Canine Infectious Cyclic Thrombocytopenia Found in Taiwan

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ABSTRACT. Here we report the first canine infectious cyclic thrombocytopenia (CICT) found in Taiwan. Platelet-specific inclusions were detected in the blood of a military working dog. To identify the etiologic agent, the patient’s blood was transmitted to three six-month-old German Shepherd dogs. The Ehrlichia platys-like inclusions were observed six to eight days after inoculation. Indirect fluorescent antibody test showed that the serum from the patient reacted specifically with the microorganisms within the platelets. Typical hematologic manifestations of E. platys infection, cyclic parasitemia and concomitant thrombocytopenia, were observed in these dogs. The prevalence of CICT in north Taiwan was also studied, and the incidence was 8.9% (4 out of 45) in civilian dogs and 97.1% (34 out of 35) in dogs from a heavily tick infested kennel. — Key words: canine infectious cyclic thrombocytopenia, Ehrlichia platys, tick-borne disease.


Canine infectious cyclic thrombocytopenia (CICT) was first described in 1978, and its pathogen, a platelet-specific rickettsia, Ehrlichia platys, was detected by observing blue-staining inclusions in the platelets of the patient’s Giemsa-stained blood smears [4]. Acute E. platys infection is characterized by cyclic parasitemia of platelets followed by severe thrombocytopenia and generalized lymphoahenomegaly [5, 10]. E. platys only invades thrombocytes, rather than mononuclear leukocytes [5, 10]. Infected platelets may contain from 1 to 3 single membrane-lined vacuoles with 1 to 8 organisms per vacuole [4].

Diagnosis of CICT can be carried out by observing basophilic inclusions (i.e. small single elements or large morulae) within platelets in Giemsa stained blood smears [4] or by using indirect immunofluorescent assay (IFA) to locate E. platys in platelets [2]. Since there is minimal serologic cross reaction among different species of ehrlichiae that commonly infect dogs [2, 5, 10], the IFA test, which evaluates species-specific antibody titer, has been widely used to diagnose canine ehrlichia infection.

E. platys infection was reported in the United States of America (U. S. A.) [4, 9] and Greece [6]. On the basis of serologic studies, CICT is widely distributed in U. S. A. [2, 7]. In this report, we confirm that E. platys exists in Taiwan, and we also describe the specific hematologic effects of the isolate on dogs, and the prevalence of CICT in the Taipei area.

In a naturally infected one-year-old military working dog, small basophilic inclusions within 10% of platelets were noted in a routine hematologic examination. Hematologic profiles of the patient revealed a normal hematogram, excepting thrombocytopenia, and the body temperature, appetite, and spirit of the patient were also normal. The etiologic agent of CICT used in this study originated from the patient dog.

Three 6-month-old male German Shepherd dogs in a healthy condition were dewormed and dipped for both internal and external parasites and quarantined for three weeks before inoculation. The dogs were housed separately and supplied with commercial dry food and adequate water. A volume of a ml of the patient’s blood was inoculated intravenously into dog no. 1, which was splenectomized on the next day after blood inoculation. Blood with 35.5% of infected platelets collected from dog no. 1 on day 20 post inoculation was mixed with an equal volume of 10% dimethyl sulfoxide in PBS (0.01 M potassium phosphate, 0.15 M NaCl, pH 7.2). Aliquots (1 ml) were frozen and stored in ampules in liquid nitrogen. These samples were thawed and used for subsequent intravenous inoculations of two additional dogs, no. 2 and no. 3.

The dogs were examined for clinical signs and their body temperatures were recorded every day. For hematologic evaluation, venous blood samples were collected daily with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Complete blood cell counts and platelet counts were performed with an electronic cell counter (Sysmex K-1000, Toa Medical Electronics Co., Ltd., Japan). Thin blood smears were examined for organisms with Giemsa stain. The ratio of infected platelets was calculated by observing 200 platelets in blood smears.

To confirm that the microorganisms within the platelets of the experimentally infected dogs were caused by donor blood inoculation, the platelets of the recipient were reacted with the serum of the cured donor. E. platys infected platelets were collected from dog no. 1 on day 20 post inoculation (platelets count 139,000/μl with 35.3% parasitized). Platelet-rich plasma, prepared by centrifuging the blood at 500 × g for 6 min, was washed with an equal volume of PBS-EDTA (5 mM EDTA in PBS) and centrifuged at 1,000 × g for 15 min at room temperature 3 times, and then were resuspended in PBS-EDTA. The platelet suspension was placed on a cover slip and fixed with a fixative (60% acetone, 40% methanol) for 10 min at -20°C. After aspirating the fixative, the antigen-coating slips were air dried and stored at -20°C.

Sera collected from patients after tetracycline treatment, from 45 civilian dogs in Taipei county and from 35 dogs...
which live in a kennel with heavy tick infestation, were heated at 56°C for 30 min. Sera diluted with PBS containing 10% fetal calf serum were allowed to react with the antigen for 30 min at 37°C. The antigen slips were washed in PBS three times (5 min each), and then were allowed to react as above with appropriately diluted fluorescein isothiocyanate conjugated rabbit anti-dog IgG antiserum (Zymed Laboratories Inc., U.S.A.). Then the slips were washed as above, and examined with a fluorescent microscope (Axioskop, Zeiss, Germany). The serum reaction was considered positive if there was a clear staining of the inclusions within platelets at a minimum serum dilution of 1:100, which has been shown to be specific for indication of active infection or previous exposure.

