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A retrospective study of the clinical hematology and the serum biochemistry tests made on canine dirofilariasis cases in an animal hospital population in Bangkok, Thailand

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Abstract

Heartworm disease, caused by the filarial nematode, *Dirofilaria immitis*, is a major, potentially life-threatening disease of dogs, with worldwide distribution and global significance. It is not only of veterinary importance but it also has zoonotic potential in many regions. It is considered as an endemic disease in Thailand, although clinical data about the disease is rarely reported. The objectives of this study were to characterize the clinical hematology and the biochemistry of canine dirofilariasis cases admitted to Chulalongkorn University, Small Animal Teaching Hospital in Bangkok, Thailand, from 2001–2003. All hematology and serum biochemistry parameter interpretations were based on reference values. A total of 1023 dogs were evaluated in this study. Dogs were divided into three groups based on their heartworm classification (microfilaremic, occult and negative). The major hematological findings in microfilaremic dogs were a mild to moderate anemia, mild to severe thrombocytopenia, marked leukocytosis, moderate to marked neutrophilia, eosinophilia and monocytosis. The most common serum biochemical abnormalities in microfilaremic dogs included increased alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase with a mean ± SD of 311 ± 299, 82 ± 76 and 50 ± 38 IU/L, respectively. It is likely that this parasitic infection led to the impairment of the hematological and biochemical status of the infected dogs. We believe that investigation of these laboratory based parameters, when associated with the clinical signs, is a very important approach to be considered in the routine clinical follow up, as well as being important for therapeutic evaluations.

Keywords: Abnormalities; *Dirofilaria immitis*; Dogs; Hematology; Retrospective study; Serum biochemistry

1. Introduction

Heartworm disease, caused by the filarial nematode, *Dirofilaria immitis*, is a major, potentially life-threatening disease of dogs, with worldwide distribution and global significance. It is not only a veterinary problem but also a zoonosis in many parts of the world (Orihel and Eberhard, 1998; Narine et al., 1999; Akst et al., 2004; Miyoshi et al., 2006). In Thailand, cases of human dirofilariasis have been reported in 1955, 1983 and 1998 (Pradatsundarasar, 1955; Sucharit and Jariya, 1983; Sukpanichnant et al., 1998). Epidemiological studies on heartworm disease in Thailand suggest that there is a high incidence of *D. immitis* both stray and domestic dogs (Sangkavoranond, 1981). Mosquitoes are the only natural vector for canine heartworm

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disease, with approximate 70 mosquito species being implicated. All dogs living in a heavily populated mosquito area are at risk. The clinical evolution of heartworm disease in dogs is usually chronic. Most infected dogs do not show any sign of the disease for months or years, depending on the worm burden, individual reactivity, and the level of exercise (Venco et al., 2005). Some dogs may never develop clinical signs if the infection is small.

Well documented radiological, serological, and echocardiographic findings are typically seen in the chronic evolution of heartworm disease (Venco et al., 2005). Radiography provides the most objective method for assessing the severity of heartworm, cardiopulmonary disease in dogs. Typical radiographic findings (nearly pathognomonic) of heartworm vascular disease, are enlarged, tortuous and often truncated, peripheral intralobar and interlobar branches of the pulmonary arteries, particularly in the diaphragmatic lobes (Litster et al., 2005; McCall et al., 2004). With echocardiography, the body wall of adult heartworms is seen to be highly echogenic and produces distinctive, short parallel-sided images with the appearance of ''equal signs'', where the imaging plane cuts across loops of the parasite. Echocardiography can also provide definitive evidence of heartworm infection, as well as providing for the assessment of the cardiac, anatomical and functional consequences of the disease (McCall et al., 2004). Moreover, with recent advances in serology, the potential now exists for veterinarians to use such serology tests in the diagnosis of canine heartworm disease (Goodwin, 1998). Heartworm assays also allow semiquantification of heartworm infection, allowing the practitioner to better plan therapy taking into consideration the disease severity. No clinicopathological finding in spontaneous canine dirofilariasis have yet been reported although, there are several hematological and biochemical tests available that are useful for determining blood component profiles. Variations in these test results compared with reference values can give information related to the overall health status.

In Thailand, although canine dirofilariasis has been identified as an endemic, mosquito-borne disease, there are few reports describing the manifestations and pathological changes in dogs which are infected with *Dirofilaria*. The aims of this study were to analyze the clinical hematology and biochemistry of canine dirofilariasis in dogs within a hospital population in Bangkok, Thailand.

2. Materials and methods

2.1. The study design and the data collection

A retrospective study on the serum biochemistry and hematology in cases of canine dirofilariasis was carried out in order to investigate the variations of each parameter. The medical records of client owned dogs admitted to Chulalongkorn University, Small Animal Teaching Hospital in Bangkok, Thailand during the period of 2001–2003 were reviewed and analyzed. Cases were only included if *D. immitis* microfilaria were diagnosed and if a full biochemistry profile was available. Cases were excluded from the study if multiple infections with other pathogens were diagnosed or if a concurrent disease was likely to affect serum biochemistry and hematology. A number of parameters of complete blood count (CBC) data were assessed: red blood cell (RBC) count, hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell (WBC) count. The biochemistry panel included the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine.

2.2. Blood sample collection

Blood sample collection was performed by a veterinarian in a standard manner. Two-milliliter samples were transferred to tubes with EDTA (in the proportion of 1 mg/ml) for blood parasite examination and hematology evaluation and 1 ml was transferred to a tube with no anticoagulant. The serum samples were stored in aliquots at −20 °C, until use in the biochemical evaluations. Blood specimen collection was only undertaken with the express willingness and permission of the owners.

2.3. Parasitological evaluations

Blood parasite examinations were performed using a light microscope for direct detection and for indirect detection using serological diagnostic tests. The diagnostic methods used were based on the cost of the test as well as on the owners ability to pay. In order to detect the parasite directly, duplicate slides of both thin blood smears and buffy coated smears were made and stained with 10% (v/v) Giemsa. The slides were then examined under a light microscope by an experienced and well trained microscopist. Heartworm microfilaria were identified morphologically in term of species, according to existing descriptions and keys such as 6×310 micrometers in size, unsheathed, slender shaped and the presence of one nucleus within a long cephalic space (Skryabin, 1991). Approximately less than 10% of the dogs in this study were confirmed as positive for heartworm antigen using the SNAP® heartworm antigen test (IDEXX Laboratories, Inc., Maine, USA). Some dogs were diagnosed as heartworm positive by using a rapid immuno-migration technique, the Agen® HW antigen test (AGEN Biomedical Limited, Queensland, Australia). The criteria used to identify occult heartworm infection were as described previously by Gossett and colleagues (1987). Briefly, it was defined as the presence of adult worms at necropsy in dogs which were negative for microfilaria detection using a modified Knott's test. The data from the necropsy records were kindly supplied by The Veterinary Pathology Unit, Department of Veterinary