The Development and Application of the Latex Agglutination Test to Detect Serum Antibodies against Japanese Encephalitis Virus

Jia Xinglin, Chen Huanchun*, He Qigai, Wang Xiang, Wu Bin, Qiu Dexin and Fang Liurong

College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, People’s Republic of China

*Correspondence

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ABSTRACT

The attenuated SA14-14-2 strain of Japanese encephalitis virus (JEV) was cultured in BHK-21 cells. The viral supernatant was purified and concentrated with PEG (MW 20000). A suitable concentration of JEV antigen was used to sensitize latex to prepare the latex antigen. The specificity, sensitivity and stability of the antigen were assessed. A latex agglutination test (LAT) was developed for rapidly detecting antibody against JEV infection. The LAT and haemagglutination inhibition (HI) assay were compared by simultaneously testing 35 porcine serum samples from five farms. Ninety per cent (20/23) of the samples were seropositive by both assays. No significant difference was found between the two methods (p > 0.05). Furthermore, when 1613 porcine sera from 120 farms were tested by LAT, the number of positive sera was 652, while that of negative sera was 961, ranging from 20% to 50% positive throughout the year. These results indicate that LAT is an appropriate candidate method for epidemiological surveys for and diagnosis of Japanese encephalitis.

**Keywords:** diagnosis, haemagglutination inhibition, Japanese encephalitis virus, latex agglutination test, pigs, serology

**Abbreviations:** BHK, baby hamster kidney (cell); CF, complement fixation; ELISA, enzyme-linked immunosorbent assay; HI, haemagglutination inhibition; IHA, indirect haemagglutination; JE, Japanese encephalitis; JEV, Japanese encephalitis virus; LAT, latex agglutination test; NT, neutralization test; PEG, polyethylene glycol; RA, radioimmunoprecipitation assay; RBC, red blood cell; TCID₅₀, 50% tissue culture infective dose

INTRODUCTION

Japanese encephalitis is a serious viral zoonosis transmitted by mosquitoes. It is harmful to both humans and animals. Encephalitis or other nervous symptoms are common in humans. Infected sows may suffer from abortion, stillborn delivery or mummified fetuses. Japanese encephalitis (JE) was first described in 1871 and 1921 and identified in 1924 and 1934 in Japan and China, respectively. Fujita (1933) and Taniguchi and colleagues (1936) isolated and identified Japanese encephalitis virus
JEV. JE has a high prevalence in some Asian countries, especially in the tropics. Konno and colleagues (1966) showed that swine are the major source of infection and play a very important role in the transmission of JE. Present serological diagnostic methods for JE included complement fixation (CF), haemagglutination inhibition (HI), the neutralization test (NT), indirect haemagglutination (IHA), enzyme-linked immunosorbent assay (ELISA), radioimmunoprecipitation (RA), the immunofluorescence test, etc. CF is only a suitable method for retrospectively confirming JE. Although the HI test can detect antibody earlier than CF, agglutination of erythrocytes by JEV is very variable, easily destroyed by an adverse pH, and time-consuming, as it necessitates removal of non-specific agglutination factors in the sera. Other methods are relatively little used clinically. Accurate and rapid diagnosis of JE is the key to its prevention and control. We developed a latex agglutination test for detecting JEV antibody to avoid shortcomings of the above assays and to provide an on-site diagnostic method.

MATERIALS AND METHODS

Materials

*Virus:* The SA14-14-2 strain, stored in our laboratory, was used.

*Cells and culture medium:* BHK-21 cells, preserved in our laboratory, were used. PRMI 1640 culture medium, commercially available from Gibco BRL, Grand Island, NY, USA, was used.

*Positive and negative sera:* A JEV positive serum was prepared in a healthy pig, that was negative for JEV by HI, by immunizing it three times with the attenuated SA14-14-2 strain of JEV cultured in primary hamster kidney cells. Its HI titre was then 1:40. Twelve negative sera came from healthy swine negative for JEV by HI.

Five sera positive for swine brucellosis and five sera positive for chlamydiosis were obtained from Lanzhou Veterinary Research Institute. Five sera positive for porcine parvovirus and five sera positive for atrophic rhinitis were prepared from healthy pigs immunized with the appropriate inactivated vaccines. Five bovine sera came from healthy cattle. All these serum samples were negative for JEV by HI.

*Other reagents:* These were 10% latex solution (Ø 0.5–0.8 nm) (Medical Examination Institute of Shanghai, China); tyrosine (Difco, USA); PBS, pH 7.4, pH 6.4 and pH 9.0; 12.5% kaolin solution; and 0.5% and 30% goose red blood cell suspension, prepared according to Denyun ZI and colleagues (1995).

*JEV HI test kit:* A kit from the Epidemiological Institute of Yunnan Province, China, was used.

*Serum samples:* Serum samples from the field were used.