

## Seroprevalence of five arboviruses in sentinel cattle as part of nationwide surveillance in South Korea, 2009–2012

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**ABSTRACT.** To investigate the possible circulation of arboviruses in South Korea, nationwide surveillance of five arboviruses was conducted in sentinel calves during 2009–2012. We used serum neutralization tests to investigate the presence of antibodies for the Aino virus, Akabane virus, bovine ephemeral fever virus, Chuzan virus and Ibaraki virus. In 2009, 2011 and 2012, the seropositive rates for these five arboviruses were all less than 14.1%. In 2010, however, the seropositive rates for Aino virus and Akabane virus were 33.2% and 40.2%, respectively. High seropositive rates were also associated with a large-scale outbreak of Akabane viral encephalomyelitis in cattle in southern Korea in 2010. Continued seroprevalence surveillance will be useful for monitoring natural arboviral diseases.

**KEY WORDS:** arbovirus, seroprevalence, South Korea

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Arthropod-borne viruses (arboviruses) are transmitted by blood-sucking arthropods, such as mosquitoes, *Culicoides* biting midges and ticks [21]. Arboviral infections generally cause not only reproductive disorders, such as abortion, stillbirth and congenital malformation, but also acute febrile disease, resulting in economic losses to the ruminant industry [10]. Aino virus (AINOV) and Akabane virus (AKAV), in the family *Bunyaviridae*, are among the arboviruses that cause disease outbreaks in cattle [1, 19]. Bovine ephemeral fever virus (BEFV) is classified into the family *Rhabdoviridae* and is known to cause an acute febrile disease called “three day sickness” [20]. Chuzan virus (CHUV) and Ibaraki virus (IBAV) belong to the family *Reoviridae* and cause reproductive disorders, fever, anorexia and deglutitive disorder [5, 21].

Isolation and antibody surveys of arboviruses, including AINOV, AKAV, BEFV, CHUV and IBAV, have previously been reported in Australia, Asia, Africa and the Middle East [3, 4, 10, 12, 18, 23]. Outbreaks of the diseases caused by these viruses have been isolated, identified and studied epidemiologically in South Korea [6, 16]. Since 2007, Akabane viral encephalitis has been reported in calves, and the genetic and pathogenic characteristics of AKAV isolated from cattle with encephalomyelitis have also been described [9, 15]. In a 2007 study of thoracic fluids from aborted calves, virus neutralization assays indicated positive rates for AINOV, AKAV

and CHUV of 11%, 14.2% and 22.8%, respectively [10]. Serosurveillance of AINOV, AKAV and CHUV in Korean native goats (*Capra hircus*) revealed positive rates of 13.3%, 5.5% and 2.0%, respectively [22].

Live vaccines for AKAV and BEFV have been commercially available in South Korea since the 1980s. Additionally, an AINOV, AKAV and CHUV trivalent vaccine was developed in 2011 [8]. However, rates of inoculation with the AKAV and BEFV vaccines are low in Korean native cattle, and the AINOV, AKAV and CHUV trivalent vaccine has received little use in the field. Therefore, it is difficult to distinguish between vaccinated and unvaccinated Korean cattle. In this study, serological monitoring of five arboviruses was conducted during 2009–2012 using a total of 4,000 blood samples, with samples collected from 500 unvaccinated sentinel cattle twice each year. This study represents the first nationwide serological survey of AINOV, AKAV, BEFV, CHUV and IBAV among sentinel cattle conducted by neutralization antibody tests.

The study was carried out in all provinces and metropolitan cities of the Republic of Korea. In May and October of each year from 2009 through 2012, blood samples were collected from 500 unvaccinated Korean calves, yielding 1,000 blood samples annually. In May, the sampled cattle were 6–7 months of age. Samples were again collected in October of each year, following the season of vector activity from June to August. These samples were compared to the samples collected from the same individuals in May of that year. The necessary sample size was calculated using Win Episcope 2.0: 475 cattle were required to analyze the nationwide arboviral prevalence, based on an error of 4.5%, 95% confidence and 50% expected prevalence. Sampling locations are shown in Fig. 1.

