

Anti-Getah ELISA Antibodies in Swine and Bovine Sera in Nagasaki 1981

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Abstract: Antibody titers against Getah virus were measured by the indirect ELISA using swine and bovine sera collected in Nagasaki Prefecture, Japan, in the year of 1981. IgG-ELISA antibody positive rate and its geometrical mean titer (GMT) in swine sera collected at a slaughter house were low until the 21st of July, and increased rapidly in the following week to 80% and 4-fold higher, respectively. Whereas, IgM antibody first became detectable on the 7th of July, coinciding to the Getah virus viremia, then, its positive rate increased to the level of 60% on the 21st of July. Compared with the result of Japanese encephalitis (JE) virus isolation and antibody appearance, circulation of Getah virus among swine population in Nagasaki Prefecture occurred 4 weeks before that of JE virus in 1981. In contrast to swine population, antibody prevalence among bovine was low (less than 27%) even at the end of the epidemic season in September.

Key words: Getah virus, ELISA, swine and bovine sera

INTRODUCTION

Getah virus, a member of genus alphavirus of family Togaviridae (Porterfield, 1980), was first isolated in Malaysia in 1955 (Elisberg & Buescher, 1963), and was shown to distribute widely in the Southeast Asia either by the virus isolation or antibody survey (Tesh *et al.*, 1975; Simpson *et al.*, 1975; Marchette *et al.*, 1978, 1980). In Japan, a strain of Getah virus, Sagiya, was isolated in 1956 by Scherer *et al.* (1962), followed by many isolates from swine sera or field mosquitoes during epidemiological survey on JE virus circulation in nature (Matsuyama *et al.*, 1968; Shichijo *et al.*, 1970; Yamamoto, 1980; Igarashi *et al.*, 1981b, c, d, e, 1982a). Recent demonstration of Getah virus as the causative agent of febrile respiratory infections with eruption and lymphadenopathy among

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racehorses in Japan (Sentui & Kono, 1980; Wada *et al.*, 1982) has attracted the attention of veterinarians and virologists resulting in several basic researches as well (Igarashi, 1983; Takashima *et al.*, 1983a, b; Morita & Igarashi, 1984). However, ecology of Getah virus still remains unclear compared with the informations on JE virus. In this report, we demonstrated the appearance of anti-Getah ELISA antibodies in relation to viremia in slaughtered swine blood collected in Nagasaki Prefecture during the summer of 1981. As a control animal, the antibody prevalence among bovine sera collected in the same Prefecture in the same year was also measured by the ELISA.

MATERIALS AND METHODS

Cell culture for virus antigen preparation: BHK21 cells were grown at 37°C with Eagle's minimal essential medium (MEM, Eagle, 1959) supplemented with 10% calf serum in 500 ml rubber-stoppered glass bottles. When the cells became semiconfluent, growth medium was removed and the cells were inoculated with 2 ml/ bottle of prototype Getah virus, strain AMM2021, at low multiplicity around 0.1 PFU/ cell. After 2 hours of adsorption at 37°C, the cells were fed with maintenance medium (cell growth medium from which serum concentration was reduced to 2 %), and incubated at 37°C for 18 hours. Getah virion was concentrated and purified from infected culture fluid by polyethylene glycol precipitation, ultracentrifugation and sucrose gradient sedimentation as described before (Morita & Igarashi, 1984), and the peak fractions of the purified virion were used as the ELISA antigen.

Test sera: Each of 20 swine sera were collected weekly at a slaughter house in Isahaya City from July 2 to September 17 by Nagasaki Prefectural Institute of Public Health and Environmental Sciences (Igarashi *et al.*, 1981c). Bovine sera were collected at 5 locations (Iki, Gotoh, Saseho, Azuma or Shimabara, Ohseto) from May to September by Central Laboratory for Animal Health of Nagasaki Prefecture (Bundo *et al.*, 1983).

