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Short Communication

EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF TRANSMISSIBLE GASTROENTERITIS VIRUS ANTIBODIES

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ABSTRACT

Hohdatsu, T., Eiguchi, Y., Ide, S., Baba, K., Yamagishi, H., Kume, T. and Matumoto, M., 1987. Evaluation of an enzyme-linked immunosorbent assay for the detection of transmissible gastroenteritis virus antibodies. Vet. Microbiol., 13: 93–97.

An enzyme-linked immunosorbent assay (ELISA) using a detergent-solubilized antigen of purified virus was developed for detection of antibody against porcine transmissible gastroenteritis (TGE) virus in swine serum. The ELISA demonstrated antibody responses in pigs immunized intramuscularly with the attenuated TO-163 strain of TGE virus and in pigs orally infected with the virulent Shizuoka strain of the virus. The results of the ELISA were well correlated with those of the neutralization test. These results indicate the usefulness of the ELISA as a serological tool for TGE virus antibody.

INTRODUCTION

Transmissible gastroenteritis (TGE) is a viral disease of swine characterized by a short incubation period, high infectivity, diarrhea and vomiting, and a high mortality in young piglets. Various serological tests for the detection of TGE virus antibodies are available. They include the neutralization test (Harada et al., 1967; Bohl et al., 1972), bentonite agglutination test (Sibinovic et al., 1966), complement fixation test (Stone et al., 1976), agar immunodiffusion test (Bohac et al., 1975; Bohac and Derbyshire, 1976; Stone et al., 1976), indirect fluorescent antibody test (Benfield et al., 1978), indirect haemagglutination test (Shimizu and Shimizu, 1977), and immunoperoxidase test (Kodama et al., 1980).
The enzyme-linked immunosorbent assay (ELISA) has been shown to be a useful serological tool for viral infections. The present study was undertaken to develop an ELISA for detection and quantitation of TGE virus antibody in swine serum.

MATERIALS AND METHODS

For preparation of viral antigen, the TO-163 strain of TGE virus (Harada et al., 1967; Furuuchi et al., 1975) was propagated in cultures of the CPK cell line derived from swine kidney (Komiya et al., 1981). Infectious culture fluid was concentrated about 10-fold by ammonium sulfate precipitation, layered onto a discontinuous sucrose density gradient (10–60%) in a Beckman SW25.2 rotor and centrifuged at 24 000 r.p.m. for 2 h. The virus bands formed were collected, mixed with an equal volume of fluorocarbon (Wako Pure Chemicals, Tokyo) and vigorously shaken for 2 min. The water phase separated by centrifugation was layered onto a linear sucrose density gradient (10–60%) in a Beckman SW25.2 rotor and centrifuged at 24 000 r.p.m. for 4 h. Fractions with densities of 1.18–1.20 g ml⁻¹ were pooled and centrifuged at 77 000 × g for 1 h. The pellets were resuspended in a 1/500 volume of NTE buffer (0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.001 M EDTA). The suspension was treated with 1% Triton X-100 at 4°C for 1 h and dialyzed against phosphate buffered saline (PBS) (0.1 M NaCl, 0.01 M phosphate buffer, pH 7.2). Supernatant fluid from CPK cell cultures was concentrated 10-fold by ammonium sulfate precipitation and used as the control antigen.

For the ELISA, virus antigen prepared as above was appropriately diluted with carbonate buffer (0.05 M, pH 9.6) and delivered in 100-μl volumes into wells of 96-well, flat-bottomed Microelisa plates (Dynatech Lab., U.S.A.). The plates were allowed to stand overnight at 4°C, washed four times with 0.85% NaCl solution containing 0.02% Tween-20, and 100 μl of the test serum diluted 100-fold with PBS containing 10% calf serum and 0.05% Tween-20 was added to each well. After incubation at 37°C for 1 h, the plates were washed, and 100 μl of optimal dilution of horseradish peroxidase-conjugated rabbit antibody against porcine IgG (Miles Lab., U.S.A.) was added to each well. After incubation at 37°C for 1 h, each well was washed, received 100 μl of substrate solution and was incubated at 25°C for 20 min in a dark room. The substrate solution was prepared by dissolving 0-phenylenediamine dihydrochloride at a concentration of 0.1 mg ml⁻¹ in 0.1 M citric acid–0.2 M Na₂HPO₄ buffer (pH 4.8) and adding 0.2 μl ml⁻¹ of 30% H₂O₂. At the end of incubation, the reaction was stopped with 3 N H₂SO₄ solution and absorbancy measurement was performed at a wave length of 492 nm. The value obtained by subtracting the absorbance of the control antigen from that of the viral antigen was taken as the ELISA value.