The AINOV strain KSA 9910 (Korea Veterinary Culture Collection (KVCC)-VR64), AKAV strain 93FMX (KVCC-

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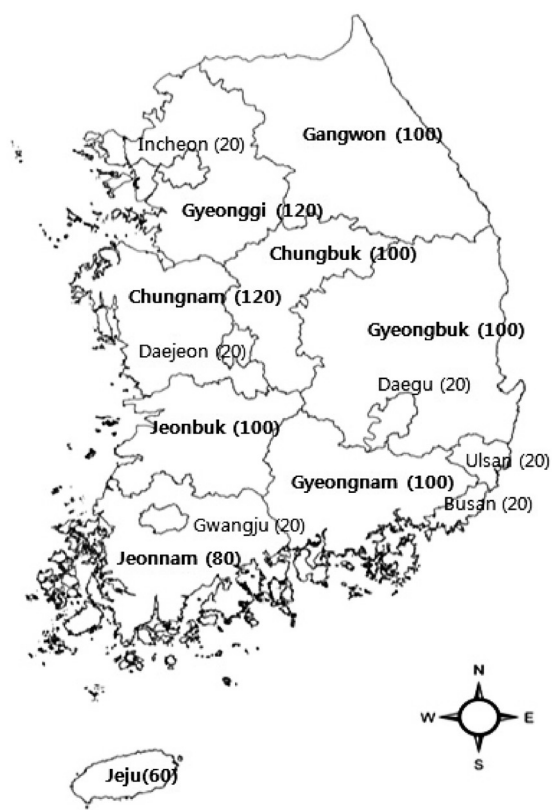


Fig. 1. Number of cattle sampled in each province of South Korea. Serum was collected from same individual in May and October of each year from 2009 to 2012 for serosurveillance of viruses. Numbers in parentheses represent sample size. Bold characteristics represent the province of South Korea.

VR63), BEFV strain TongRae (KVCC-VR41), CHUV strain YongAm (KVCC-VR66) and IBAV strain 08220 (KVCC-VR65) were used for serum neutralization tests (SNT) [8, 10, 13]. Vero cells (ATCC, C-1586) were maintained in alpha-minimum essential medium (Gibco, Grand Island, NY, U.S.A.) containing 5% fetal bovine serum and antimycotic-antibiotics (Gibco). SNT against AINOV, AKAV, BEFV, CHUV and IBAV were performed in flat-bottomed 96-well plates. Briefly, two-fold serial dilutions of sera were mixed with equal volumes of virus containing 200 TCID<sub>50</sub>/0.1 ml and inoculated with Vero cells [8, 11]. The plates were microscopically examined after 3 and 5 days for evidence of virus-specific cytopathic effects (CPE). Antibody titers were expressed as the reciprocal of the highest serum dilution at which CPE was inhibited. A titer of 1:4 or greater was considered to be positive.

Seropositive animals were those that were antibody-negative in May, but antibody-positive in October of the same year. Therefore, the seroprevalences for the five viruses in this study represent the proportions of the 500 sampled individuals that tested positive for the antibody in each year. Collecting serum samples from the same individuals before and after the vector season provided reliable epidemiologi-

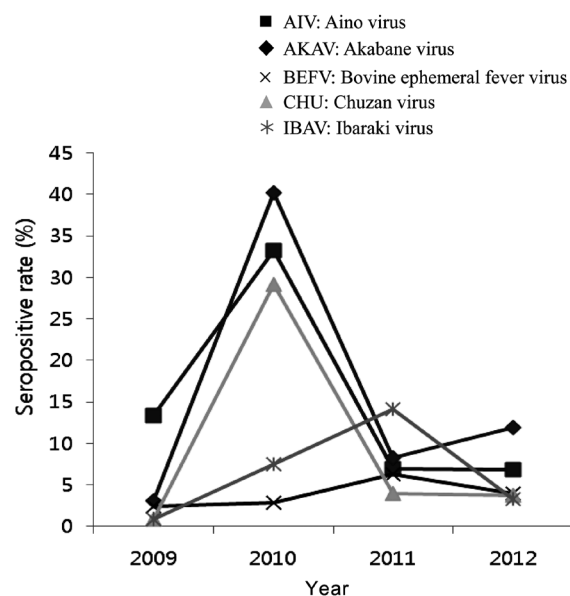


Fig. 2. Nationwide seropositivity rates for five arboviruses in South Korea from 2009 through 2012.

cal data for arboviral infection.

