Prevalence of Canine Infectious Endocarditis and Possible Association with *Bartonella* spp. in Bangkok, Thailand

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Abstract

Infectious endocarditis (IE) is a heart valve or endocardial disease. *Bartonella* spp. are considered one of the causes of IE. The objective was to study the prevalence of canine IE in Bangkok, Thailand with an emphasis on *Bartonella* spp. infections. A review of the reports between January 1999 to December 2009 of 3,545 necropsied dogs was performed. Cardiac tissue blocks from 11 dogs were studied for the presence of eubacterial DNA and *Bartonella* spp. DNA by fluorescence in situ hybridization (FISH) and for *Bartonella* spp. DNA by conventional polymerase chain reaction (PCR). The prevalence of canine IE was 0.65%. The cardiac tissues from 2 of 11 dogs were positive for eubacteria DNA by FISH. None of the dogs was positive for *Bartonella* spp. by FISH or conventional PCR. The prevalence of canine IE was low in this population and *Bartonella* spp. DNA was not detected in any dog tested with these techniques.

Keywords: *Bartonella* spp., dogs, endocarditis, heart, Thailand, valve

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Introduction

Infectious endocarditis (IE) is a heart valve or an endomyocardial disease caused by bacterial infection. The incidence of this disease in dogs is reported to be approximately 0.06-6.6% (Sisson and Thomas, 1984). A recent report from western United States showed an incidence of IE 0.9% (MacDonald et al., 2004). In the past, Staphylococcus aureus, Streptococcus spp., Escherichia coli, Pseudomonas aeruginosa, Erysipelothrix rhusiopathiae, and Corynebacterium spp. were the most common bacteria associated with IE (Kittleson 1998; Peddle and Sleeper, 2007).

Recently, Bartonella spp. have been recognized as a cause of IE in dogs (MacDonald et al., 2004; Pesavento et al., 2005). Bartonella vinsonii var. berkhofti was first described as a cause of canine IE in 1995 (Breitschwerdt et al., 1995). Subsequently, IE has been associated with B. rochalimae (Henn et al., 2009), B. claridgeiae (Chomel et al., 2001), B. quintata (Kelly et al., 2006), B.henselae (Fenimore et al., 2011), B.koehlerae (Ohad et al., 2010) and B.washoensis (Chomel et al., 2003). In addition, 28% of dogs affected by IE in western United States had antibody titers against Bartonella spp. including B. vinsonii berkhoftii, B. claridgeiae, and B. claridgeiae-like (MacDonald et al., 2004).

Seroreactivity to Bartonella spp. has been found in 38% of stray dogs (Suksawat et al., 2001) in Thailand. The species found were B. henselae, B. claridgeiae and B. vinsonii supsp. Berkhoftii (Breitschwerdt et al., 1995; Henn et al., 2001). Dogs in Thailand were also found to be frequently bacteremic with rodent Bartonella species (Kosoy et al., 2010). To our knowledge, the prevalence of IE from any cause is unknown in dogs in Thailand and because of the high seroprevalence of Bartonella spp., we hypothesized that Bartonella spp. IE occur in dogs in Thailand. The objectives of the study were firstly to determine the prevalence of IE in dogs in Bangkok, Thailand based on necropsy descriptions of the lesions. The second objective was to determine whether bacterial DNA could be amplified from formalin fixed paraffin embedded cardiac tissues using a polymerase chain reaction (PCR) for Bartonella spp. DNA and fluorescence in situ hybridization (FISH) assay for eubacterial and Bartonella spp. DNA.

Materials and Methods

Study population: Necropsy reports of the Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University were evaluated for diagnosis of IE from January 1999 to December 2009. Presence of vegetation on any valve leaflets as well as bacteria and inflammatory cells within vegetative leaflets or endocardium were used as the criteria for the diagnosis of IE (Fig 1). All Hematoxylin and Eosin (H&E) stained slides of dogs reported with IE were retrieved and reviewed under light microscope by the same pathologist. Dogs that had lesions in leaflets and endocardium were diagnosed with valvular endocarditis and mural tissue endocarditis, respectively. All dogs affected with IE must have bacteria and/or inflammatory reaction seen in the vegetative leaflets or the endocardium on histopathologic sections. Age, gender, breed, clinical findings, and cause of death were recorded for all dogs with IE. The prevalence of IE was calculated by dividing the total cases of IE by the total number of necropsy evaluations performed during the time period X100.