ELISA: Indirect micromethod (Voller *et al.*, 1976) was used with modifications as described before (Igarashi *et al.*, 1981a; Bundo *et al.*, 1982). Plastic microplate (Immulon U) was coated with Getah virus antigen diluted in coating buffer at 4°C overnight. The plate was washed with PBS-T, and was reacted with test sera diluted to 1:100 or 1:1000 in PBS-T. Standard positive serum of known endpoint titer was serially diluted in 2-fold steps starting from 1:100 up to 1:2560 and run in parallel with test sera on the same plate. Plate was washed with PBS-T followed by the reaction with peroxidase-conjugated anti-swine IgG, anti-bovine IgG or IgM goat IgG (Cappel Laboratories, USA) diluted in PBS-T, in order to measure swine IgG, bovine IgG or IgM ELISA titers, respectively. In order to measure swine IgM ELISA titer, the plate was reacted with anti-swine IgM rabbit serum (Miles, USA) followed by the reaction with peroxidase-conjugated anti-rabbit IgG goat IgG (Cappel Laboratories, USA). After washing with PBS-T, peroxidase

reaction was carried out with *o*-phenylenediamine and H₂O₂ to develop colored product. The reaction was stopped by adding H₂SO₄ to 1.7 N and optical density at 490 nm was measured by a Micro ELISA Autoreader (Dynatech, USA) with 630 nm as a reference wavelength. The titer of each test serum was calculated by a computer system using the standard curve obtained by serial dilution of a standard serum (Morita *et al.*, 1982).

Mosquito specimens: Mosquitoes were collected by Nagasaki Prefectural Institute of Public Health and Environmental Sciences using light traps at Aino Town (Igarashi *et al.*, 1981c). Female *Culex tritaeniorhynchus* were pooled not exceeding 100 mosquitoes/pool and 20 pools were used for virus isolation every week from July 2 to September 17.

Virus isolation: Inoculation to *Aedes albopictus*, clone C6/36, cells (Igarashi, 1978) was followed as described (Igarashi *et al.*, 1981b, c, d, e, 1982, b). The cells were prepared at 28°C in rubber-stoppered culture tubes with 2 ml/tube of growth medium which contained 10 % heat-inactivated fetal calf serum and 0.2 mM each of nonessential amino acids in Eagle's minimal essential medium (Eagle, 1959). One-tenth ml of each swine serum was inoculated to cultured cells without removing cell growth medium, and after 2 hours adsorption, the medium was replaced with the maintenance medium (cell growth medium from which serum concentration was reduced to 2 %). Each pool of mosquito specimens was homogenized with 2 ml of PBS containing 0.2% of bovine plasma albumin and centrifuged, and the resulting supernatant was filtrated through 0.22 μm filter. Two-tenth ml of the filtrate was inoculated to a tube culture of C6/36 cells after removing the cell growth medium and adsorption was carried out for 2 hours. The cells were fed with the maintenance medium and were incubated at 28°C for 7 days. The presence of infectious virus was screened by inoculating the infected culture fluid to C6/36 cells prepared on 8-chamber slides (Miles, USA), which were harvested 3 days later in order to reveal intracellular viral antigens by the immunoperoxidase method (Okuno *et al.*, 1977).

RESULTS

Viremia and Anti-Getah Antibodies in Swine Sera

The upper panel of Fig. 1 shows the result of Getah virus isolation from mosquitoes and swine sera, and the lower panel of Fig. 1 shows percent positives of IgM- and IgG-anti-Getah antibodies as well as GMT of IgG antibodies in swine sera collected at a slaughter house from July 2 to September 17, 1981. Single peak of Getah virus viremia in swine was recorded on July 7 with 20% positivity (4/20), however, virus isolation from *Cx. tritaeniorhynchus* were negative. IgG-ELISA antibody positive rate was low before July 21 and increased rapidly over 70% in the following week of July 27, together with approximately 4-fold increase in its GMT. While IgM-ELISA antibody became first detectable on July 7 coinciding to the viremia peak, and its positivity increased to 50-60% on July 21. The data indicate that Getah virus infection in swine population caused transient