Among the regions and years studied, Gwangju had the highest seropositivity for AINOV and AKAV at 100% and 66.7%, respectively, in 2010. Gyeongnam had the highest seropositivity for CHUV at 61% in 2010. The highest seropositivity rate for BEFV was 35.7% in Daegu, while that for IBAV was 33.3% on Jeju Island, both in 2011. In 2009, the first year of monitoring, the same individuals could not be sampled a second time in Chungbuk and Jeonnam (Table 1). Therefore, the seropositivity rates for these provinces are not shown in Table 1. No significant association was found between seropositivity and region. Figure 2 shows the seropositivity rates for each virus in each year. In 2009, no virus had a seropositivity rate greater than 13.3%. However, in 2010, the overall seropositivity rates were 33.2% for AINOV, 40.2% for AKAV, 2.9% for BEFV, 29.1% for CHUV and 7.5% for IBAV. In 2011 and 2012, all seropositivity rates were less than 14.1% (Fig. 2). We found a high seropositivity rate for AKAV in 2010, suggesting that AKAV infection may have caused disease outbreaks in cattle. Indeed, a large-scale outbreak of Akabane viral encephalomyelitis in cattle aged 4–72 months occurred in the southern part of Korea from late summer to late autumn in 2010. Most of the affected cattle were found in Jeonbuk and Jeonnam provinces [14]. These data were supported by a previous report indicating that arboviral disease has been suspected to be sporadic outbreaks [17]. Although elevated antibody prevalence also against AINOV in 2010 was founded, AINOV which was associated with the large-scale outbreak including samples of the southern part was not detected.

Significant seroconversions against AKAV, AINOV and CHUV were observed in 2010. However, no clinical cases were reported in the field for any virus, except for AKAV

Table 1. Percentage of seropositivity from Korean sentinel cattle to each five arboviruses in 2009–2012, presented by region

Virus	Aino virus				Akabane virus				Bovine ephemeral fever virus				Chuzan virus				Ibaraki virus			
	'09	'10	'11	'12	'09	'10	'11	'12	'09	'10	'11	'12	'09	'10	'11	'12	'09	'10	'11	'12
Busan	0.0	0.0	0.0	0.0	0.0	0.0	0.0	30.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0
Chungbuk	–	15.4	3.4	3.6	–	20.8	11.9	10.9	–	4.0	3.4	0.0	–	34.0	10.2	9.1	–	14.3	13.6	3.6
Chungnam	43.6	20.0	17.8	7.4	0.0	39.3	13.3	13.0	0.0	2.0	6.7	13.0	0.0	21.9	0.0	5.6	1.8	11.6	11.1	3.7
Daegu	0.0	27.3	0.0	27.3	0.0	14.3	0.0	0.0	0.0	0.0	35.7	0.0	0.0	10.0	0.0	9.1	0.0	0.0	0.0	0.0
Daejeon	10.0	75.0	0.0	0.0	0.0	0.0	20.0	10.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gangwon	2.3	40.0	2.9	0.0	2.3	25.0	5.7	5.9	0.0	0.0	2.9	5.9	0.0	15.0	0.0	2.0	0.0	5.4	14.3	0.0
Gwangju	0.0	100.0	0.0	14.3	0.0	66.7	6.7	14.3	0.0	0.0	13.3	0.0	0.0	37.4	0.0	0.0	10.0	0.0	13.3	0.0
Gyeongbuk	0.0	36.0	8.0	6.0	2.0	24.0	2.0	5.0	10.0	12.8	6.0	0.0	0.0	34.1	0.0	2.0	4.0	5.0	18.0	2.0
Gyeonggi	12.7	40.0	10.7	8.0	3.2	44.1	21.4	20.0	0.0	1.7	3.6	0.0	1.6	20.8	7.1	2.0	1.6	7.7	10.7	4.0
Gyeongnam	22.5	51.9	8.0	8.0	11.3	31.7	8.0	8.6	2.8	5.4	8.0	0.0	1.4	61.0	4.0	2.0	1.4	11.5	20.0	2.0
Incheon	0.0	28.6	0.0	0.0	0.0	36.3	0.0	7.7	0.0	0.0	0.0	0.0	0.0	21.4	0.0	0.0	0.0	0.0	0.0	7.7
Jeonbuk	9.1	46.2	2.4	3.3	0.0	34.8	9.5	5.0	0.0	0.0	0.0	6.7	3.0	28.1	2.4	1.7	0.0	3.2	21.4	3.3
Jeonnam	–	42.3	16.3	11.4	–	56.3	2.3	8.6	–	0.0	16.3	17.1	–	38.5	11.6	8.6	–	5.6	7.0	0.0
Jeju	10.0	26.7	3.7	3.3	3.3	14.3	11.1	26.7	0.0	0.0	0.0	6.7	3.3	0.0	7.4	3.3	3.3	7.4	33.3	3.3
Ulsan	0.0	66.7	0.0	10.0	0.0	20.0	0.0	10.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0

