Experimental Infection of Japanese Encephalitis Virus in Dogs

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ABSTRACT. A previous serosurvey of Japanese encephalitis virus (JEV) among dogs suggested that dogs are well suited for use as sentinels for assessing the risk of JEV transmission to humans. To examine the clinical symptoms and duration of anti-JEV antibodies in dogs, three dogs were experimentally challenged with JEV. All JEV-infected dogs did not show any clinical signs or abnormal blood tests, except for C-reactive protein. Virus-neutralization titers rapidly increased and were maintained until 70 days postinfection, and neither the virus nor the viral genome was detected in blood. Thus, since dogs live in close proximity to humans as companion animals, they are well suited for use as sentinels for surveying the human risk of JEV infection.

KEY WORDS: dog, Japanese encephalitis virus, sentinel.

Japanese encephalitis virus (JEV), which is transmitted to a variety of hosts by mosquitoes, mainly by Culex tritaeniorhynchus, causes serious acute encephalitis in horses and humans. JEV is endemic to Southeast Asia and the Western Pacific region [6], where the annual incidence of the disease is approximately 50,000 human cases and 10,000 deaths [4]. In Japan, the majority of Japanese encephalitis (JE) cases have been reported in the west of the country where most domestic pigs are seropositive for JEV in summer, suggesting that the risk of JEV infection to humans in this region is high [1]. However, because pig farms are generally far from residential/urban areas in Japan, it is possible that serosurveys in pigs may not accurately reflect the risk of JEV infection to humans.

Since dogs, and not pigs, live commensally with their owners, the seroprevalence of JEV in dogs is likely to be a more sensitive indicator of human risk than that in pigs. We previously reported that 25% of dogs had virus-neutralizing (VN) antibodies against JEV, with particularly high seropositivities detected in the Kyushu (47%) and Shikoku (61%) districts of western Japan [11]. Despite the relative importance in sero-epidemiological studies, the duration of anti-JEV antibodies after JEV infection in dogs remains unknown. Furthermore, several canids with encephalitis and myocarditis, possibly associated with West Nile virus, which belongs to Japanese encephalitis serocomplex, have been reported in U.S.A. [2, 5, 9, 10]. To examine the duration of anti-JEV antibodies and clarify the clinical symptoms and viremia in dogs, several dogs were experimentally infected with JEV in the present study.

Three female beagles (2 months old) (NARC, Japan) were intraperitoneally and subcutaneously inoculated with 5 × 10⁶ plaque-forming units (PFU) of JaOH0566, a genotype III strain isolated from a JE patient in 1966 (kindly provided by Dr. Ishikawa (Biken, Kanonji, Japan)). Virus infectivity was measured by the plaque formation assay using Vero9013 cells (JCRB number; JCRB9013) [8, 11]. While no clinical signs or increase in body temperature were observed during the observation period, transient decreases in body weight was observed on day 2 postinfection (dogs No. 1 and No. 2) and from day 1 to 3 postinfection (dog No. 3). Among the various blood parameters assayed, including a complete blood count and biochemical tests, only C-reactive protein (CRP; an index of inflammation) levels in sera were abnormally high. The CRP levels reached 2.35 mg/dl (dog No. 1–day 2), 5.6 mg/dl (dog No. 2–day 1) and 1.05 mg/dl (dog No. 3–day 2) before recovering to less than 1.0 mg/dl by day 6 postinfection.

VN titers of JaOH0566 were measured as described previously [8, 11]. The results showed that until day 21 postinfection in dogs No. 1 and No. 2, and day 28 in dog No. 3, VN titers kept increasing before being maintained at similar levels for 70 days after challenge (Table 1). The sera of all animals exhibited cross-VN activity to JEV/sw/Chiba/88/2002, which belongs to genotype I (data not shown).

To examine the presence of JEV in blood, sera and peripheral blood mononuclear cells collected on days 1 to 3, 7, 10, 14 and 17 postinfection were inoculated onto Vero9013 cells. Furthermore, collected sera were intracerebrally inoculated to suckling mice (BALB/c, 3 days old). To detect viral genome, sera were examined by RT-PCR using one-step RT-PCR kit (Qiagen, Valencia, CA, U.S.A) with primers, JEV1F (5’-GGA ACA GCA TGC AAA TCG AAA-3’) and JEV2R (5’-ACC AGA AGG CCC AGC TGA AAA-3’); the detection limit of the RT-PCR was 100 PFU/ml. As a result, neither the virus nor the viral genome was detected in blood by these methods (data not shown).

Ideally, sentinels for serological surveys should be susceptible to infection, capable of surviving from the infection, develop detectable antibodies, pose no risk of infection

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to handlers, and never attain sufficiently high levels of viremia to infect vector mosquitoes [3]. In this study, we reported that dogs are susceptible to JEV-infection without developing clinical signs, having high antibody titers, or detectable viremia. Even when JEV occurred at epidemic levels in Japan, no JE cases were reported in domestic cats or dogs [7]. Dogs therefore appear to be well suited for use as sentinels for surveying the potential risk of human infection with JEV, especially in residential/urban areas.

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