be transmitted by *Rhipicephalus sanguineus* (Inokuma et al., 2003).

*Anaplasma platys* (formerly, *Ehrlichia platys*) is an obligatory intracellular bacterium of platelets and is the etiologic agent of canine infectious cyclic thrombocytopenia. The acute phase of infection is characterized by cyclic thrombocytopenia, but infected dogs are not severely ill and rarely show significant hemorrhage. Follicular hyperplasia of lymph nodes and plasmacytosis has been observed in the acute phase of infection and some organs, such as spleen, may develop hemorrhage (Huang et al., 2005).

Canine babesiosis is an infectious disease caused by protozoans of the genera *Babesia* that is characterized by fever, anemia and hemoglobinuria. The species *Babesia gibsoni* and *Babesia canis* are able to promote natural infections in dogs, with large geographic distribution. The latter is yet grouped into three subspecies: *Babesia canis canis*, found in Europe; *Babesia canis vogeli*, in North and South Africa, North America and Brazil; and *Babesia canis rossi*, in South Africa. The nomenclature *B.canis*, *B.rossi* and *B.vogeli* are becoming increasingly used in the literatures (Duarte et al., 2007).

*Hepatozoon canis* is characterized by fever, anorexia, weight loss, anemia, ocular discharge and weakness of the hind limbs and signs of chronic debilitating disease (O’Dwyer et al., 2000).

*Dirofilaria immitis*, a filarial nematode, is the causative agent of canine heartworm disease. It is well known in dogs that mature female *D. immitis* release microfilaria into the blood and the density of microfilaria in the peripheral venous blood changes in relation to the time of day (Nogami et al., 2000). The adult worms are thin, almost thread like. Males are 12 to 30 cm long and females are 25 to 31 cm long, and are found in the right ventricle of heart and less often in the right auricle, pulmonary artery and vena cava. The male and female copulate in these sites. The viviparous female releases highly motile microfilariae, which circulate in the blood. They are taken up from cutaneous circulation by certain biting mosquitoes, in whose bodies. They undergo stages of development. The infective microfilariae then enter into the tissues of final host through the bite of intermediate host. The filarial larvae undergo further development in muscles, subcutaneous, and adipose tissues of the new host. When they reach a length of about 5 cm, they enter into veins and are carried to right heart. Transplacental infection of the
foetal pups with microfilariae may occur. However these do not develop into adult and disappear after two months (Gadahi et al., 2008).

The aim of this study was to investigate present status of hemoparasites in dogs in Nongchok, Bangkok, Thailand.

Materials and Methods

Experimental animals and site: Blood samples of 1,104 dogs, regardless of their age, sex and breed were examined to determine the prevalence of hemoparasites. The samples were collected from The Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Nongchok districts, Bangkok, Thailand were conducted.

Collection of blood samples: From each animal 1 ml blood was collected aseptically with the help of disposable syringe from cephalic vein and EDTA was used as anticoagulant.

Examination of blood samples: Following techniques were used for the examination of blood samples for hemoparasites.

Preparation of blood smears: A drop of blood was taken near one end of the clean glass slide and another slide used to prepare the blood smear. The edge of second slide, held at an angle of about 45° was touched with the drop spreading it on either side. Then the slide was moved in forward direction allowing the blood to spread as thin layer on the surface of the slide. The smear was allowed to air dry.

Fixing and Staining: The dried blood smears were fixed in methyl alcohol for 5 minutes and allowed to dry. The dry smear were placed in a glass staining jar containing eosin stain and new methylene blue stain for 10 minutes. After the smears were take out and washed with distill water to remove excess stain. The slide were allowed to dry in air and then examined under oil immersion lens of the microscope. The blood smears were examined to find hemoparasites as infectious in blood.

Results and discussion

Prevalence of hemoparasites was studies for a period of 12 months in Nongchok district. A total of 1,104 blood samples of dogs were examined for hemoparasites. One hundred and fifty samples (13.59%) were found to have at least one kind of the hemoparasites from total blood sample one thousand, and hundred and four blood samples of dog. The overall prevalence was 1.27% (14/1,104) for Babesia vogeli, 8.7% (96/1,104) for Ehrlichia canis, 2.45% (27/1,104) for Hepatozoon canis, 0.82% (9/1,104) for Anaplasma platys and 0.36% (4/1,104) for the microfilaria of Dirofilaria immitis (see the Table 1) The present study suggested the high prevalence of Ehrlichia canis infection. The vectors of Ehrlichia canis, Hepatozoon canis, Anaplasma platys and
Table 1 The prevalence (%) of hemoparasites in 1,104 dogs.