– : Chungbuk and Jeonnam had no the same individual's data to analyze.

during the study period. Many other factors may be involved in this result, including characteristics of subclinical signs in most arbovirus-infected cattle. In AINOV and CHUV infections of cattle, *in utero* infections of pregnant cows are common during outbreaks. Therefore, laboratory-confirmed cases are rare in many countries. Additionally, a sporadic outbreak in the field may not be reported. Although unlikely, cross reactivity with other related arboviruses in the virus neutralization test cannot be eliminated as a factor. Therefore, further studies are necessary to reveal the relationship between disease incidence and the seroconversion of sentinel animals.

In this study, we established a statistically reliable sampling strategy for the serological surveillance of arboviruses in South Korea. The sample size used in this study was optimal for the surveillance of herd immunity to arboviruses across the country.

Baseline serological studies of animals, such as the current study, can be used to determine antibody seropositivity rates. The determination of seropositivity rates often leads to an understanding of virus circulation dynamics and is useful in the formulation of disease control measures [2]. Therefore, quarantines and the summer control of vectors could be better implemented in those provinces with elevated seropositivity rates. Indeed, after the large-scale outbreak in 2010, the Animal and Plant Quarantine Agency (QIA) recommended preventive vaccination in all regions of South Korea.

In South Korea, Shin *et al.* monitored bovine arboviral diseases using a method for detecting viral genes from arthropod vectors, such as *Culicoides* species, in 2006–2008 [17]. However, as data from the continued monitoring of arthropod vectors have not been reported since 2009, we are unable to speculate regarding the epidemiological relationship between the viral genes from these arthropod vectors

and the seropositivity rates observed in the present study. The species of the biting midge of the genus *Culicoides* (Diptera: Ceratopogonidae) most commonly collected on cattle farms was *Culicoides (C.) punctatus*, followed by *C. arakawae* [13]. This result is consistent with those of previous seasonal abundance observations of cowsheds conducted in the southern part of the Republic of Korea [7]. However, the isolation of bovine arboviruses from *Culicoides* biting midges and the identification of the main vector species remain subjects for a future study. The results of this seroprevalence study may serve as a basis for future epidemiological studies of arboviral infection.

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## REFERENCES

1. Akashi, H., Kaku, Y., Kong, X. G. and Pang, H. 1997. Sequence determination and phylogenetic analysis of the Akabane bunyavirus S RNA genome segment. *J. Gen. Virol.* **78**: 2847–2851. [Medline]
2. Batista, P. M., Andreotti, R., Chiang, J. O., Ferreira, M. S. and Vasconcelos, P. F. 2012. Seroepidemiological monitoring in sentinel animals and vectors as part of arbovirus surveillance in the state of Mato Grosso do Sul, Brazil. *Rev. Soc. Bras. Med. Trop.* **45**: 168–173. [Medline] [CrossRef]
3. Jagoe, S., Kirkland, P. D. and Harper, P. A. 1993. An outbreak of Akabane virus-induced abnormalities in calves after agistment in an endemic region. *Aust. Vet. J.* **70**: 56–58. [Medline] [CrossRef]
4. Jun, Q., Qingling, M., Zaichao, Z., Kuojun, C., Jingsheng, Z., Mingxing, M. and Chuangfu, C. 2012. A serological survey of Akabane virus infection in cattle and sheep in northwest China. *Trop. Anim. Health Prod.* **44**: 1817–1820. [Medline] [CrossRef]
5. Jusa, E. R., Inaba, Y., Kadoi, K., Kurogi, H., Fonseca, E. and

