Journal of Virological Methods 187 (2013) 85–89

Contents lists available at SciVerse ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Development and application of an indirect enzyme-linked immunosorbent assay for serological survey of Japanese encephalitis virus infection in dogs

Hiroshi Shimoda[a], Natnaree Inthong[b], Keita Noguchi[a], Yutaka Terada[a], Yumiko Nagao[a], Masayuki Shimojima[a], Tomohiko Takasaki[c], Worawut Rerkamnuaychoke[b], Ken Maeda[a,*]

[a] Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan
[b] Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand
[c] Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan

Article history:
Received 16 February 2012
Received in revised form 6 September 2012
Accepted 10 September 2012
Available online 6 October 2012

Keywords:
Japanese encephalitis virus (JEV)
Enzyme-linked immunosorbent assay (ELISA)
Serological survey
Dog

ABSTRACT

Japanese encephalitis virus (JEV) causes serious acute encephalitis in humans and horses. Although dogs are good sentinels for assessing the risk of JEV infection to humans, a virus neutralization test has been the only method available for measuring the levels of JEV antibody in dogs. In this study, an indirect enzyme-linked immunosorbent assay (ELISA) using purified viral particles as an antigen, was developed for serological survey of JEV infection in dogs. In dogs inoculated experimentally with JEV, the ELISA detected anti-JEV IgM 3 days after infection, with IgM levels peaking 7 days after infection. Anti-JEV IgG was detected 14 days after infection and peaked on 21–28 days after infection. Virus neutralization titers correlated with anti-JEV immunoglobulins measured by the ELISA. To test the utility of the new assay, the seroprevalence of JEV infection among 102 dogs in Kyushu, Japan, was examined by IgG ELISA and by virus neutralization. The correlation coefficient between the IgG ELISA and virus neutralization was 0.813 ($p < 0.001$); comparison of the IgG ELISA and virus neutralization showed a sensitivity and specificity of 82% and 98%, respectively. The IgG ELISA was used to survey dogs in Bangkok, Thailand and 51% of these dogs were found seropositive for JEV. These data suggest that in the capital city of Thailand, the risk of infection with JEV remains high.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Japanese encephalitis virus (JEV) is a member of genus *Flavivirus*, family *Flaviviridae*. JEV is transmitted primarily by *Culex tritaeniorhynchus* vector mosquitoes, and pigs play a main role as amplifiers. The virus is widely endemic in nations of Southeast Asia and the Western Pacific, including Japan and Thailand (Mackenzie et al., 2001), with an annual incidence of approximately 50,000 cases and 10,000 deaths in humans (Erlanger et al., 2009).

In Thailand, 1500–2500 JE cases had been reported annually throughout the 1970s and 1980s (Olsen et al., 2010). In contrast, between 2005 and 2010, the annual number of encephalitis cases in Thailand ranged from 322 to 431, including 36–78 confirmed JE cases per year (Thailand Ministry of Public Health). This dramatic reduction in the number of JE cases over the past 20 years has been the result of an effective human vaccination program by the Thailand Ministry of Public Health. However, a recent (2009) serological survey showed that 39% of pigs are seropositive for JEV in Thailand; the seroprevalence of JEV was especially high (67%) in pigs in Chiang Mai province, which includes the second biggest city of Thailand (Prompiram et al., 2011). In addition, the number of JE cases in Thailand still exceeds those in other countries where a JEV vaccine is used, such as Japan and Korea (Erlanger et al., 2009). However, recent human risk of JEV infection in the capital city, Bangkok, remains unknown.

In Japan, serological surveys for JEV infection among pigs have been performed annually. However, it is unclear whether serological surveys among pigs accurately reflect the risk of JEV infection to humans, since pig farms typically are separated from urban areas in Japan. Therefore, another method for monitoring JEV infection risk in humans in urban areas is necessary. In the previous serological survey of JEV, one fifth of dogs in urban and residential areas in Japan were infected with JEV (Shimoda et al., 2010). In addition, experimental infection of JEV in dogs showed that dogs infected with JEV do not exhibit any symptoms or detectable viremia, although dogs do develop high JEV antibody titers (Shimoda et al., 2011). These data indicate that dogs are good sentinels to assess the risk of human infection with JEV.

Serological tests such as virus neutralization and hemagglutination inhibition (HI) have been the primary assays used to detect

* Corresponding author at: Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan. Tel.: +81 83 933 5887; fax: +81 83 933 5887.
E-mail address: kmaeda@yamaguchi-u.ac.jp (K. Maeda).

0166-0934/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jviromet.2012.09.022

JEV-specific antibodies in serum (Anderson et al., 2011; Chiou et al., 2007; Mall et al., 1995; Ting et al., 2004; Watanabe et al., 2008). The HI requires a large volume of serum, while the virus neutralization requires a special facility (e.g., biosafety level-2 or -3) and a high level of technical skill. In contrast, enzyme-linked immunosorbent assay (ELISA) does not require the use of live JEV, and the procedure is simple and requires only a small amount of sample. Other laboratories have reported the use of an indirect IgG ELISA for JEV serological surveys among pigs and bats (Cui et al., 2008; Hamano et al., 2007; Nidaira et al., 2007; Xinglin et al., 2005; Yang et al., 2006).

In the present study, an indirect ELISA to detect JEV antibodies in dogs was developed using sera of dogs infected experimentally with JEV and domestic dogs in Kyushu and Hokkaido, Japan. The test was then applied for a serological survey of dogs in Thailand to assess the risk of human infection with JEV.

