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Sero-survey on Aino, Akabane, Chuzan, bovine ephemeral fever and Japanese encephalitis virus of cattle and swine in Korea

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Vector-borne arboviruses produce mild to severe symptoms in domestic animals. Bovine ephemeral fever (BEF), Akabane, Aino, and Chuzan virus have been primarily attributed to reproductive disorders or febrile diseases in cattle, and Japanese encephalitis virus (JEV) is mainly associated with reproductive failures in swine. We investigated antibody titers from domestic swine against four bovine arboviruses (BEF, Akabane, Aino, and Chuzan virus) and from cattle against JEV in Korea. While the positive rates for Akabane and BEF were 37.4% and 15.7%, the positive incidence of Chuzan and Aino were relatively low, with positive rates of 3.04% and 0.4%, respectively, based on a virus neutralization assay. Antibody titers against more than one virus were also frequently detected in domestic swine. The incidence of JEV was 51.3% among domestic cattle. In addition, one positive case was detected in the thoracic fluids from 35 aborted calves, based on the hemagglutination inhibition test. Our results indicate that swine are susceptible hosts of bovine arboviruses without showing clinical symptoms in a natural environment. Moreover, we confirmed that JEV could be associated with reproductive failure in pregnant cattle, as were other vector-borne bovine arboviruses assessed in this study.

Key words: arboviral infection, cattle, sero-incidence, swine

Introduction

The arthropod-borne viruses (arboviruses) are a diverse group of RNA viruses that replicate in hematophagous arthropods (vectors) prior to transmission to human or animals. The incidence of arbovirus infection is widely distributed throughout the world [17]. The majority of these viruses are usually transmitted by arthropod vectors, and are maintained in nature by a propagative cycle involving blood-feeding arthropods, causing significant morbidity in humans and economic loss in the animal industry. Although there is no scientific evidence that arboviruses can be directly spread from one animal to another susceptible animal, it is well-known that the vector insects can transmit viruses to susceptible animals as amplifying hosts during blood feeding cycles [8,22].

In general, arboviral infections produce diverse symptoms in affected animals, and some of them cause reproductive disorders such as abortion, stillbirth, and congenital malformation in domestic animals [1,2,9,15,27].

Aino and Akabane virus are members of the Simbu serogroup, genus *Orthobunyavirus*, family *Bunyaviridae* [1-3,23], and Chuzan virus belongs to the Palym serogroup within the genus *Orbivirus* in the family *Reoviridae* [16].

Bovine ephemeral fever virus (BEFV) is an arthropod-borne rhabdovirus, genus *Ephemerovirus*, family *Rhabdoviridae* [14]. While BEFV primarily induces acute fever, Aino, Akabane, and Chuzan virus cause reproductive disorders in pregnant cattle [10,11,21,25].

Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*, family *Flaviviridae*, is an arthropod-borne viral disease of public health importance [20]. Although Aino, Akabane, Chuzan, and BEFV have been primarily attributed to reproductive disorders or febrile disease in cattle, JEV is mainly associated with reproductive failures in swine as an amplifying host. However, the exact mode of transmission, including the host-range and possible amplifying source of virus within livestock animals, has not yet been completely investigated [7,12,19,24].

In this study, we conducted a serological survey on bovine arboviral infections in domestic swine and JEV infection in cattle in Korea.

Materials and Methods

Cells and viruses

Vero cells (ATCC C-1586) were regularly maintained in alpha-MEM (GibcoBRL, USA) supplemented with 5% fetal bovine serum, penicillin (100 unit/ml), streptomycin (100 unit/ml), and amphotericin (0.25 g/ml).

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Korean isolates, designated KSA9910 strain of Aino, 93FMX strain of Akabane, and YoungAm strain of Chuzan virus, were plaque-purified once at the sixth passage and used for a virus neutralization assay. Attenuated BEFV from commercially-available vaccine was also propagated in Vero cells as indicated above. For JEV, the Nakayama strain of JEV was inoculated into the brains of suckling mice for the preparation of hemagglutination antigen (HA) according to the standard procedures described by Morimoto *et al.* [13].

Virus neutralization assay (VNA)

In order to conduct VNA, all of the test sera were inactivated at 56ºC for 30 min. Two-fold serial dilutions of sera in alpha-MEM (GibcoBRL, USA) were mixed with an equal volume of virus containing 200 $TICD_{50}$/0.1 ml. After incubation at 37ºC for 1 h, 100 ml of each mixture was dispensed in duplicate into 96-well microplates prior to the addition of the same volume of Vero cells (20,000 cells/well) against Aino, Akabane, Chuzan, and BEFV. Each plate included standard wells for virus titration from 200 $TICD_{50}$ to 2 $TICD_{50}$ and cell control.

The plates were incubated at 37ºC for 7 days in a humidified incubator with 5% CO_2. The titer from VNA was determined to the highest dilution of sample that inhibited the cytopathic effects by 50% compared to control wells. A titer with a ratio of less than 1 : 4 in VNA was taken to be negative in this screening experiment.

