CASE REPORT

CANINE *ORIENTIA TSUTSUGAMUSHI* INFECTION: REPORT OF A CASE AND ITS EPIDEMICITY

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Abstract. A lethargic household dog was referred to a private hospital in Japan. Diagnosis was carried out by the polymerase chain reaction (PCR) method developed for human *Orientia tsutsugamushi* infection using the dog's anticoagulated peripheral blood. Karp, Kato and Kuroki-type genomes were detected and the dog was diagnosed with *O. tsutsugamushi* infection. These findings demonstrate that dogs can act as a host for *O. tsutsugamushi* and the PCR method developed for human beings can be used for the diagnosis of canine *O. tsutsugamushi* infection. A concurrent epidemiological study examined 10 asymptomatic dogs that were fed in the same area as the sick dog. Kuroki-type genome in all dogs, Gilliam-type genome in 6 dogs and Kawasaki-type genome in 3 dogs were detected. These results provide further evidence that dogs can be naturally infected with *O. tsutsugamushi* outdoors and that dogs play a role as a host in the lifecycle of *O. tsutsugamushi*.

Keywords: dog, epidemicity, genus *Haemaphysalis*, *Orientia tsutsugamushi* infection, PCR

INTRODUCTION

Orientia tsutsugamushi is the etiological agent of *O. tsutsugamushi* infection, also known as tsutsugamushi disease or scrub typhus. It is an acute, mite-borne, febrile human rickettsial illness that oc-

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curs in Asia-Pacific region (Takahashi and Tanaka, 1995; Kelly *et al*, 2009). The three major presenting symptoms of *O. tsutsugamushi* infection are reported as eschars, fever and rash (Ogawa *et al*, 2001a). Other symptoms include localized lymphadenopathy, cephalalgia and malaise (Ogawa *et al*, 2001a; Yoshida, 2003). Disseminated intravascular coagulation (DIC) develops in some cases when appropriate treatment is delayed, indicating that tsutsugamushi infection can be life threatening (Ogawa

et al, 2001b). For this reason, early diagnosis and treatment are important to prevent serious complications associated with the disease.

Serodiagnosis is generally used to diagnose this disease. However, in the early stages of the illness, diagnosis is difficult by this method as a positive result merely indicates the presence of antibodies. To date, over 20 antigenically distinct strains have been reported, including prototypic strains Karp, Gilliam, Kato, Kawasaki, and Kuroki (Furuya *et al*, 1993; Ogawa *et al*, 2001a; Yoshida, 2003). Improvement in the polymerase chain reaction (PCR) method has resulted in sensitivity sufficient to detect *O. tsutsugamushi* (Yoshida 2003; Razak *et al*, 2010).

Studies have found that some dogs have positive serum against *O. tsutsugamushi*, raising the possibility that dogs may act as the host (Huxsoll *et al*, 1977; Shirai *et al*, 1979). However, these investigations did not test for the genome.

The present study investigated the potential of the PCR method as described by Furuya *et al* (1993) to identify each genomic type of *O. tsutsugamushi* in a canine case of suspected *O. tsutsugamushi* infection. Furthermore, 10 asymptomatic household dogs were tested by the same PCR method to study the epidemiological situation in the local dog population.

CASE REPORT

A 4-year-old male mixed breed dog of body weight 15.1kg had been kept outdoors in a rural mountainous area with abundant plant life. It was referred to a private hospital in Japan for evaluation of anorexia, lethargy and infestation by ticks that had continued for 10 days. The dog's general condition indicated potentially life-threatening symptoms

and physical and laboratory examinations were performed.

The dog had a temperature of 39.1°C. Complete blood counts revealed 120x10^4/ l red blood cells, 6% hematocrit, 524x10^2/ l white blood cells and 91x10^3/ l platelets. Blood biochemical tests showed the following abnormal results: aspartate aminotransferase, 440 U/l; alanine aminotransferase, 829 U/l; alkaline phosphatase, 522 U/l; blood urea nitrogen, 105.9 mg/dl; creatinine, 2.1 mg/dl. Bilirubin was within normal limits.