2. Materials and methods

2.1. Cells

Vero cells (JCRB number JCRB9013), which are derived from African green monkey, were purchased from the Health Science Research Resources Bank (HSRRB, Tokyo, Japan). Vero cells were cultured at 37 °C and 5% CO_2 in Eagle's minimum essential medium (EMEM; GIBCO, Grand Island, NY, USA) supplemented with 5% heat-inactivated fetal calf serum (FCS; J R Scientific, Woodland, CA, USA), 1 mM sodium pyruvate, 100 U/ml of penicillin, and 100 μg/ml of streptomycin (GIBCO). Mosquito-derived C6/36 cells (JCRB number IFO 50010) were cultured at 28 °C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 10% heat-inactivated FCS, 100 U/ml of penicillin, and 100 μg/ml of streptomycin.

2.2. Virus

JEV/sw/Chiba/88/2002 was originally isolated from the serum of a healthy pig in 2002, and has been genetically classified into genotype I (Nerome et al., 2007). The virus was propagated in C6/36 cells grown at 28 °C in DMEM supplemented with 2% FCS, and was stored at −80 °C until use.

2.3. Dog serum samples

Sera of dogs infected experimentally with JEV were collected sequentially and stored at −80 °C in a previous study (Shimoda et al., 2011). Briefly, three female dogs were inoculated intraperitoneally and subcutaneously with 5×10^6 plaque-forming units (PFU) per dog of JaOH0566, a viral strain (kindly provided by Dr. Ishikawa (Biken, Kagawa, Japan)) that was originally isolated from a JE patient in 1966. Serum samples, which had been collected at a series of time points, were used for ELISA and virus neutralization. The animal experiments were approved by Animal Research Ethics Board of Faculty of Agriculture, Yamaguchi University.

For the serological survey, a total of 183 family dog sera were collected from domestic dogs in Kyushu and Hokkaido districts, Japan, and Bangkok, Thailand. These samples were collected from veterinary hospitals in Japan, or by visiting the owners of the examined dogs in Thailand. All owners were informed of the research objectives before sampling and data collection. To classify the dogs examined in this study, the owners also were asked to provide data about the sex, age, breed, and housing of their dogs. The definitions of outdoor (group A) and indoor dogs (group B) in this study are always (100%) staying outside of the house and, staying inside of the house more than 80% of the time, respectively. Note that all sera used for the virus neutralization were inactivated by incubation at 56 °C for 30 min to prevent the inactivation of JEV by complement in serum samples, and then stored at −20 °C until use.

2.4. Virus neutralization

To determine the presence in sera of virus neutralizing antibodies against JEV, an 80% plaque reduction virus neutralization was carried out as described previously (Ohno et al., 2009; Shimoda et al., 2010, 2011). Briefly, sera were diluted 1:5 and then were serially two-fold diluted in EMEM containing 2% FCS. The diluted sera or medium alone were mixed with equal volumes of virus solution containing 100 PFU of JEV/sw/Chiba/88/2002, and the mixtures were incubated at 37 °C for 90 min. After incubation, the mixtures were inoculated to subconfluent Vero cells and incubated at 37 °C for 90 min. After two washes with EMEM, the infected cells were overlaid with 0.8% agarose (Lonza, Rockland, ME, USA) in EMEM containing 5% FCS. Following 4 days incubation at 37 °C in 5% CO_2, the cells were fixed with 10% buffered formaldehyde for 1 h, and the agarose layer was removed. After staining with crystal violet, plaques were counted. Sera that reduced the number of plaques by more than 80% in comparison with the mean number of plaques in control wells were considered to be JEV antibody positive.

2.5. ELISA

For ELISA, inactivated JEV originating from the Beijing 01 strain was selected as the antigen. This strain had been propagated in Vero cells, inactivated with formaldehyde, and purified by ultracentrifugation, and the product was intended for use as a vaccine antigen in humans. The inactivated JEV was diluted to 5 μg/ml with adsorption buffer (0.05 M carbonate–bicarbonate buffer, pH 9.6), and distributed at 100 μl per well into 96-well microplates (Maxisorp; Nunc, Roskilde, Denmark). Control wells received an equivalent volume of adsorption buffer without antigen. After incubation at 37 °C for 2 h, plates were placed at 4 °C overnight. The wells were washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween20 (PBS-T) and then incubated with 100 μl per well of 0.1% bovine serum albumin (BSA) (Fraction V; Sigma, St. Louis, MO, USA) in PBS at 37 °C for 30 min. Test sera were diluted with PBS-T containing 10% FCS. Wells were washed three times with PBS-T, and then diluted sera were added to duplicate wells, and plates were incubated at 37 °C for 30 min. Next, the wells were washed three times with PBS-T and incubated with 100 μl per well of diluted peroxidase-conjugated sheep anti-dog IgG or goat anti-dog IgM antibody (Bethyl Laboratories, Montgomery, TX, USA) at 37 °C for 30 min. Following washing three times with PBS-T, 100 μl of horseradish peroxidase substrate kit (Bio-Rad, Hercules, CA, USA) was added to each well. After incubation at room temperature for 30 min, the enzymatic reaction was stopped by adding 100 μl of 2% oxalic acid to each well. The absorbance was measured by a spectrophotometer (Bio-Rad) with a 405 nm filter. All results were corrected by the value of the non-antigen control.

2.6. Optimization of concentration of ELISA antigen

The optimal concentration of the antigen was determined by cross-titration using the serially diluted antigen and the serially diluted serum from one mouse infected with JEV as the primary antibody. Based on the results of this preliminary study, an optimal antigen concentration of 5 μg/ml was selected (data not shown).

2.7. Statistical analysis

Chi-square test was performed to assess statistically the seroprevalence of JEV among dogs using IgG ELISA, and Pearson's correlation coefficient was calculated to determine the correlation

Download English Version:

https://daneshyari.com/en/article/6134463

Download Persian Version:

https://daneshyari.com/article/6134463

Daneshyari.com