Seroprevalence of bovine arboviruses belonging to genus Orthobunyavirus in South Korea

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Running head: SEROPREVALENCE OF BOVINE ARBOVIRUSES
ABSTRACT

Arboviruses, belonging to the Simbu serogroup of the genus *Orthobunyavirus*, often cause congenital malformations and reproductive loss in cattle. The recent occurrences of such reproductive problems suggest that new arboviruses have emerged in Japan. However, there is no information on the presence of these viruses in South Korea. The aim of this study was to determine the presence of antibodies for Akabane, Aino, Peaton, Sathuperi, and Shamonda viruses in four regions, namely Gyeonggi, Jeollabuk, Jeollanam provinces, and Jeju Island of South Korea by a serum neutralization test. Antibody positivity against Akabane, Aino, Peaton, Sathuperi, and Shamonda viruses was detected in the country, with average seropositive rates of 10.4%, 4.5%, 1.1%, 4.9%, and 5.6%, respectively.

KEYWORDS: ainvirus, akabanevirus, peatonvirus, sathuperivirus, shamondavirus
Arthropod-borne viruses (arboviruses) are transmitted among vertebrates by blood-sucking arthropods, such as mosquitoes, biting midges, and ticks [34, 35]. There are more than 520 known arboviruses, and about 25 of these are known to cause diseases in livestock [35].

Representatives of problematic arboviruses for cattle include Akabane virus (AKAV) and Aino virus (AINOV) belonging to the Simbu serogroup of the genus *Orthobunyavirus*. Arboviruses can infect any species or sexes of cattle; however, symptoms remain in apparent unless the cow is pregnant, in which the pregnancy often results in stillbirth, abortion, premature birth, or congenital malformation of calves. Thus, farmers suffer significant economic losses from such outbreaks [15].

AKAV infections have been reported in Australia [14], Japan [15, 33, 37], the Middle East [31], South Korea [18, 23], and Taiwan [19]. AINOV infections have also been reported in Japan [6, 40]. Vaccines have also been developed. Large-scale outbreaks of AKAV and AINOV infections no longer occur in vaccinated regions; however, their occurrence continues in other regions.

The arbovirus-infected arthropod vectors are spreading further north each year possibly due to global warming, resulting in the expansion of infected regions [34, 35]. Moreover, with diversification of international transportation and logistics, there is an increased opportunity for arthropod vectors to encounter livestock, and thereby cause the spread of arbovirus infections [5].

In Japan, mainly in the Okinawa and the Kyushu regions, new arboviruses were found in the blood of cattle and biting midges after the late 1990s, confirming their entry. Peaton virus (PEAV) [21] and Sathuperi virus (SATV) [38] were found in 1999, while Shamonda virus (SHAV) [41] was found in 2002. They all belong to the genus *Orthobunyavirus* and the family *Bunyaviridae*. 
Pregnant cattle that are suspected to have PEA V has also been reported to undergo abnormal labor [30]. Although no damage to cattle by SHAV infection has been reported, genetic analyses have shown that Schmallenbergvirus (SBV) is a possible ancestor of SHAV [7] or is likely are assortment between SATV and SHAV [39]. SBV first emerged in Europe in 2011 and caused outbreaks in cattle, goats, and sheep, causing fever, stillbirth, abortion, fetal malformation, diarrhea, and reduced milk yield. It also increased the importance of Orthobunyavirus in livestock worldwide [4].

PEAV, SATV, and SHAV were isolated one after another in Japan, suggesting the extent of damage to livestock. However, PEAV, SATV, and SHAV have not yet been reported in Korea. In order to clarify the state of infiltration of new arboviruses in cattle, the first epidemiological survey of new arboviruses belonging to the genus Orthobunyavirus was performed in Korea through this study.

From October 2013 to February 2015, blood samples were collected from 268 cattle that were between 6 months and 14 years of age (unvaccinated) from 7 farms (A to G) in Gyeonggi, Jeollabuk, Jeollanam provinces, and Jeju Island in Korea (Fig. 1). Required sample size was calculated using EZR (Easy R; Saitama Medical Center, Jichi Medical University, Saitama, Japan), with 10% precision, expected prevalence of 15% based on our preliminary survey data 95% confidence interval (CI). Minimum sample size was 196. Blood samples were centrifuged at 2,000 rpm for 10 min and sera samples were stored at -20°C until use. AKAV strain JaGAr39 [26], AINOV strain JaNAr28, PEAV strain KSB-1/P/06, SATV strain KSB-2/C/08, and SHAV strain KSB-6/C/02 [11] were cultured in hamster lung (HmLu-1) cells and used for the serum neutralization test.

