Serological Survey of Arthropod-Borne Viruses among Wild Boars in Japan

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Abstract. A total of 90 blood samples were collected from wild boars in the Kyushu region of Japan, and a seroepidemiological survey for 7 arthropod-borne viruses was performed using hemagglutination inhibition tests. The individual seropositive rates for each virus were 52.2% for Japanese encephalitis virus (JEV), 47.8% for Getah virus (GETV), 13.3% for Akabane virus, 10.0% for Aino virus and less than 5% for Bluetongue virus, Chuzan virus and Ibaraki virus. The results indicated that among the 7 viruses, JEV and GETV infections were prevalent among the wild boars and that the animals were involved in the natural transmission cycle of JEV and GETV in Japan. It is necessary to consider the participation of wild animals for the control of arthropod-borne virus infections.

Key Words: arthropod-borne virus, Japanese encephalitis virus, wild boar.

There are many epizooties transferred by arthropods in Japan [5, 8, 16]. The epidemiologies of these diseases are essentially elucidated between domestic animals and arthropod vectors. However, there are many wild animals in Japan, and their participation must be considered in control of diseases. Among the wild animals of Japan, the wild boar is a species close to the pig and could be sensitive to a majority of the microbiological pathogens of the pig. Wild boars are distributed in the area south of Fukushima Prefecture, and their numbers have tended to increase recently. Therefore, the existence of the wild boar is an important factor in control of swine diseases, particularly epidemics transferred by arthropods. The present study was undertaken to assess the extent of dissemination of arthropod viruses among wild boars by testing for hemagglutination inhibition (HI) antibodies.

A total of 90 blood samples were collected from wild boars captured by hunting in and around the city of Amakusa in Kumamoto Prefecture, in the Kyushu region of Japan during 2000 and 2001, and a seroepidemiological survey for 7 arthropod-borne viruses was performed using HI tests. Blood samples were absorbed in filter paper strips (Blood Sampling Paper; Toyo Roshi, Tokyo, Japan) and dried at room temperature. About 0.1 ml of blood (equivalent to 40 μl of serum) was absorbed into the strips. Blood samples were extracted from the strips, which were cut into four or five pieces and dipped into 0.4 ml of borate-buffered saline solution (BBS) at 4°C overnight. They were then mixed with an equal volume of 25% kaolin solution in BBS at room temperature for 30 min and centrifuged at 1,200 × g for 20 min. To absorb isohemagglutinins, 0.1 ml of washed goose or bovine erythrocytes was added to the supernatant fluid. Adsorption took place at room temperature for 20 min with occasional shaking and centrifugation for 10 min at 1,000 × g. The supernatant fluid was then ready for use in the HI test at a 1:20 dilution.

Akabane virus (AKAV), Aino virus (AIV), Japanese encephalitis virus (JEV), Getah virus (GETV), Ibaraki virus (IBAV), Chuzan virus (CHUV) and Bluetongue virus (BTV) were subjected to the tests. Outbreaks of diseases caused by these viruses have been occasionally reported in Japan and are of concern to farmers and veterinarians in the domestic animal hygiene field. Among these viruses, IBAV, CHUV and BTV are classified in genus Orbivirus, and their pathogenicity in the pig has not been reported. However, pigs antibody-positive for some orbiviruses have been reported, though rarely [6]. Wild animals have more opportunities to come into contact with arthropods as their conditions are different from those of domestic animals. The sources of these viruses are shown in Table 1.

Hamster lung (HL) cells were used for cultivation of these viruses in Eagle MEM containing 5% fetal calf serum, 0.3% tryptose phosphate broth and antibiotics (100 u/ml penicillin and 100 μg/ml streptomycin). After virus inoculations, the infected cells were placed in maintenance medium containing 0.1% bovine serum albumin instead of fetal calf serum. The culture fluids of infected cells were used as hemagglutinating (HA) antigen directly or after concentration as described by Tokuhisa et al. [14].

The HI tests of these viruses were carried out in 96-well plastic trays using BBS containing 0.1% bovine serum albumin as the diluent solution. Four HA units of antigen in 0.025 ml of BBS were added to each 0.025 ml serial 2-fold dilution of serum. After incubation at 4°C overnight, 0.05 ml of a 0.5% erythrocyte suspension in phosphate-buffered saline solution (PBS) was added. The optimal pH value and salt density for hemagglutination of each virus were examined previously and adjusted for each test. The plates were then kept at room temperature for 2 hr, and the wells were
observed for agglutination. The HI titer was expressed as a reciprocal of the serum dilution that inhibited HA activity. The procedure for these HI tests was reported previously [2, 3, 14].

The results of the HI tests are shown in Table 2. The samples that had HI titers of more than 1:40 were regarded as positive. A high positive rate was observed for JEV and GETV, and the individual seropositive rates for these viruses were 52.2% and 47.8%, respectively. The samples were classified into 2 groups (young and adult) according to the ages of the animals based on reports from the hunters. When the positive rate was compared between young and adult animals, it increased in number with age (Fig. 1). This phenomenon was considered to be due to the epidemic style of the pathogen, transferred by *Culex* and *Aedes*, which have wide host ranges and are prevalent every year. There was no significant difference in the positive rate between males and females.

The positive reaction rates for AKAV and AIV were 13.3% and 10.0%, respectively, and did not increase with age. This was due to transmission by *Culicoides*, which have a limited host range, and the periodic appearance of the disease at several-year intervals. The positive rates were less than 5% for BTV, CHUV and IBAV. This was due to the fact that the pig and the wild boar are not the natural hosts of these viruses, which are transmitted by *Culicoides*. There are many serotypes in the *Orbivirus* group, especially for BTV. Therefore, there might be more BTV-positive animals infected with different serotypes of viruses. However, the possibility that the HI antibodies detected in present test may have been produced by infection with different viruses cannot completely be excluded because there are some viruses in the *Orbivirus* group that partially react with the viruses used in the present test.

The present study indicated that the wild boar has antibodies to arthropod-borne viruses that are prevalent among pigs [6]. Therefore, it is quite likely that an epidemic between pigs and arthropods could also form a natural transmission cycle between the wild boar and arthropods. Among the epidemics associated with pigs and arthropods,
Japanese encephalitis is important as a zoonosis in Japan [1]. Hamano et al. reported that 68% of wild boars in Hiroshima Prefecture had antibodies to JEV [4], and our present investigation in the Kyushu region also produced similar results. Therefore, there seems to be a high rate of JEV infection among wild boars in the Chugoku, Shikoku and Kyushu regions. Unlike domestic animals, wild animals are affected by various kinds of arthropods, such as ticks, mosquitoes, midges and lice. Therefore, an unknown infection cycle may be formed between wild animals and arthropods. It is thus necessary to consider the participation of wild animals for the control of arthropod-borne virus infections among domestic animals.

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REFERENCES