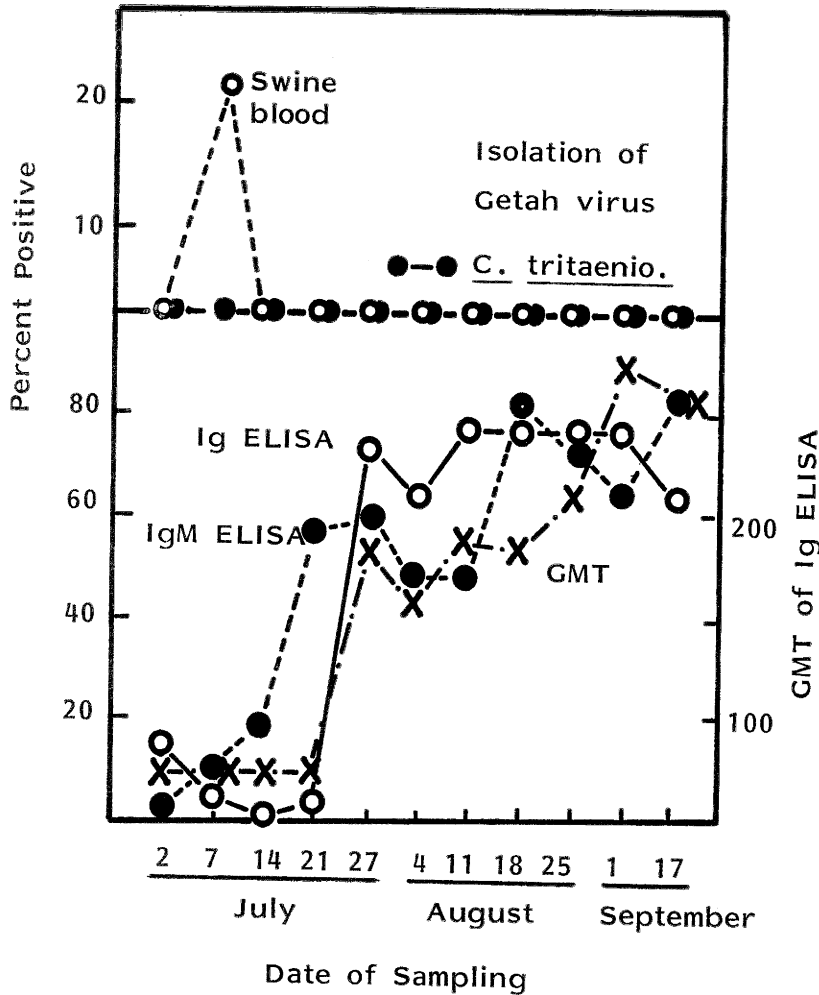


Fig. 1. Isolation of Getah virus from *Cx. tritaeniorhynchus* and swine blood and changes of ELISA titer in swine sera in Nagasaki, 1981. Every week from July [2 to September 1981, 20 pools of *Cx. tritaeniorhynchus* and 20 individual swine sera were treated for virus isolation, and ELISA titer in swine sera were measured as described in the Materials and Methods.

viremia which was followed by the appearance of IgM- and IgG-antibodies reaching their peaks 2 and 3 weeks later, respectively, in Nagasaki Prefecture in the year of 1981.

Anti-Getah Antibodies in Bovine Sera

Table 1 shows prevalence of anti-Getah antibodies among bovine sera collected from June to September at 5 locations in Nagasaki Prefecture in the year of 1981. The antibody positive rate at the beginning of summer in June was generally low; only 10% on Iki Island, 5% in Saseho, and 0% in Goto, Azuma, and Ohseto. The rate did not significantly increase during July (15% in Goto), and August (5-11% in Saseho, Goto, & Azuma). Even at the end of the summer in September, the highest antibody positive rate was only 26% in Saseho. The results indicate that bovine is not an efficient vertebrate host of Getah virus.