<table>
<thead>
<tr>
<th>Hemoparasites species</th>
<th>Number of dogs</th>
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<tbody>
<tr>
<td>Babesia vogeli</td>
<td>14 (1.27%)</td>
</tr>
<tr>
<td>Ehrlichia canis</td>
<td>96 (8.7%)</td>
</tr>
<tr>
<td>Hepatozoon canis</td>
<td>27 (2.45%)</td>
</tr>
<tr>
<td>Anaplasma platys</td>
<td>9 (0.82%)</td>
</tr>
<tr>
<td>Microfilaria of Dirofilaria immitis</td>
<td>4 (0.36%)</td>
</tr>
</tbody>
</table>

*Babesia vogeli* were ticks while the vector of *Dirofilaria immitis* was mosquito. (see the Figures 1, 2, 3, 4, 5)

In the present study, hemoparasites recovered from dogs in Nongchok district were *Babesia vogeli*, *Hepatozoon canis*, *Ehrlichia canis*, *Anaplasma platys* and microfilaria of *Dirofilaria immitis*. In this area

Figure 1 Hemoparasites in blood smear. Normal RBCs (1a), *Babesia vogeli* pear shaped within RBCs (1b), *Ehrlichia canis* morulae in cytoplasm of monocyte (1c), *Hepatozoon canis* gamont in cytoplasm of leukocyte (1d), *Anaplasma platys* morulae within thrombocyte (1e) and microfilaria of *Dirofilaria immitis* in blood smear (1f).
*Rhipicephalus sanguineus* is present, *Babesia vogeli*, *Hepatozoon canis*, *Ehrlichia canis and Anaplasma platys* often occur, and transmitted by the bite of infected ticks. Another hemoparasites transmitted by the same tick species is *Hepatozoon canis*. It is one of the rare examples of a hemoparasite which infects its host by oral ingestion. *Dirofilaria immitis* have transmitted microfilariae to dogs by bite of mosquitoes.

A thin blood smear is prepared in the same manner as a blood smear prepared for a white blood cell differential count. The area of the smear faethest from the original drop of blood should be the thinnest part of the smear. This region is known as the feathered edge of the smear. Because of the large relative size of microfilaria, they are not carried into the feathered edge. The entire slide is fixed and stained with Diff-Quick. Protozoans such as *Babesia* and *Theileria* species can be seen with in RBCs. Rickettsiae such as *Anaplasma* and *Ehrlichia* species may be seen on the surface of the RBCs and leukocytes.

The diagnosis species of hemoparasite used for blood smear by the microscopy. *Babesia canis* is an intracellular parasite.
Figure 3 Babesia vogeli in blood smear. Babesia vogeli pear shaped within RBCs (3a, 3d, 3f). Pear-shaped organisms within RBCs in stained blood smear (3b, 3c). Babesia vogeli round shaped within RBCs (3e).

found within the RBCs of dogs. It has been referred to as the canine piroplasma. The parasite demonstrates pear-shaped organisms within canine RBCs. Diagnosis is by observing basophilic, pear-shaped organisms within RBCs in stained blood smears. Hepatozoon canis is an intracellular, malarialike parasite affecting dogs. The gamonts of these protozoan parasites are found in the leukocyte. Leukocytes containing gamonts of
**Figure 4** *Ehrlichia canis* in blood smear. *Ehrlichia canis* morulæ in cytoplasm of monocytes (4a, 4b, 4c, 4d, 4e) and *Anaplasma platys* morulæ within thrombocyte (4f).

*Hepatozoon canis* are common in peripheral blood smear (Hendrix et al., 2006).

The hemoparasite diagnosis is based on the typical clinical signs and results of special blood tests. The ehrlichiosis diagnosis two blood tests that detect the dog’s antibodies to *Ehrlichia* are available. One is called the indirect immunofluorescent antibody (IFA), and the other is known as an ELISA test. A veterinarian cannot rely solely on these tests to make a diagnosis. A newer diagnostic test called the PCR tests for the presence of the organism itself, not antibodies to it. Sometimes, the organism can be seen inside
cells on a blood smear. To find them, a small drop of blood is spread over a microscope slide, stained and examined under the microscope. The organism can only be found in the bloodstream for a few days during the acute phase of the disease. So this method of diagnosis could miss some cases of the disease.

A diagnosis of *Hepatozoon canis* infection is made by microscopically examining the blood and finding the parasite in particular white blood cells called neutrophils. A great increase in the number of this certain type of white blood cell is a characteristic sign of this disease. Finding the parasite in a muscle biopsy is a very reliable method of diagnosing this disease.

The Babesiosis can be diagnosed by examining blood or tissues under the microscope and finding the parasite. This can sometimes be difficult, so often a serologic test is performed.

In the present study, we did not observe differences in the prevalence of hemoparasites infection the age. Ezeokoli et al. (1983) also found *Hepatozoon canis* infection distributed amongst all ages, although it was most prevalent in young dogs.
Mundim et al. (1994) found that infection was most prevalent in dogs under 1 year of age. Baneth and Weigler (1997) found infected dogs in all age groups, however, the prevalence was higher in animals less than 6 months of age and in animals from 5 to 10 years old. Our results indicated that animals of all ages are equally infected. Nevertheless, some researchers found a higher frequency in young animals that were probably in an acute phase of the disease when more gametocytes could be detected in the peripheral blood.

The present study suggested a widespread transmission of tick in dogs from this area. So, Tick control is the main method to prevent hemoparasite, such as ehrlichiosis, babesiosis and hepatoplasmosis. Products which repel and kill ticks such as those containing permethrins are excellent choices. Sometimes in conjunction with permethrin products in those areas with high tick infestations. If a large number of cases of ehrlichiosis are diagnosed in an area, some veterinarians recommend placing dogs on low doses of tetracycline or doxycycline during the tick season.

References