Hemagglutination inhibition (HI) test

HI test for JEV (Nakayama strain) was carried out by a modified method of Clarke & Casals [4]. Test sera were inactivated at 56ºC for 30 min. The heat-inactivated sera were then treated with 25% kaolin solution and absorbed with washed gander RBC to remove nonspecific inhibitors and hemagglutinins.

Two-fold dilutions from 1 : 10 sera and thoracic fluids were serially conducted with borate-buffered saline (0.4% bovine serum albumin, pH 9.0) in 96-well microtiter plates and mixed with an equal volume of JEV containing 8 HA units. After incubation at 37ºC for 1 h, 50 ml of each mixture was dispensed in 96-well U-form microplates prior to the addition of the same volume of 0.033% goose erythrocytes. The plates were incubated at 37ºC for 30 min in a humidified incubator. The highest dilution of sample that inhibited the agglutination of red blood cells was determined. Samples with titers greater than 20 HI were considered to show positive results compared with reference positive serum.

Field samples

A total of 230 porcine sera were collected from 15 farms throughout the country. None of these farms had any history of vaccination against Aino, Akabane, Chuzan, and BEFV when the study was conducted.

A total of 144 bovine sera were randomly collected from a slaughterhouse located at Anyang city in Korea during the same period of time. In addition, 35 thoracic fluids from aborted calves were collected at a late stage of gestation (> 6 months) from farms across the country. All of the aborted calves were initially submitted for etiological findings to the National Veterinary Research and Quarantine Service, Korea.

Results

Sero-prevalence of Aino, Akabane, Chuzan, and BEFV in swine

A total of 230 porcine sera from fifteen farms throughout the country were investigated. Among the 15 farms screened, the positive incidence of Akabane was 100%, and those of BEF, Aino, and Chuzan were 86.7%, 6.7%, and 20.0%, respectively (Fig. 1). Distribution of virus neutralization (VN) titer against Aino, Akabane, Chuzan, and BEF virus revealed 100 out of 130 positive swine sera had VN titer 4 (Fig. 2).

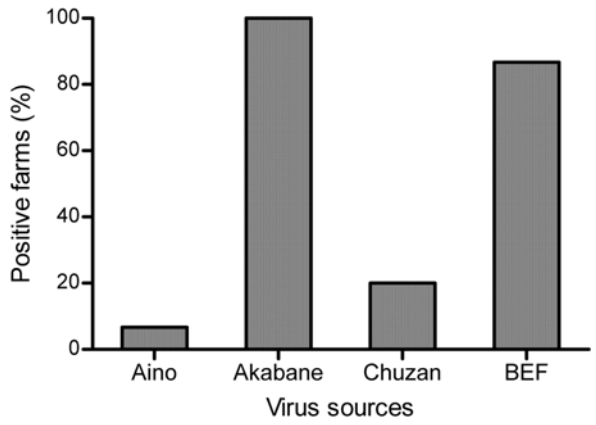

Fig. 1. Sero-positive rates against Aino, Akabane, Chuzan, and BEF virus on fifteen swine farms.

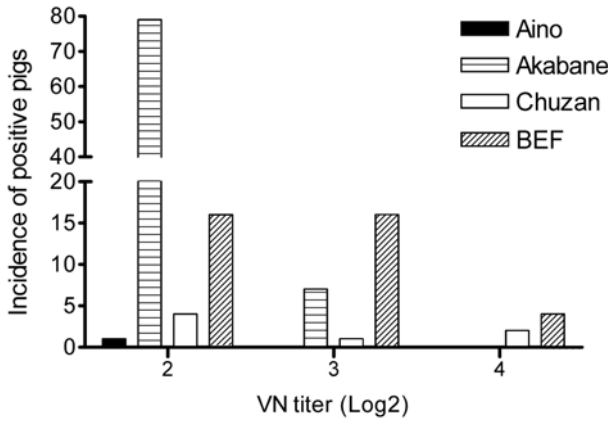

Fig. 2. Incidence of virus neutralization (VN) titers of 130 positive sera in swine sera against Aino, Akabane, Chuzan, and BEF virus.

Table 1. Sero-incidence of Akabane, BEF, Chuzan, and Aino virus by VNA in swine

Designation	Prevalence [No. positive / No. tested (%)]			
	Akabane	BEF	Chuzan	Aino
Akabane	86/230 (37.4)			
BEF	22/86 (25.5)*	36/230 (15.7)		
Chuzan	3/86 (3.5)*	1/36 (2.8)*	7/230 (3.04)	
Aino	1/86 (1.2)*			1/230 (0.4)

*Sero-positive incidence of more than one virus.