Bovine sera were inactivated at 56°C for 30 min and serially diluted (two-fold) in Eagle’s minimal essential medium (EMEM; Nissui, Tokyo, Japan) supplemented with 2%
fetal bovine serum (FBS) and a mixture of antibiotics in round-bottom 96-well microplates.

Each serum dilution was mixed with equal volume of 200 TCID$_{50}$/0.05ml of virus and incubated at 37°C for 1 hr. Then 0.05ml of the mixture was inoculated with HmLu-1 cells at 37°C for 1 hr. After inoculation, the mixture was removed and 0.1ml of EMEM was added to each well. After incubation in a humidified 5% CO$_2$ atmosphere at 37°C for 7 days, antibody titer was determined as the reciprocal of the highest serum dilution showing complete inhibition of cytopathic effect. Samples with titers of 1:4 or greater were considered to be positive [13].

Seroprevalence and their corresponding 95% CI and statistical analysis were calculated using EZR. Chi-square test was used to analyze differences in seroprevalence between provinces, ages, sexes, production type, and rearing form. A $p$-value of less than 0.05 was considered statistically significant.

Serum neutralization test confirmed the presence of antibodies for AKAV, AINOV, PEA V, SATV, and SHA V in Gyeonggi, Jeollabuk, and Jeollanam provinces and Jeju Island. Their average seropositive rates were 10.4% (28/268, 95% CI: 7.1-14.7%), 4.5% (12/268, 95% CI: 2.3-7.7%), 1.1% (3/268, 95% CI: 0.2-3.2%), 4.9% (13/268, 95% CI: 2.6-8.2%), and 5.6% (15/268, 95% CI: 3.2-9.1%), respectively. Only the presence of anti-AKAV antibodies was confirmed in Gyeonggi province, with a positive rate of 2.0% (2/98, 95% CI: 0.2-7.2%). In Jeollabuk province, antibody positivity against AKAV, AINOV, and SATV was detected, with positive rates of 12.0% (6/50, 95% CI: 4.4-24.6%), 4.0% (2/50, 95% CI: 0.5-14.0%), and 2.0% (1/50, 95% CI: 0.0-10.9%), respectively. In Jeollanam province, antibody positivity against AKAV, AINOV, and SHA V was detected, with positive rates of 8.0% (4/50, 95% CI: 2.1-19.5%), 6.0% (3/50, 95% CI: 1.2-16.8%), and 4.0% (2/50, 95% CI: 0.5-14.0%), respectively. In contrast, in Jeju Island, antibodies against all the 5 viruses (AKAV, AINOV, PEA V, SATV, and SHA V) were detected, with
Infiltration rates of viruses tested were higher in Jeju Island than those in Gyeonggi, Jeollabuk, or Jeollanam province. The differences in seroprevalence among the 4 regions for each virus were investigated and found to be significant ($p < 0.05$).

In 4 out of 7 farms, serum antibody positivity for arbovirus was detected. The seropositive rates were 6.7% (2/30, 95% CI: 8.0-22.1%) for farm A, 18.0% (9/50, 95% CI: 8.6-31.4%) for farms E and F, and 66.0% (33/50, 95% CI: 51.2-78.8%) for farm G.

Samples were collected from dairy cattle Holsteins, beef cattle Hanwoo (Bostaurus coreanae), and black cow, and variation among production type and between sexes was investigated; however, no significant differences were noted. Samples used for the test were collected from cattle that were aged between 6 months and 14 years, and seropositive rate was investigated by age. Cattle were divided into those less than one year old and those over one year old, based on whether or not they had experienced their first summer after birth. When the rate of antibody positivity was compared between these two groups, a significant ($p < 0.05$) difference was noted, except for PEA V. In cattle that were over one year old or had experienced summer at least once, we analyzed their sera by further dividing them into 4 stages: 1 year, 2 to 4 years, 5 to 7 years, and over 8 years of age. Antibodies against AKAV, AINOV, and SHAV were noted in all stages, and against PEAV and SATV were noted in the stages of 1 year, 2 to 4 years, and over 8 years (Table 2). This suggests that the presence of new arboviruses in cattle is due to a recent infection in South Korea.