All the experimentally infected dogs did not appear clinically ill. These dogs were afebrile, with fair spirits and good appetites during clinical evaluation. Only a slight increase in rectal temperatures (39.0–39.5°C) was noted during the initial parasitemic episode. Basophilic inclusions were observed within platelets of experimentally infected dogs on day 6–8 post inoculation (Fig. 1). These inclusions were found only in thrombocytes, but not in leukocytes and erythrocytes, and they appeared in the form of small single elements of large morulæ, resembling those observed in the blood smears of the naturally infected dog.

Cyclic parasitemia and concomitant thrombocytopenia were observed in all the dogs. When the infected dog appeared in peripheral blood smears, there was a steep reduction in the total platelet counts, and then the organisms were no longer seen (Fig. 2). Thereafter, the platelet count increased rapidly and reached normal values in 4 to 6 days (Fig. 2B). Splenectomy did not alter the periodicity of parasitemia and thrombocytopenia, but did cause some other differences. In each parasitemic episode, the ratio of infected platelets of the splenectomized dog was higher than that of the nonsplenectomized dogs. In the splenectomized dog, the percentage of infected platelets at peak of every parasitemic episode always reached 30% or higher, and the platelet counts were always below the range of the normal dog’s value (Fig. 2A). Intrathrombocytic parasitemia and concomitant cycles of thrombocytopenia recurrent at 1–2-week intervals (Figs. 2A, 2B).

Three weeks of tetracycline therapy regimen was applied to the naturally infected dog (22 mg/kg, PO, tid). Dog no. 3 was also treated with tetracycline hydrochloride for 2 weeks from the day parasitemia appeared. The signs of cyclic thrombocytopenia disappeared after administering tetracycline hydrochloride to both dogs. After giving tetracycline, the total platelet counts of dog no. 3 returned to and kept in a normal range in ongoing monitors, however, a few organisms appeared occasionally in platelets of Giemsa stained blood films (Fig. 2C).

The serum collected from the patient reacted specifically with the inclusions in platelets of the experimentally infected dogs (Fig. 3), and the antibody titer was 1:1,280. Antibody titers to E. platys higher than 1:100 were detected in sera of 4 of 45 (8.9%) civilian dogs in Taipei area, and 34 of 35 (97.1%) dogs from a heavily tick infested kennel which the patient lives in.

As reported here, the E. platys infection in Taiwan was confirmed by the following facts: (1) the dogs experimentally inoculated with the blood of a dog suspected to be infected with E. platys developed cyclical appearances of platelet specific inclusions and thrombocytopenia; (2) the cured dogs developed a specific antibody against the organisms; (3) the isolated organism was sensitive to tetracycline.

Dogs with CICT are not usually clinically ill and rarely show signs of significant hemorrhage, even with severe thrombocytopenia [10]. We made the same observation in our study, and a mild increase in rectal temperatures (39.0–39.5°C) was also noted at the time of the initial parasitemias. These results indicate that our isolate is similar to the strain isolated by Harvey et al. in 1978 [4], although more virulent strains of E. platys may exist in nature. One report indicates that another strain which causes illness (fever, depression, and weight loss) exists in Greece [6]. Another report shows that there is a case of E. platys associated with overt signs of hemorrhage in Michigan, U. S. A. [9].

Harvey et al. reported that the cyclic nature of the appearance of parasitized platelets diminishes during chronic infection [4]. In contrast, our study showed that the later parasitemias also appeared with a relatively high infected platelet proportion, especially in the splenectomized dog. In some cases of CICT, transient decreases in total leukocyte
counts and packed cell volume occurred concomitantly with the appearance of parasitized platelets, but these values were seldom below the normal range of dogs [3, 10]. One previous report indicates that a mild normocytic, normochromic anemia developed on day 7 post inoculation, which may have been attributable to the syndrome of anemia of inflammation [1]. In our experiments, a cyclic decrease of total leukocyte counts following parasitemias was observed, but these values were still within the normal range. Mild normocytic and normochromic anemia was also noted in all three dogs, however, the relationship between these observations and cyclic episodes of parasitemia and thrombocytopenia was obscure.

IFA test appears to be a specific method in identifying dogs that have been exposed to E. platys. The IFA titers may rise 7 to 17 days after initial infection to titers of 1:5,120 [5, 10]. Serum antibody titers against E. platys higher than or equal to 1:100 by the IFA test indicate prior exposure [5, 10]. In our experiment, serum collected from the cured patient with a titer of 1:1, 280 suggests that the patient was exposed to the etiologic agent of CICT.

Although the mode of E. platys transmission between dogs and the reservoir in nature has not yet been proved, CICT is suspected of being transmitted among dogs primarily by the brown dog tick (Rhipicephalus sanguineus), the same as Ehrlichia canis is [5, 10]. An earlier report demonstrated that the brown dog tick may not serve as a biologic vector of E. platys [8]. In our study, we found the positive rate of IFA test was much higher (97.1%) in the sera collected from a kennel with a heavy tick infestation. Inasmuch as brown dog ticks are predominant here, we conclude that the transmission of CICT is possibly carried out by R. sanguineus.

Antibodies against E. platys with titers greater than 1:100
were detected in sera of 8.9% civilian dogs in the northern part of Taiwan. The observed infection rate among dogs in Taipei county shows that the agent and its vector are well established in Taiwan.

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REFERENCES