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Development and evaluation of a PCR assay for the detection of *Cytauxzoon felis* DNA in feline blood samples

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Abstract

Cytauxzoonosis is an emerging tick borne infectious disease of domestic cats in the United States, caused by the organism *Cytauxzoon felis* (*C. felis*). In naturally infected domestic cats the disease is almost always fatal. Currently there are no commercially available molecular or serologic tests to facilitate the antemortem diagnosis of *C. felis* infection. Clinical and pathological diagnosis of cytauxzoonosis is based on microscopic identification of parasites in tissues or on blood smears. We have developed and evaluated the sensitivity and specificity of a polymerase chain reaction (PCR) based assay for the diagnosis of *C. felis* infections in feline blood samples. The assay is sensitive enough to detect one copy of a cloned fragment of the *C. felis* 18S rRNA gene. This PCR assay can be used for the rapid clinical diagnosis of cytauxzoonosis and for epidemiological studies that will better define the geographic distribution of *C. felis* infection in cats.

Keywords: Cytauxzoonosis; Piroplasmosis; Pancytopenia

1. Introduction

Cytauxzoon felis is an emerging tick-transmitted apicomplexan protozoal parasite that infects domestic cats (*Felis catus*). It is presumed to be transmitted to domestic cats via ticks from wild felids such as bobcats (*Lynx rufus*) and cougars (*Felis concolor*). In domestic cats cytauxzoonosis is characterized by fever, pancytopenia, multi-organ failure and often results in the death of affected cats (Criado Fornelio et al., 2004; Greene et al., 1999; Hoover et al., 1994; Meier and Moore, 2000; Meinkoth et al., 2000). Due

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to progressive multi-organ system failure, most domestic cats either die or are euthanized within 1 week of the onset of clinical signs. In contrast, chronic *C. felis* infection is better tolerated by wild felids in which fatal cytauxzoonosis appears to be a rare occurrence. In recent years, this devastating infection has been recognized with increased frequency in many areas of the United States including areas where *C. felis* has not been previously documented (Birkenheuer et al., in press). There are also several more recent reports of cats surviving *C. felis* infections (Greene et al., 1999; Meinkoth et al., 2000). It is unknown whether non-fatal *C. felis* infection in cats represents a change in the biological behavior of the organism (less pathogenic strains) or its tick vectors or the establishment of infection in new reservoir species.

Despite the original disease description in cats from Missouri almost 30 years ago, little to no information exists about the epidemiology of *C. felis* (Wagner, 1976). The current cumulative *C. felis* knowledge base is derived from individual case reports, small case series and experimentally induced infections. The absence of user friendly, sensitive and specific tests for *C. felis* presents a substantial barrier for future epidemiological studies. The clinical diagnosis of cytauxzoonosis has relied on the light microscopic detection of parasites in erythrocytes or macrophages, and due to the low sensitivity of blood smear examinations, infection is frequently only documented at necropsy (Kier et al., 1977). Serologic testing for anti-*Cytauxzoon* antibodies has been described experimentally, but a serological test is not available commercially. In addition, perpetuation of an antigen supply requires infection of live cats since *C. felis* has never been cultured in vivo and recombinant diagnostic antigens have not been produced. Due to the acute onset and rapid clinical course of illness, anti-*Cytauxzoon* antibodies may not be detectable during the early stages of infection or at the time of death. Recently the polymerase chain reaction (PCR) has been used to amplify *C. felis* 18S rRNA gene sequences (Allsopp et al., 1994; Meinkoth et al., 2000). These broad-range PCR assays were designed to amplify 18S rRNA genes from nearly all piroplasms. Genus and species confirmation required DNA sequencing to confirm the identity of the parasite. A more recent study used PCR to amplify *C. felis* DNA in pooled tick samples, but the investigators did not report the limit of detection for their assay (Bondy et al., 2005). As ticks would be expected to harbor a much higher concentration of *C. felis* than would be found in a feline blood sample, the limit of DNA detection becomes critical when a PCR assay is used diagnostically. Currently, no PCR assay is readily available to facilitate a clinical diagnosis of feline cytauxzoonosis by practitioners.

The clinical diagnosis of *C. felis* infection based on the identification of merozoites in red blood cells has several potential limitations. Despite substantial differences in DNA sequences, the merozoites of hemoprotozoan parasites are often morphologically indistinguishable via light microscopy (Caccio et al., 2002; Kjemtrup et al., 2000; Zahler et al., 2000). For example, morphologically, *C. felis* merozoites are indistinguishable from *Babesia felis* merozoites. The use of PCR tests for the diagnostic detection of hemoprotozoan parasites is well documented and is more sensitive than light microscopic identification of parasites on stained blood smears (Almeria et al., 2001; Birkenheuer et al., 2003; Calder et al., 1996). In addition, the specificity of PCR facilitates the detection of genetic differences between morphologically indistinguishable organisms (Birkenheuer et al., 2003; Calder et al., 1996; Gubbels et al., 1999). Polymerase chain reaction based tests can detect parasitemias that are up to 1000-fold lower than the limit of detection that can be achieved by light microscopic observation of thin stained blood smears (Almeria et al., 2001; Birkenheuer et al., 2003; Krause et al., 1996). Due to the limitations of currently available diagnostic tests for *C. felis* we sought to develop a PCR assay for cytauxzoonosis. The development of a sensitive and specific PCR assay for the detection of *C. felis* in feline blood samples will be beneficial for the clinical diagnosis of feline cytauxzoonosis and can be also used to study the epidemiology of *C. felis* in wild and domestic felids.

2. Materials and methods

2.1. Patient samples

C. felis: Whole blood samples were collected from six cats from North Carolina, four cats from Florida, and two cats from Tennessee. By microscopy