Neutralization (NT) tests were carried out by the serum dilution method in CPK cell cultures prepared in flat-bottomed microplates (Corning Glass
 Works, Corning, NY). In wells of a transfer plate, 25 μl of each of serial 2-fold dilutions of the serum inactivated at 56°C for 30 min was mixed with 25 μl of maintenance medium containing 200 TCID$_{50}$ of virus. Four wells were employed for each serum dilution. The virus–serum mixtures were incubated at 37°C for 60 min, transferred into wells of a microplate containing CPK cell cultures, and incubated in an atmosphere of 5% CO$_2$ in air at 37°C for 5 days. The antibody titre was expressed as the reciprocal of the antibody dilution showing 50% neutralization calculated by the method of Kärber.

RESULTS

In preliminary experiments with a swine antiserum with a NT titre of 256 against the TO-163 strain, specific reaction was observed when each well of the Microelisa plates received 10–2.5 μg protein in the virus antigen, as determined by Lawry’s method. In the following experiments, 2.5 μg protein was delivered in each well.

Serum samples were collected from 78 specific pathogen-free pigs on a

![Fig. 1. ELISA and NT tests on sera collected at intervals from pigs inoculated with two intramuscular doses of the TO-163 strain of TGE virus.](image-url)
Fig. 2. ELISA values and NT titres in pigs orally infected with the virulent Shizuoka strain.

farm where no TGE outbreaks were experienced in the past. These serum samples had an average ELISA value of 0.131 with a standard deviation of 0.051. In order to give an ample safety margin, ELISA values of 0.284 (0.131 + 3 × 0.051) or higher were taken as positive.

Serial 2-fold dilutions of four swine antisera having NT titres of 16000, 2048, 256 and 32 were tested by the ELISA. The end point dilutions of these serum samples were 1:25600, 1:5000, 1:600 and 1:400, respectively, being closely correlated with their NT titres.

Serum samples were collected at intervals from four initially seronegative, 2-month-old pigs inoculated with two intramuscular doses of about 10⁷ TCID₅₀ each of the TO-163 strain and tested by the ELISA and NT test. The results are summarized in Fig. 1. The ELISA value and the NT titre increased gradually after the first inoculation, and rapidly after the second one.

Fifteen initially seronegative, 2-month-old pigs were infected by the oral route with the virulent Shizuoka strain of TGE virus (Harada et al., 1967). Serum samples were collected before infection and 21 or 28 days after infection, and tested for ELISA value and NT titre. All the animals produced antibodies after infection, ELISA values and NT titres ranging from 0.64 to > 2.00 and from 2 to 256, respectively. ELISA values of individual pigs were closely correlated with their NT titres (Fig. 2). The correlation coefficient was 0.911 (P > 0.01).

DISCUSSION

In the present study an ELISA using detergent-solubilized antigen of purified virus was developed for detection of antibody against TGE virus in swine serum. Nelson and Kelling (1984) reported an ELISA for TGE virus antibody in swine serum.
Antibody responses were demonstrated by the ELISA and NT test in pigs immunized intramuscularly with the attenuated TO-163 strain and in pigs orally infected with the virulent Shizuoka strain. These results seem to indicate the usefulness of the ELISA as a serological tool. The ELISA may be applied with advantage to testing large numbers of sera for TGE virus antibody.

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