The ticks that had infested the dog were identified as *Haemaphysalis hystricis* (3 adult males, 2 adult females), *H. flava* (2 adult males) and genus *Haemaphysalis* (15 nymphs and 9 larvae) on the first day of referral to hospital.

Samples for detection of antibodies against leptospirosis, antibodies against ehrlichiosis and antigen of Q fever were sent to Monolis, and PCR of *Babesia gibsoni* was sent to Zenyaku Kougyou, both are commercial veterinary laboratories in Japan. Samples for antibodies against Japanese spotted fever and *O. tsutsugamushi* infection were tested using immune peroxidase test at the Animal Care and Management Center of the Tokushima Prefectural Government.

The tests for infectious diseases conducted by the commercial veterinary diagnostic laboratories entailed a waiting period of several days, during which time the dog was administered atovaquone, azithromycin and clindamycin, as the dog was also suspected of being infected with *Babesia gibsoni* which was endemic in its home area.

On the 15th day, tests confirmed that antibodies against Kato, Karp and Gilliam types of *O. tsutsugamushi* were negative. However, Kawasaki type (1:320) and Ku-

roki type (1:320) were positive. Other tests were all negative.

To detect the *O.tsutsugamushi* genome in the dog's blood and in the ticks attached to the dog, PCR was performed as described by Furuya *et al* (1993). In brief, genomes derived from blood with EDTA or homogenized ticks were prepared using a DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany). The amplification of approximately 1,000 base pairs of oligonucleotides corresponding to N-terminus of a mature 56-kDa protein in *O. tsutsugamushi* by PCR system with Primer 34 (5'-TCAAGCT-TATTGCTAGTGCAATGTCTGC-3') and Primer 55 (5'-AGGGATCCCTGCTGCT-GTGCTTGCTGCG-3') was performed. The amplified fragment was purified by MiniElute® Gel Extraction Kit (Qiagen, Hilden, Germany), which was used as a template for the second PCR to determine the strain of *O. tsutsugamushi* from among Gilliam, Karp, Kato, Kawasaki, and Kuroki. The primer sets, Primer 10 (5'-GATCAAGCTTCCTCAGCCTAC-TATAATGCC-3') and Primer G (5'-CTT-TATATCACTATATATCTT-3') for detection of Gilliam, Primer 10 and Primer KP (5'-ACAATATCGGATTTATAACC-3') for detection of Karp, Primer 10 and Primer KT (5'-GAATATTTAATAGCACTGGA-3') for detection of Kato, Primer 10 and Primer KR (5'-CACCGGATTTACCATCATAT-3') for detection of Kuroki, and Primer KW (5'-ATGCTGCTATTGATACAGGC-3') and Primer 11 (5'-CTAGGGATCCCGA-CAGATGCACTATTAGGC-3') for detection of Kawasaki were used. Positive controls used in the present study were provided by Dr Y Furuya, Department of Biology, Kanagawa Prefectural Institute of Public Health, Kanagawa, Japan.

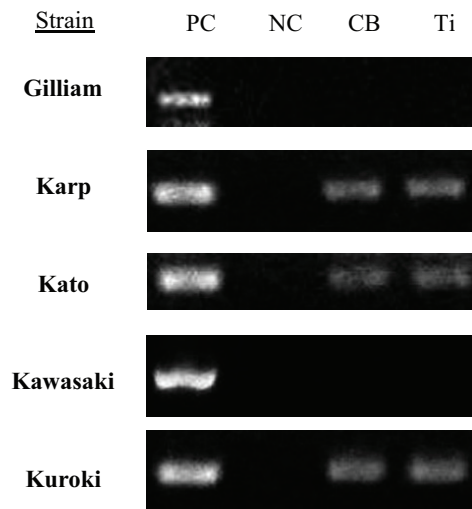Karp, Kato, Kuroki types were found to be positive, while Gilliam and Kawa-