Tissue archives were searched for stored paraffin blocks of all dogs with IE. Paraffin embedded tissue blocks from 11 dogs were shipped to Colorado State University for further evaluation.

Molecular diagnostics: The FISH protocols used were adapted from a previous report (Kornreich et al., 2012). The 4 µm formalin fixed paraffin embedded cardiac tissue sections were deparaffinized by passage of graded alcohol and air-dried. Each slide was initially screened for eubacteria using a combination of an eubacteria probe (EUB-338 Cy3: GCTGCGCTCCGTAAGGAGT) and a non-eubacterial control (non-EUB338 6 FAM ACTCTACGGGAGCGAAGCC). The slides were subsequently screened using a probe directed against Bartonella spp. (ALF 98: GCTGAAGTTCTGCGCGTT) to evaluate for the presence of Bartonella spp. DNA.
FISH probes (Integrated DNA Technologies, Coralville, IA) were reconstituted with sterile water and diluted to a working concentration of 5 ng/µl with a hybridization buffer (20 mM Tris, 0.9 mol/l NaCl, 0.1 sodium dodecyl sulphate, 40% formamide, pH 7.2). Each section was hybridized with 30-50 µl of hybridization buffer and DNA probe in a humid chamber at 46°C overnight. Washing was done with a washing buffer (20 mM Tris, 0.9 mol/l NaCl, pH 7.2) at 48°C for 20 min. The slides were rinsed with sterile water, air-dried and counterstained with ProLong Antifade Gold with DAPI (Molecular Probes Inc., Eugene, Oregon, USA). All slides were examined under an Olympus BX51 epifluorescence microscope (Olympus America, Melville, New York, USA). Images were taken with a DP-70 camera and DPManager (Olympus America, Center Valley, Pennsylvania, USA). Sensitivity of these assays for detection and identification of bacteria was 87.5% and specificity was 100% (Kornreich et al., 2012).

A conventional Bartonella spp. PCR assay was performed using a previously published protocol (Jensen et al., 2000). Total DNA was extracted from 200 µg of formalin fixed paraffin embedded cardiac tissues from 6 valves and 8 myocardium samples using a commercially available kit (QIAamp DNA Mini Kit, QIAGEN Inc, Valencia, California). The PCR amplifications were performed in 50 µl that contained 10 mM Tris, pH 8.3, 50 mM KCl, 3.5 mM MgCl2, 200 mM each dATP, dCTP, and dGTP, 400 µM dUTP, 1 µM each primer, and 2.5 units Taq polymerase (AmpliTaq Gold DNA polymerase, PE Applied Biosystems, Foster City, Calif). The amplifications were performed in an automated thermal cycler using a protocol including 10 min incubation at 20°C followed by 2 - min denaturation at 95°C then 45 cycles of 1 min at 95°C, 1 min at 60°C, and 30 second at 72°C. PCR amplification products were identified after electrophoresis in 3% agarose gels. Positive and negative samples were included as controls. Sensitivity of this assay in detection of Bartonella spp. was 100% of blood samples with 50 to 100 CFU/ml, 85% of blood samples with 30 CFU/ml, and 75% of blood samples with 10 to 20 CFU/ml (Jensen et al., 2000). The sensitivity when used with formalin fixed tissues was unknown.

**Results**

**Study population:** Data of 3,545 necropsied dogs were analyzed. Twenty-three were diagnosed with IE giving a prevalence of necropsy cases of 0.65%. Twelve dogs were female and 11 were male. The mean age of dogs was 7.3 ± 3.5 years. Golden retriever was the most common breed (5/23). Other breeds of dogs were 2 German shepherds, 2 Cocker spaniels, 5 mixed breed dogs and one of each Boxer, Collie, Doberman pinscher, Rottweiller, Thai, Mastiff, Siberian, Jack Russell terrier and Poodle.