The cattle rearing form was also surveyed. Cattle were reared by feedlot in Gyeonggi, Jeollabuk, and Jeollanam provinces. They were reared by either feedlot or grazing in Jeju
Island. Differences in the seropositive rates for the five arboviruses due to differences in the cattle rearing form were investigated only in Jeju Island. The rates for antibody positivity for AKAV, SATV, and SHAV were significantly ($p < 0.05$) higher in cattle reared by grazing compared to that in cattle reared by feedlot (Table 1).

The newly emerged arboviruses, PEA V, SATV, and SHA V are classified into the Simbu serogroup of the genus *Orthobunyavirus*, similar to AKAV and AINOV. Antibody cross-reactivity exists for some arboviruses of the Simbu serogroup. However, cross reactivity among AKAV, AINOV, PEA V, and SHA V was not observed in cross-neutralization tests [28, 41], and that between SATV and SHA V has not been reported, suggesting that each antibody positivity against these viruses observed in the 4 regions was due to introduction by AKAV, AINOV, PEA V, SATV and SHA V, respectively.

Arboviruses were isolated in countries with a tropical or subtropical climate. PEA V was initially isolated in Australia in 1976 [28]. SATV was initially isolated in India in 1957 [3]. SHA V was initially isolated in Nigeria in 1965 [2]. It is difficult for adult midges to survive winter in countries that have four seasons, such as Japan and Korea. They do not become indigenous owing to interruption of their infection cycle. Therefore, it is assumed that they might have been brought in from tropical and subtropical regions [32, 36, 40]. Recently, it has been reported that SBV can overwinter, but the exact mechanism remains unknown [4].

Among the five arboviruses surveyed, only anti-AKAV antibody positivity was confirmed in Gyeonggi province, farm A. Gyeonggi province is located at 37° North latitude and 127° East longitude. It belongs to a cold temperate zone with a dry winter climate based on Koppen-Geiger climate classification. After 2000, 25 cases caused by AKAV infection were reported in Gyeonggi province [1]. In Japan, AKAV infection-induced diseases occurred in Hokkaido and Tohoku regions [25, 29, 35].
belonging to cold temperate zones. This suggests that the activity area of
arbovirus-infected arthropod vectors has been expanding northward. From 2006 to 2011,
outbreaks of AKAV encephalomyelitis have been reported in Korea and Japan [10, 15, 23].
Calves and breeding cows exhibited neurological signs, including locomotor ataxia, astasia,
and hypersensitivity after postnatal infection. These are different from other typical AKAV
infection symptoms, such as abortion, stillborn calves, and congenital malformation in
pregnant cattle. They might have originated from a new virus that invaded Korea and
Japan from overseas.

Biting midges are vectors of bovine arboviruses. However, their distributed type species
diffs among epidemic countries. In Japan, AKAV and SATV were isolated from
_Culicoides(C.) oxystoma_ [17], and PEAV was isolated from _C. jacobsoni_ [12]. In Australia,
PEAV was isolated from _C. brevitarsis_ [28]. In South Africa, SHAV was isolated from _C.
imicola_ [22]. In Korea, the AKAV genome was detected in _C. punctatus, C. arakawae, C.
maculatus_, and _C. oxystoma_ [24]. In cattle farms in Jeju Island, where antibodies against
AKAV, AINOV, PEAV, SATV, and SHAV were detected, biting midges were collected
using a light trap, and _C. homotomus_ and _C. punctatus_ were captured. However, no
arbovirus antigen was detected in these biting midges.

Recent outbreaks of arbovirus infection have been characterized by an increase in
frequency and expansion of outbreak regions due to temperature elevation and
diversification of the causative virus [35]. Since 2000, loss of cattle due to arbovirus
infection, including AKAV, has occurred every year in Korea and Japan. It is known that
the outbreak of AKAV infection tends to occur at intervals of 5-6 years. It has also occurred
in regions without previous outbreaks [1, 9].