Fig 1–PCR method was performed to detect DNA. Karp, Kato, Kuroki type were positive, while Gilliam and Kawasaki type were negative. PC, positive control; NC, negative control, CB, canine blood; Ti, tick.

saki type were negative in the sick dog's blood. In addition, the ticks infesting the dog were identified as having the same genome types as the dog's blood (Fig 1). However, as the genome type of the dog's blood was not in agreement with its serum antibody type, sequencing of secondary PCR products was performed. Sequencing of these three PCR products, Karp, Kato, and Kuroki type from canine blood sample agreed with standard nucleotide sequence (provided by Dr Y Furuya, Department of Biology, Kanagawa Prefectural Institute of Public Health) 100%, 97.9%, and 100%, respectively. Thus, these data demonstrated that this individual was infected with these three strains of *O. tsutsugamushi*.

According to PCR analysis, this dog was diagnosed with *O. tsutsugamushi* infection, and following administration

Table 1
Detection of DNA by PCR method in 10 asymptomatic household dogs.
Kuroki type was positive in all dogs, Gilliam type was positive in 6 dogs
and Kawasaki type was positive in 3 dogs.

Dog no.	Dog breed	Age (Years)	Strain		
			Kuroki	Gilliam	Kawasaki
1	Labrador retriever	6	+	+	+
2	Labrador retriever	3	+	+	+
3	American cocker	5	+	+	-
4	Chihuahua	6	+	+	-
5	Mixed breed	15	+	+	-
6	Miniature schnauzer	4	+	+	-
7	Akita-inu	3	+	-	+
8	Shiba-inu	6	+	-	-
9	Labrador retriever	4	+	-	-
10	Mixed breed	6	+	-	-

Karp and Kato types were negative in all dogs. +, positive; -, negative.

of oral minocycline, 100 mg, every 12 hours daily for 7 days, the general and laboratory findings for the dog showed improvement.

Ten asymptomatic dogs from the same area of the infected dog were tested to ascertain whether they had been infected or not (Table 1). These dogs were studied by the same PCR method that was used for the suspected case. Kuroki type was found to be positive in all 10 asymptomatic dogs, Gilliam type was positive in 6 dogs and Kawasaki type was positive in 3 dogs. In contrast, Karp and Kato types were negative in this group.

DISCUSSION

The common symptoms of *O. tsutsugamushi* infection include fever, cephalalgia, eschar, rash, malaise and lymphadenopathy (Razak *et al*, 2010; Ogawa *et al*, 2001b). It is impossible to identify cephalalgia and malaise as subjective symptoms in sick dogs. The sick dog's fur may make it difficult to detect eschars and rash; identifying this condition in dogs may be problematic. In this case it took 10 days from referral to a private hospital to identify the causative organism. The prepatent period of *O. tsutsugamushi* infection has been reported to be 7 to 10 days, and thus identification took 2 to 3 weeks from when the dog was initially infested by chiggers. For this reason, enlargement of lymph nodes in which the pathogens primarily multiply might not be found in the dog. An absence of significant lymphadenopathy may be a common finding in cases at an advanced stage of the illness (Kobayashi *et al*, 1992; Kwon *et al*, 2013).

Tetracyclines are effective for *O. tsutsugamushi* infection (Furuya *et al*, 1997; Chen *et al*, 2000; Jayakrishnan *et al*, 2011). However, the condition is sometimes complicated with hemophagocytic syndrome, DIC and systemic inflammatory response syndrome, leading to severe multiple

organ failure if early diagnosis and appropriate treatment are delayed (Kwon *et al,* 2013; Kobayashi *et al,* 1992; Chen *et al,* 2000). Therefore, not only severe anemia, elevated ALT, AST and ALP but also elevated BUN and creatinine values may be evident. Leukocytosis is reported in complicated cases (Furuya *et al,* 1997; Chen *et al,* 2000; Jayakrishnan *et al,* 2011; Kwon *et al,* 2013), and this case may have developed a secondary bacterial infection because of the advanced stage of the illness and the dog had been kept outdoors.