Clinical findings included heart murmur (26.1%), fever (26.1%), dyspnea (26.1%), vomit (13.0%), anorexia (13.0%), depression (8.7%), diarrhea (8.7%), icterus (4.3%), pale (4.3%), cyanosis (4.3%), cough (4.3%), and exercise intolerance (4.3%). Based on necropsy findings, lesions were noted in the mitral valve alone (30.4%), aortic valve alone (8.7%), both mitral and aortic valves (17.4%), the mural tissue endocarditis alone (26.1%), and the mural tissue endocarditis concurrent with mitral valve endocarditis (17.4%). Of the tissues evaluated by histopathology, bacteria were noted in 17 of 23 cases (73.9%). None of the tissues had been cultured. Suppurative inflammation of other organs was identified by histopathologically and included pneumonia (3.5%), myocardiitis (26.1%), lymphadenitis (21.7%), nephritis (17.4%), endometritis (13.0%), enteritis (8.7%), cellulitis (8.7%), hepatitis (8.7%), myositis (4.3%), splenitis (4.3%), peritonitis (4.3%), pancreatitis (4.3%), pleuritis (4.3%), meningoencephalitis (4.3%), and arthritis (4.3%). Five dogs had endocarditis without other organ inflammation. Most of the dogs died from suspected septicemia (78.3%) thought to result from several causes including cutaneous open wound, gastrointestinal rupture, suppurative pneumonia, urogenital infection, and cancer with secondary bacterial infection. Emboli were seen in 3 dogs (13.0%), causing pulmonary thromboembolism in 2 dogs (8.7%) and myocardial infarction in one dog. Twenty of the 23 (86.9%) dogs had ante-mortem antimicrobial therapy. Ten of the 23 dogs (43.5%) were treated with anti-inflammatory drugs including aspirin tolfenamic acid and caprofen.

![Figure 2](image-url) FISH results for paraffin blocks of 2 dogs positive for eubacteria; myocardium (left) and heart valve (right). Eubacteria was hybridized with the EUB-338-Cy3 probe (red). Non-eubacteria was hybridized with the non-EUB338-6 FAM probe (green). DAPI staining for unspecific DNA was revealed as background fluorescence (blue).
Molecular assays: Paraffin blocks from 11 dogs were available for further evaluation. Bacteria were present based on review of H&E stained sections of 7 blocks. The positive and negative controls in all assays performed as expected. Eubacterial DNA was detected by FISH in 2 of 11 dogs (Fig 2). Bartonella spp. DNA was not detected by PCR assay or FISH in any tissue.

Discussion

The results of the present study found that the prevalence of canine IE was low in Bangkok, Thailand. These findings are similar to a previously reported prevalence study of dogs from western United States (MacDonald et al., 2004). Almost equal number of males and females were affected in the present study. This is in contrast to previous studies which mostly reported a male: female ratio of 2:1 (Wall et al., 2002; Sykes et al., 2006). The majority of cases were middle-aged, large breeds of dogs. The most affected breed was the Golden retriever. This finding is in agreement with previous studies which found German shepherd, Boxer, Golden retriever, and Labrador retriever as over represented breeds (Wall et al., 2002; Sykes et al., 2006).

Most of the dogs with endocarditis present a combination of non-specific signs including depression, weakness, lethargy, weight loss, anorexia, or fever. These clinical signs are consistent with the findings in this study. The present study demonstrated that 26.1% of the dogs had a heart murmur which is in agreement with a previous study (33%) (Peddle et al., 2009). Thus, auscultation of heart murmur is not a sensitive way to diagnose endocarditis in dogs. This may relate in part to the presence of lesions only on the mural endocardium without involvement of valve leaflets. In this study, only 26.1% of dogs had mural endocarditis alone. Due to the absence of heart murmur and the lack of echocardiographic evidence of disease, mural endocarditis is rarely reported in humans and dogs (Kearney et al., 2004; Miller et al., 2004).