Table 1. Prevalence and (geometrical mean titer) of anti-Getah virus antibodies in bovine sera in Nagasaki Prefecture, 1981

Sampling place	Sampling time			
	June	July	Aug.	Sep.
Iki Is.	2/20 (153)	nt	0/20 (448)	nt
Goto Is.	0/20 (110)	3/20 (152)	1/20 (132)	0/20 (147)
Saseho	1/21 (194)	0/20 (213)	1/16 (327)	4/15 (206)
Azunma	0/19 (179)	0/20 (208)	2/18 (175)	nt
Shimabara	nt	nt	nt	0/20 (162)
Ohseto	0/10 (50)	0/9 (91)	0/10 (70)	nt

Positive limit of antibody: 400

DISCUSSION

In Japan, JE virus is practically the only arbovirus related with human diseases, and extensive field and laboratory studies have been performed to elucidate its nature. During field survey to isolate JE virus from mosquitoes collected in nature, several "non-JE" viruses were isolated including Getah virus. Until recent reports on its association with an infectious disease among racehorses (Sentsui & Kono, 1980; Wada *et al.*, 1982), however, the pathogenicity of Getah virus remained unclear although serological studies demonstrated its prevalence among humans or animals (Tesh, *et al.*, 1975; Simpson *et al.*, 1975; Marchette *et al.*, 1978, 1980) and animal experiments demonstrated its teratogenicity in mice (Sentsui & Kono, 1981). Our data showed that during the summer of 1981 in Nagasaki Prefecture, Getah virus infection in swine population resulted in a transient viremia followed by the production of IgM- and IgG-antibodies. Compared with the data of JE virus (Igarashi *et al.*, 1981c), Getah virus circulation appeared to occur approximately 4 weeks before that of JE virus. However, attempted isolation from *Cx. tritaeniorhynchus* could not detect Getah virus, in contrast to the case of JE virus which showed the peak of mosquito infection rate 1 week after the swine viremia (Igarashi *et al.*, 1981c). Although apparent *Culex*-swine cycle was not demonstrated for Getah virus in this study, the result support the applicability of the ELISA for seroepidemiological survey on the virus infection among swine and bovine populations. In Nagasaki Prefecture, Getah virus has been isolated from *Aedes vexans* (Shichijo *et al.*, 1970) and *Armigeres subalbatus* (Igarashi *et al.*, 1982a) besides *Cx. tritaeniorhynchus* in 1980 (Igarashi *et al.*, 1981b.)

These mosquitoes were shown to support the growth of Getah virus by laboratory experiments (Igarashi, 1983; Takashima *et al.*, 1983a, b). As shown by the antibody survey, bovine were less susceptible to Getah virus compared with swine. The result is similar to the case of JE virus reported previously (Bundo *et al.*, 1983).

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1982年長崎県のブタとウシ血清のゲタウイルスに対する ELISA 抗体価

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1981年 長崎県で採取されたブタとウシ血清のゲタウイルスに対する ELISA 抗体価を測定した。7月21日以前に屠殺場で採取されたブタ血清の IgG-ELISA 抗体陽性率と抗体価の幾何平均値は低かったがその後速やかに各々80%と4倍以上に上昇した。一方, IgM 抗体はウイルス血症と同じ日の7月7日に初めて検出された後陽性率は上昇し7月21日には60%に達した。日本脳炎ウイルスの分離と抗体保有率の経移と比較すると1981年長崎県のブタの間ではゲタウイルスが日本脳炎ウイルスよりも約4週間早くその感染が起こっていた事になる。ブタと比べてウシの抗体保有率は流行期末の9月でも27%と低値であった。

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