Regarding arboviruses used in this survey, mainly in Okinawa and the Kyushu regions
in Japan, PEAV was isolated in 1999, 2005, and 2006, SATV was isolated in 1999, 2006,
2007, and 2008, and SHA V was isolated in 2002 and 2007 [11]. Stillbirth and abortion in cattle with suspected PEA V has been reported over the past several years [20, 21,30]. The pathogenicity of SHA V in ruminants has not been confirmed; however, malformation induction has been observed in an experiment using chick embryos [22], suggesting that more attention must be paid to this virus. Outbreaks of SBV have occurred in Europe and caused considerable damage to livestock farms rearing cattle, goats, and sheep [7, 39]. Therefore, it may be necessary to reinforce information collection on arbovirus infection occurring worldwide and conduct surveys of new arbovirus types.

Sera of the cattle were positive to one or more of 5 viruses, but not all. Some of the reasons we could speculate are due to very short period of viremia (AKAV, AINOV and SBV in cattle last for less than 1 week) and low prevalence of these viruses. Antibody positivity against all 5 viruses was confirmed in Jeju Island. Jeju Island is located at 33° North latitude and 126° East longitude, in the southernmost region of Korea. Its climate is classified as temperate humid. From June to October of 2014, the average temperature in Jeju Island was between 19.1 and 25.1°C (average maximum temperature: 22.1-27.8°C, average minimum temperature: 16.3-23.5°C), and average humidity was 69.7 to 84.3% [16]. Insects such as mosquitos and biting midges can readily grow in large numbers in such temperate climates with high humidity. In addition, an increase in transportation has been observed due to a rise in foreign tourists visiting Jeju Island. Moreover, many farms are rearing cattle by grazing. All these factors can increase the chance of cattle encountering virus-carrying biting midges, thereby increasing the risk of arbovirus infection.

In our study, high seropositive rates for the arboviruses were detected in farm G, but not in farm C. Arbovirus can easily infect animals when all factors are met, including biting midges, immune state of host livestock, and viral properties. Therefore, it is difficult to
conclude why there was no infection in farm C. We assume that one of the reasons is the influence of the geographical condition. Jeju Island has a big mountain called Hallasan in the central area, and farm C is located in the eastern part of the mountain. Adult midges might have been brought in from tropical and subtropical regions by eastbound monsoons. However, farm C may be less affected owing to its geographical advantage.

The outbreaks of AKA V infection in 1989-90 [8] and Chuzan virus (belonging to the family Reoviridae) infection in 1993 [27] have been reported in Jeju Island. However, no arbovirus infection has been reported in Jeju Island after that, according to Korea Animal Health Integration System (KAHIS) of the Animal and Plant Quarantine Agency [1]. Since the development of arbovirus vaccines in Korea, outbreaks of arbovirus infection have reduced significantly; thus, vaccination rates have decreased. Cattle that have acquired immune antibodies through vaccination will not be infected again. Due to the high outbreak risk in Jeju Island, it is essential to vaccinate young reproductive cattle that have not received prior vaccination in the entire southern region of the Korean peninsula, including Jeju Island.

Arboviruses can infect animals when all factors are conducive, including the distribution and activity of biting midges, population and immune state of host livestock, and viral properties [34, 35, 37]. Although no case of arbovirus infection has been reported in Jeju Island after 2000, it may be important to perform arbovirus-related surveys in Jeju Island, a temperate region in Korea, in order to predict the risk of infection in Korea. In addition to continuous survey of the distribution of biting midges in cattle farms with high positive rates, it may be necessary to isolate arboviruses and perform genetic identification for these viruses.
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REFERENCES


Figure legend

Fig. 1. Sampling regions, including farms, and the number of samples tested in South Korea. Blood samples were collected from dairy and beef cattle from October 2013 to February 2015. Number in parentheses represents sample size. b: beef cattle, d: dairy cattle.
Fig. 1
### Table 1. Analysis for the association between potential risk factors and seroprevalence of arboviruses using $\chi^2$ test