Table 2. Sero-prevalence of JEV in domestic cattle and positive incidence against four arboviruses in aborted calves

Designation	Cattle [No. positive / No. tested (%)]	Aborted fetuses [No. positive / No. tested (%)]			
	JEV	JEV	Akabane	Aino	Chuzan
HI*	74/144 (51.3)	1/35 (2.9)	NT	NT	NT
VNA‡	NT†		5/35 (14.2)	4/35 (11.4)	8/35 (22.8)

*Hemagglutination inhibition test.
†Virus neutralization assay.
‡Not tested.

However, the overall positive incidences from swine were 37.4% against Akabane and 15.7% against BEF, followed by Chuzan (3.04%) and Aino (0.4%), as indicated in Table 1. In addition, while the sero-positivity of more than one type of virus within a herd was 25.5% against Akabane and BEF, the positive cases for the Akabane-Chuzan viruses (3.5%), BEF-Chuzan (2.8%), and Akabane-Aino viruses (1.2%) were relatively less frequent compared to Akabane and BEF (Table 1).

Incidence of HI titer against JEV in cattle and aborted fetuses

Using > 20 HI titer of antibodies as positive, the incidence of positive cases appeared to be 74 out of 144 (51.3%), indicating that JEV infections are quite frequent in domestic cattle (Table 2). The distribution of HI titers against JEV revealed that half of JEV positive cattle (38.2%) had HI titers of less than 160, but the rest of the cases (12.47%) showed HI titers higher than 160, suggesting repeated exposures to JEV, as indicated in Fig. 3. Table 2 shows the positive cases from 35 aborted calves. By using thoracic fluids from aborted fetuses, positive findings of antibodies against arboviruses were found to be Akabane (14.2%), Aino (11.4%), Chuzan (22.8%), and JEV (2.9%).

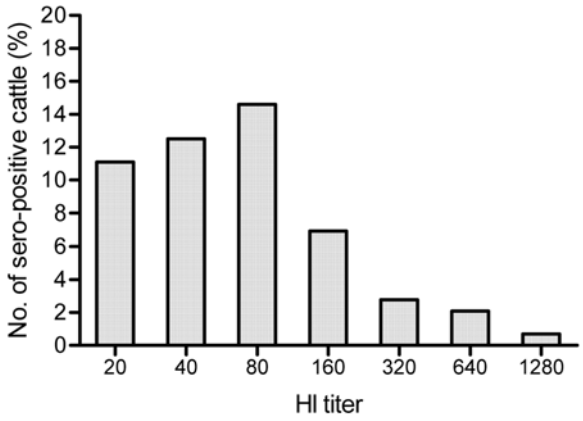

Fig. 3. Distribution of HI titers against JEV in positive bovine sera.

Discussion

Although Aino, Akabane, Chuzan, and BEFV have ruminant animals as their primary hosts in the natural environment, thorough epidemiological studies were not performed in swine until now. Akabane virus was recently isolated from swine and aborted fetuses in Taiwan, indicating that pigs were susceptible to Akabane virus with 75% sero-positivity [6].

In this study, we conducted a serological survey on bovine arboviruses in swine and JEV in cattle. Although BEF and Akabane are known to be natural hosted in ruminants like cattle, infection with BEF and Akabane was also found to be quite prevalent in domestic swine. In addition, the present results suggest that domestic swine were exposed to more than one bovine arbovirus, indicating that herds were repeatedly exposed to different arboviruses, as reported previously [21]. However, the average antibody titers were found to be relatively low, from 4 to 16 for each virus. One of possible explanation for these results may presumably be related to the ages of pigs and environmental factors such as temperature and mosquito population. In this study, serum samples were collected from finishing pigs in farms rather than covering sows and newborn piglets, as compared to a

previous study [6]. Nevertheless, it is worth noting that we could not determine any positive incidence in aborted swine against those arboviruses (data not presented here). For this reason, the present results also indicate that pigs can act as silent hosts without clinical symptoms in the virus-host-vector circulating cycle [6].

A recent study showed that C. *oxystoma* and C. *arakawae* were both closely associated with rice fields and *Culicoides* biting midges, which are capable of transmitting all of the bovine arboviruses [26]. Therefore, the present results were not surprising, but rather expected, if we consider the fact that one susceptible pig can be exposed to transmitting vectors like *Culicoides* spp. and mosquitoes.

JEV is an important arboviral pathogen that causes encephalitis in humans. While it is well-confirmed that the swine acts as an amplifying host, the epidemiological role of cattle, especially in relation to transmission of the virus, still is not clearly understood [18,20].

Previously, cattle were shown to possess antibodies to JEV, but the exact role of cattle as amplifiers of JEV remains rather controversial, in part because the cattle did not show viremia after experimental infection and cattle did not circulate the virus at titers high enough to infect mosquitoes [5,19]. Interestingly, we confirmed that JEV infections are quite common in domestic cattle, and are also associated with reproductive failure in pregnant cattle like other vector-borne arboviruses. In fact, we were able to detect the short period of viremia after experimental inoculation in cattle using quantitative RT-PCR (data not presented here). Therefore, further study will be necessary to investigate the epidemiological performance of these arboviruses both in swine and cattle, including the duration of viremia in the natural environment.

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