The detection of IE lesions most frequently on the mitral valves is similar to previous studies (Sykes et al., 2006; Peddle et al., 2009). The mitral valves are more affected presumably because these valves have higher risk to be damaged secondarily to an encounter with higher resting pressures for longer length of time (Lepeschkin et al., 1952). The aortic valve was the second most commonly affected valve. Fever was present in only 26.1% of the dogs in the present study indicating that dogs affected with endocarditis may present normal body temperature. Fever may sometimes be obscured by concurrent use of anti-inflammatory drugs (Sykes et al., 2006; Peddle et al., 2009). Almost half of dogs (43.5%) with endocarditis in the present study were treated with anti-inflammatory drugs. These results support those of Peddle et al. (2009), who suggested that a lack of fever was not a good criterion to rule out IE because only half (56%) of the dogs with endocarditis had fever. Based on the results of this study, IE is difficult to diagnose clinically. Only clinical signs and findings from physical examination fail to provide a definitive diagnosis. Although echocardiographic examination can be non-invasively used to reveal lesions within the heart, it has limitations to identify mural lesions and to distinguish between endocarditis and endocardiosis (i.e. degenerative valves) (Boon, 2011). To date, the standardized criterion for assessing patients with IE in veterinary medicine has not been established. Thus, the definitive diagnosis is mostly based on clinical signs including new murmur heart sound and/or fever, laboratory data including positive blood culture results and echocardiographic evidence of endocardial involvement.

In this dog population, suppurative inflammation was seen in several organs including the lungs, lymph nodes, kidneys, uterus, intestines, livers, spleen, muscles, pancreas, peritoneum, pleura, joints and brain. Only 21.7% of the dogs had endocarditis without other organ inflammation. In contrast to a previous report which found 11.84% of dogs with endocarditis to concurrently have arthritis, arthritis was only detected in 4.4% of the IE dogs described here (Peddle et al., 2009). The arthritis associated with IE is thought to occur secondary to deposition of immune complex within the joint, sepsis or emboli (Kittleson, 1998). The dog with arthritis in this study was affected by septic emboli in the joints. Septic emboli are commonly reported in dogs affected with endocarditis and can result in several non-specific clinical signs of disease such as seizure from emboli in brain, diarrhea from intestinal ischemia or sudden death from myocardial infarction (Miller et al., 2004). Emboli were observed in 13.0% in the dogs in the present study and all were detected in the cardiopulmonary system. In another study, the common sites were lungs, kidneys and distal portion of the aorta (Peddle et al., 2009).

The majority of dogs in this study (78.3%) died secondary to sepsis resulting from open wounds, gastrointestinal rupture or diseases with secondary bacterial infection. The remaining dogs presented without septicemia and had no known underlying cause of bacteremia. In most studies, the organisms most commonly associated with IE included Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginasa and Erysipelothrix rhusiopathiae (Kittleson, 1998). Common associations include S. aureus from pyoderma, E. coli from the gastrointestinal or urinary tracts, P. aeruginasa from chronic wound infections, and E. rhusiopathiae from the oral cavity (Calvert and Wall, 2006).

Multiple Bartonella spp., including B. vinsonii var. berkoffii, B. rochalimae (Henn et al., 2009), B. clarridgeiae (Chomel et al., 2001), B. quintata (Kelly et al., 2006), B. henselae (Fenimore et al., 2011), and B. koehlerae, have been grown or amplified from dogs with IE (Breitschwerdt et al., 1995; Henn et al., 2009; Chomel et al., 2001; Kelly et al., 2006; Fenimore et al., 2011). These agents are generally vector borne but may also be transmitted by direct contact like bites or scratches. For example, B. henselae DNA has been amplified from dog saliva (Duncan et al., 2007a). DNA of several Bartonella spp. has been amplified from Chenocephalides felis and Pulex spp. collected from dogs (Yore et al., 2012). Ticks are also suspected as vectors. Fleas and ticks are common on dogs in Thailand and Bartonella spp. antibodies have been detected in stray dogs, thus, exposure to Bartonella spp. in the dogs described herein.
would be expected (Nithikhathkul et al., 2005; Sukawat et al., 2001).

The present study used previously described FISH protocols to attempt to identify eubacterial or Bartonella spp. DNA in the formalin fixed tissues contained in archived blocks from 11 dogs (Kornreich et al., 2012). When compared to H&E staining, one study reported 87.5% sensitivity and 100% specificity of FISH for detection of bacteria in archival heart valve sections (Kornreich et al., 2012). The advantage of FISH over H&E staining and microscopic examination of tissues is the ability to identify the species of causative bacteria. Moreover, FISH can be used to localize bacteria within histological sections while conventional PCR techniques cannot (Moter and Göbel, 2000).