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>AKAV</th>
<th>AINOV</th>
<th>PEAV</th>
<th>SATV</th>
<th>SHAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>2/98 (2.0%)</td>
<td>0/98 (0.0%)</td>
<td>0/98 (0.0%)</td>
<td>0/98 (0.0%)</td>
<td>0/98 (0.0%)</td>
</tr>
<tr>
<td>JB</td>
<td>6/50 (12.0%)</td>
<td>2/50 (4.0%)</td>
<td>0/50 (0.0%)</td>
<td>1/50 (2.0%)</td>
<td>0/50 (0.0%)</td>
</tr>
<tr>
<td>JN</td>
<td>4/50 (8.0%)</td>
<td>3/50 (6.0%)</td>
<td>0/50 (0.0%)</td>
<td>0/50 (0.0%)</td>
<td>2/50 (4.0%)</td>
</tr>
<tr>
<td>JJ</td>
<td>16/70 (22.9%)</td>
<td>7/70 (10.0%)</td>
<td>3/70 (4.3%)</td>
<td>12/70 (17.1%)</td>
<td>13/70 (18.6%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2/16 (12.5%)</td>
<td>0/16 (0.0%)</td>
<td>0/16 (0.0%)</td>
<td>0/16 (0.0%)</td>
<td>0/16 (0.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>26/252 (10.3%)</td>
<td>12/252 (4.8%)</td>
<td>3/252 (1.2%)</td>
<td>13/252 (5.2%)</td>
<td>15/252 (6.0%)</td>
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<tr>
<td>p-value</td>
<td>2.29E-04</td>
<td>0.020</td>
<td>0.035</td>
<td>7.20E-07</td>
<td>7.41E-07</td>
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<td>Production type</td>
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<td></td>
<td></td>
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<tr>
<td>Beef</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
</tr>
<tr>
<td>Dairy</td>
<td>28/248 (11.3%)</td>
<td>12/248 (4.8%)</td>
<td>3/248 (1.2%)</td>
<td>13/248 (5.2%)</td>
<td>15/248 (6.0%)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.227</td>
<td>0.657</td>
<td>1.000</td>
<td>0.611</td>
<td>0.531</td>
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<tr>
<td>Rearing form (Jeju only)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Feedlot</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
<td>0/20 (0.0%)</td>
</tr>
<tr>
<td>Grazing</td>
<td>16/50 (32.0%)</td>
<td>7/50 (14.0%)</td>
<td>3/50 (6.0%)</td>
<td>12/50 (24.0%)</td>
<td>13/50 (26.0%)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.010</td>
<td>0.186</td>
<td>0.641</td>
<td>0.040</td>
<td>0.029</td>
</tr>
</tbody>
</table>

GG: Gyeonggi province, JB: Jeollabuk province, JN: Jeollanam province, JJ: Jeju island

### Table 2. Analysis for the association between cattle age and seroprevalence of arboviruses using $\chi^2$ test

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>AKAV</th>
<th>AINOV</th>
<th>PEAV</th>
<th>SATV</th>
<th>SHAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1y</td>
<td>3/88 (3.4%)</td>
<td>0/88 (0.0%)</td>
<td>0/88 (0.0%)</td>
<td>0/88 (0.0%)</td>
<td>0/88 (0.0%)</td>
</tr>
<tr>
<td>1y ≤</td>
<td>25/180 (13.9%)</td>
<td>12/180 (7.2%)</td>
<td>3/180 (1.7%)</td>
<td>13/180 (7.2%)</td>
<td>15/180 (8.3%)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.015</td>
<td>0.030</td>
<td>0.549</td>
<td>0.023</td>
<td>0.012</td>
</tr>
</tbody>
</table>

(Age stages 1y ≤)

| 1y          | 11/51 (21.6%) | 7/51 (13.7%) | 1/51 (2.0%) | 8/51 (15.7%) | 1/51 (2.0%) |
| 2-4y        | 9/95 (9.5%) | 2/95 (2.1%) | 1/95 (1.1%) | 4/95 (4.2%) | 7/95 (7.4%) |
| 5-7y        | 3/25 (12.0%) | 1/25 (4.0%) | 0/25 (0.0%) | 0/25 (0.0%) | 4/25 (16.0%) |
| 8y ≤        | 2/9 (22.2%) | 2/9 (22.2%) | 1/9 (11.1%) | 1/9 (11.1%) | 3/9 (33.3%) |