Serological method, isolation of the agents, and DNA detection are used as diagnostic technologies for *O. tsutsugamushi* infection. However, *O. tsutsugamushi* is an obligate parasitic bacterium in the family Rickettsiaceae, therefore isolation of this agent requires fresh sample and injection into live mice, a process requiring skill and experience. Furthermore, at least 1 to 3 months is required for identification, and success of all tests is not guaranteed (Yoshida, 2003). In contrast, PCR methods could provide rapid and sensitive identification, even if the amount of samples were little and/or had been placed in storage. Furuya *et al* (1991, 1993) reported a method which can simultaneously identify five standard serovariants, the Kato, Karp, Gilliam, Kawasaki, Kuroki type, which are prevalent in Japan. Yoshida *et al* (1994) have developed a nested PCR method which can identify each genome type of *O. tsutsugamushi* DNA even only 2 copies are available. In the present study, this method was applied for canine peripheral blood with EDTA and homogenized ticks, enabling detection of each type of genome of *O. tsutsugamushi* from the samples, thus demonstrating the usefulness of this method in small animal clinics.

Although the results of antibody and PCR of this case did not match, a previous study of Katayama *et al* (2006) reported that serum antibody by immunofluorescence (IF) test and PCR were both positive for 43 of 58 samples, only IF method was positive for 13 samples and only PCR method was positive for 2 samples. Furthermore, Furuya *et al* (1997) also reported that 3 cases were PCR positive but antibody detection was negative. The reason for this discrepancy is depended on the progress of the infection and/or the inadequate immune response of the host.

The fact that three types of genome were detected from the sick dog may be explained by the dog being kept outside and thus exposed to several strains for a long period. Although chiggers were not detected on the dog, it is suggested that *Leptotrombidium pallidum*, which could transmit Karp-type *O. tsutsugamushi*, *L. scutellare*, which could transmit Kuroki-type *O. tsutsugamushi*, and *L. akamushi*, which could transmit Kato-type *O. tsutsugamushi* are present in the area. If dogs supply nutrition for chiggers and act as hosts, they may thus play an important role in the maintenance of the *O. tsutsugamushi* life cycle. According to an epidemiological survey (Ogawa *et al,* 2001a) of human serum antibody conducted in 1998, no cases were found in Tokushima Prefecture. However, in Kagoshima Prefecture, which had the most cases, there were 95 cases: 5 cases of Kato type, 4 cases of Karp type, 5 cases of Gilliam type, 51 cases of Kawasaki type, 18 cases of Kuroki type, and 12 cases of undetermined type. From this report, it may be suggested that chiggers of genus *Leptotrombidium* have a lifecycle in the field in Japan and it is necessary not only for dogs but also for humans to take care not to be bitten by these small-sized mites.

The same genome types were detected from the dog's blood and homogenized

ticks, indicating that ticks ingested the dog's blood when the dog had bacteremia. These ticks are not the original vector of *O. tsutsugamushi*, and it may thus be supposed that pathogens are not transmitted stage to stage in the ticks. However, given that *Haemaphysalis hystricis* and *H. flava* are suggested as vectors of Japanese spotted fever, they may transmit *O. tsutsugamushi*. Furthermore, as Little *et al* (2007) showed for *Rhipicephalus sanguineus* and Burgdorfer *et al* (1989) showed by Ixodid ticks, if a tick ingests blood from more than one animal during the same stage without ecdysis, it is possible that ticks become an incidental vector for the pathogen not only to dogs but also to human beings.

Though virulence for dogs of every genomic type has not been reported, it is suggested that *O. tsutsugamushi* can multiply in canine peripheral blood. Since all genomic types tested were detected in dogs, it is possible that dogs can act as a reservoir of this parasite for human beings. From this case report it is suggested that *O. tsutsugamushi* is prevalent in the dog population and dogs may play a role in the life cycle of *O. tsutsugamushi*.

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