While 7 of the 11 dogs had bacteria visualized on H&E stained sections, eubacteria were only detected in 2 dogs by FISH. The positive tissues were endocardium for one case and valve tissue for the other case. Of these 2 cases, bacteria were also seen histopathologically. The failure to document eubacterial DNA by FISH in the other 5 histopathologically positive samples suggest that the bacteria visualized were not actually bacteria or that the FISH was falsely negative. None of the cases were evaluated by blood or tissue culture and so further information to aid in determining which possibility is true is not available. All 11 dogs were treated with antibiotics before death which may have affected the results of both assays. In future studies, it would be optimal to collect fresh tissues for culture as well as molecular assays.

None of the dogs in the present study was positive for Bartonella spp. by FISH or conventional PCR performed on the archived formalin fixed tissues. Bartonella spp. DNA can be amplified from formalin fixed cardiac tissues from dogs and the conventional Bartonella spp. assay used is relatively sensitive (Fenimore et al., 2011; Jensen et al., 2000). These results suggest that Bartonella spp. were not the cause of IE in this population of dogs. Another recent report from mid-Atlantic United States also failed to detect Bartonella spp. in any archival valve sections from dogs with suspected IE (Kornreich et al., 2012). These findings may indicate that biological behavior of Bartonella spp. strains may vary regionally as has been documented with B. henselae infection in cats. While most B. henselae strains in cats induce only subclinical infections, one strain studied in experimentally exposed cats induced cardiac disease in 2 of 6 cats (Bradbury and Lappin, 2010). In one study, Bartonella spp. was mostly found at aortic valves (Pesavento et al., 2005), however, most of the dogs in this study had mitral valve endocarditis which may have also influenced the results. A larger study that combines more sensitive techniques should be performed using blood and fresh tissues. The use of pre-enrichment liquid culture followed by PCR has been shown to be more sensitive than other methods for detection of Bartonella spp. infections in dogs (Duncan et al., 2007b; Bai et al., 2010).

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References


บทคัดย่อ

ความชุกของโรคเยื่อบุหัวใจอักเสบติดเชื้อในสุนัขและความเป็นไปได้ของการติดเชื้อบาโทเนลลาในเขตกรุงเทพมหานคร ประเทศไทย

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โรคเยื่อบุหัวใจอักเสบติดเชื้อเป็นโรคของลิ้นหัวใจหรือผนังเยื่อบุหัวใจชั้นใน เชื้อบาโทเนลลาเป็นสาเหตุหนึ่งของการเกิดโรคเยื่อบุหัวใจอักเสบติดเชื้อ วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาความชุกของโรคเยื่อบุหัวใจอักเสบติดเชื้อในสุนัข ในเขตกรุงเทพมหานคร ประเทศไทย โดยนับความเสี่ยงต่อการติดเชื้อบาโทเนลลา การศึกษาโดยทบทวนรายงานผลชันสูตรซากสุนัขจำนวน 3545 ตัว ระหว่างเดือนมกราคม 2542 ถึงเดือนธันวาคม 2552 และศึกษาการปรากฏของดีเอ็นเอของยูแบคทีเรียในกล้ามเนื้อหัวใจ จากชิ้นเนื้อหัวใจจำนวน 11 ชิ้น โดยวิธีฟลูอินไซต์ไฮบริดเดนซ์ และปฏิกิริยาลูกโซ่โพลิเมอเรส พบความชุกของโรคเยื่อบุหัวใจอักเสบติดเชื้อจากการติดเชื้อในสุนัขร้อยละ 0.65 พบยูแบคทีเรียดีเอ็นเอในกล้ามเนื้อหัวใจ 2 จาก 11 ตัวอย่าง ไม่พบผลบวกต่อเชื้อบาโทเนลลาด้วยการตรวจด้วยฟลูอินไซต์ไฮบริดเดนซ์ และปฏิกิริยาลูกโซ่โพลิเมอเรส ไม่พบข้อสรุปความชุกของโรคเยื่อบุหัวใจอักเสบติดเชื้อในสุนัขค่อนข้างต่ำในประชากรสุนัขที่ทำการศึกษา และไม่พบการปรากฏของดีเอ็นเอของเชื้อบาโทเนลลาจากการวิเคราะห์ผลการศึกษาในครั้งนี้

คำสำคัญ: เชื้อบาโทเนลลา สุนัข ลิ้นหัวใจอักเสบ หัวใจ ประเทศไทย ลิ้นหัวใจ

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