Enzyme-linked Immunosorbent Assay for the Serological Survey of Getah Virus in Pigs

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Getah virus, a member of the genus Alphavirus, was shown to cause outbreaks of acute febrile illness with urticarial rash and edema in the hind legs in Japanese race horses [2, 6]. Later the virus was isolated from healthy pigs [4] and newborn piglets dying of peracute illness [7]. The virus produced a febrile illness in experimentally inoculated pigs [3, 5] and transplacental infection in pregnant pigs [1].

The neutralization test [2, 4, 5, 7], complement-fixation test [6] and hemagglutination-inhibition test [1, 6] have been used to assay Getah virus antibodies. The present study was undertaken to develop an enzyme-linked immunosorbent assay (ELISA) for the detection of Getah virus antibodies in swine sera.

The HmLu-1 cells derived from hamster lung was grown in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum, 10% tryptose phosphate broth (TPB) and 100 units/ml Kanamycin. The maintenance medium was MEM containing 10% TPB and Kanamycin as above. The Kanagawa strain [7] of Getah virus was propagated in HmLu-1 cells and assayed in microplate cultures of HmLu-1 cells.

The antigen for ELISA was prepared from culture fluid of HmLu-1 cells infected with the Kanagawa strain. Supernatant culture fluid was mixed with 0.02 M zinc acetate adjusted to pH 7.4 with 1 N NaOH and stirred at 4°C for 20 min. The resulting material was centrifuged at $9,300 \times g$ for 20 min and 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 applied on a discontinuous sucrose density gradient (20–60%) in a Beckman SW28 rotor and centrifuged at 26,000 rpm for 4

h. The virus bands formed were collected and centrifuged at $80,000 \times g$ for 2 h. The pellets were resuspended in NTE buffer, mixed with an equal volume of fluorocarbon and vigorously shaken for 2 min. The aqueous phase was separated by centrifugation, treated with 1% Triton X-100 at 4°C for 1 h and dialyzed against PBS (0.1 M NaCl, 0.01 M phosphate buffer, pH 7.2). Supernatant fluid from uninfected HmLu-1 cell cultures was treated with zinc acetate as described above, and used as the control antigen. The protein content of antigens was determined by the method of Lawry. The antigens were stored -80°C until use.

For the ELISA the virus and control antigens were 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. The plates were allowed to stand at 4°C overnight and washed 4 times with 0.85% NaCl solution containing 0.02% Tween 20. The test serum was diluted 100-fold with PBS containing 10% calf serum and 0.05% Tween 20 and delivered in 100 μl volumes to antigen-coated wells. 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 swine IgG (Miles Lab., USA) was added to each well. After incubation at 37°C for 30 min, each well was washed and received 100 μl of substrate solution. The plates were incubated at 25°C for 20 min in a dark room. The substrate solution was prepared by dissolving o-phenylene dihydrochloride at a concentration of 0.1 mg/ml in 0.1 M acetic acid, 0.2 M Na_2PO_4 buffer (pH 4.8) and adding 0.2 $\mu\text{l}/\text{ml}$ of 30% H_2O_2 . At the end of incubation the reaction was stopped with 3 N H_2SO_4 solution and absorbancy measurement was performed at a wave length of 492 nm. The value obtained by subtracting the absorbance of

Fig. 1. Results of ELISA and HI test of serial serum samples from pigs experimentally infected with Getah virus.

Fig. 2. Correlation of ELISA value and HI titer of 30 random swine serum samples.

the control antigen from that of the viral antigen was taken as ELISA value.

Hemagglutination-inhibition (HI) tests were carried out by the microtiter method. The serum was treated with acetone to remove non-specific inhibitors and with packed goose erythrocytes and inactivated at 56°C for 30 min. The mixtures of serum and antigen were incubated at 4°C overnight, mixed with 0.33% goose erythrocyte suspension and incubated at 37°C for 1 h. The HI titer was expressed as the reciprocal of the highest serum dilution showing complete inhibition of agglutination with 4 units of hemagglutinin. HI titers of 10 or higher were taken as positive.

In preliminary experiments with a swine anti-serum with a HI titer of 160 to the Kanagawa

Fig. 3. Geographical distribution of Getah virus antibody detected in Japanese pigs by ELISA. The number of serum samples tested in each district is shown in parentheses.

strain, ELISA values were highest when wells were coated with 1 or 2 μ g protein of virus antigen. In the following experiments 1 μ g protein of virus antigen was used for coating of wells.

A total of 64 swine serum samples negative for HI antibodies to the Kanagawa strain were tested by ELISA. These serum samples had an average ELISA value of 0.125 with a standard deviation of 0.046. In order to give a sample safety margin, ELISA values of 0.263 ($0.125 + 3 \times 0.046$) or higher were taken as positive.

Serial serum samples were obtained from 2 initially seronegative pigs after subcutaneous

inoculation with 10^6 TCID₅₀ of the Kanagawa strain and examined by ELISA and HI test. The results are illustrated in Fig. 1. The 2 pigs showed similar patterns of antibody development after virus inoculation. ELISA values began to rise earlier than HI titers.

Thirty random swine serum samples were tested by ELISA and HI test. As shown in Fig. 2, their ELISA values were well correlated with their HI titers. Fifteen of these sera were negative in both of the tests. The remaining 15 sera were positive by ELISA values of 0.360 to 2.0 and by HI test with titers of 20 to 320.

The serum samples were collected from fattening pigs in various districts of Japan (37~254 samples each, totally 1,313 samples) over the period from June to October of 1980 and examined by ELISA for Getah virus antibodies. The results are illustrated in Fig. 3. Getah virus antibodies were detected among pigs in all the districts. The positive rate ranged from 2.7% (1/37) to 19.1% (40/209), the lowest in Hokkaido and the highest in Kanto.

These results indicate the ELISA developed in

this study to be useful for testing large numbers of swine sera for Getah virus antibody.

REFERENCES

1. Izumi, A., Takuma, H., Inagi, S., Kubota, M., Hirahara, T., Kodama, K., and Sasaki, N. 1988. *Jpn. J. Vet. Sci.* 50: 679-684.
2. Kamada, M., Ando, Y., Fukunaga, Y., Kumano-mido, T., Imagawa, H., Wada, R., and Akiyama, Y. 1980. *Am. J. Trop. Med. Hyg.* 29: 984-988.
3. Kawamura, H., Yago, K., Narita, M., Imada, T., Nishimori, T., and Haritani, M. 1987. *Jpn. J. Vet. Sci.* 49: 1003-1007.
4. Kumano-mido, T., Fukunaga, Y., Ando, Y., Kamada, M., Imagawa, H., Wada, R., Akiyama, Y., and Tanaka, Y. 1982. *Bull. Equine Res. Inst.* 19: 89-92.
5. Kumano-mido, T., Wada, R., Kanemaru, T., Kamada, M., Hirasawa, K., and Akiyama, Y. 1988. *Vet. Microbiol.* 16: 295-301.
6. Sentsui, H. and Kono, Y. 1980. *Res. Vet. Sci.* 29: 157-161.
7. Yago, K., Hagiwara, S., Kawamura, H., and Narita, M. 1987. *Jpn. J. Vet. Sci.* 49: 989-994.

要 約

ゲタウイルス感染豚の血清疫学的調査のための Enzyme-linked immunosorbent assay (ELISA 法) (短報) : 宝達 勉・井出誠弥¹⁾・山岸郭郎¹⁾・永口良雄¹⁾・長野秀樹¹⁾・前原信敏¹⁾・田中義夫¹⁾・藤崎優次郎¹⁾・田口邦史²⁾・井上剛光²⁾・矢後啓司³⁾・松本 稔⁴⁾(北里大学獣医学部獣医微生物学教室, ¹⁾北里研究所附属家畜衛生研究所, ²⁾農林水産省動物医薬品検査所, ³⁾神奈川県家畜病性鑑定所, ⁴⁾北里研究所研究部ウイルス)——豚血清中の Getah ウイルス抗体検出のため精製ウイルス可溶化抗原を用いた ELISA 法を開発した。ELISA 法は、赤血球凝集抑制(HI)反応と同様、Getah ウイルス接種豚に抗体産生を示した。ランダムに採取した豚血清サンプルの ELISA 値と HI 価はよく相関していた。ELISA 法を用いた全国的抗体調査の結果、Getah ウイルスが日本の豚の間に広く伝播していることが示された。これらの結果は、ELISA 法が豚の Getah ウイルス感染の血清疫学研究に有用であることを示している。