- Shope, R. E. 1994. Identification of Kagoshima and Chuzan viruses of Japan as Kasba virus, an orbivirus of the Palyam serogroup. *Aust. Vet. J.* **71**: 57. [Medline] [CrossRef]
6. Kang, W. C., Kim, E. J., Hyun, K. J., Cheon, C. I., Kim, H. S. and Lee, D. S. 2000. Isolation, identification and epidemiological study of akabane virus on Jeju-do. *Korea. J. Vet. Serv.* **12**: 93–102.
  7. Kim, H. C., Bellis, G. A., Kim, M. S., Chong, S. T., Lee, D. K., Park, J. Y., Yeh, J. Y. and Klein, T. A. 2012. Seasonal abundance of biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae), collected at cowsheds in the southern part of the Republic of Korea. *Korean J. Parasitol.* **50**: 127–131. [Medline] [CrossRef]
  8. Kim, Y. H., Kweon, C. H., Tark, D. S., Lim, S. I., Yang, D. K., Hyun, B. H., Song, J. Y., Hur, W. and Park, S. C. 2011. Development of inactivated trivalent vaccine for the teratogenic Aino, Akabane and Chuzan viruses. *Biologicals* **39**: 152–157. [Medline] [CrossRef]
  9. Lee, J. K., Kim, J. H., Park, B. K., Yoo, H. S., Lee, B. C. and Kim, D. Y. 2007. Akabane viral encephalitis in calves in South Korea. *Vet. Rec.* **161**: 236–238. [Medline] [CrossRef]
  10. Lim, S. I., Kweon, C. H., Tark, D. S., Kim, S. H. and Yang, D. K. 2007. Sero-survey on Aino, Akabane, Chuzan, bovine ephemeral fever and Japanese encephalitis virus of cattle and swine in Korea. *J. Vet. Sci.* **8**: 45–49. [Medline] [CrossRef]
  11. Lim, S. I., Kweon, C. H., Yang, D. K., Tark, D. S. and Kweon, J. H. 2005. Apoptosis in Vero cells infected with Akabane, Aino and Chuzan virus. *J. Vet. Sci.* **6**: 251–254. [Medline]
  12. Metselaer, D. and Robin, Y. 1976. Akabane virus isolated in Kenya. *Vet. Rec.* **99**: 86. [Medline] [CrossRef]
  13. Oem, J. K., Chung, J. Y., Kwon, M. S., Kim, T. K., Lee, T. U. and Bae, Y. C. 2013. Abundance of biting midge species (Diptera: Ceratopogonidae, *Culicoides* spp.) on cattle farms in Korea. *J. Vet. Sci.* **14**: 91–94. [Medline] [CrossRef]
  14. Oem, J. K., Lee, K. H., Kim, H. R., Bae, Y. C., Chung, J. Y., Lee, O. S. and Roh, I. S. 2012. Bovine epizootic encephalomyelitis caused by Akabane virus infection in Korea. *J. Comp. Pathol.* **147**: 101–105. [Medline] [CrossRef]
  15. Oem, J. K., Yoon, H. J., Kim, H. R., Roh, I. S., Lee, K. H., Lee, O. S. and Bae, Y. C. 2012. Genetic and pathogenic characterization of Akabane viruses isolated from cattle with encephalomyelitis in Korea. *Vet. Microbiol.* **158**: 259–266. [Medline] [CrossRef]
  16. Park, B. K., Lee, J. C., An, S. H., Moon, H. K., Kim, K. S. and Son, D. S. 1993. An outbreak of Chuzan disease in Korea and the immunogenicity of binary ethylenimine-treated Chuzan virus vaccine in cattle. *Kor. J. Vet. Publ. Hlth.* **17**: 301–305.
  17. Shin, Y. K., Oem, J. K., Yoon, S. R., Hyun, B. H., Cho, I. S., Yoon, S. S. and Song, J. A. 2009. Monitoring of Five Bovine Arboviral Diseases Transmitted by Arthropod Vectors in Korea. *J. Bacteriol. Virol.* **39**: 353–362. [CrossRef]
  18. Taylor, W. P. and Mellor, P. S. 1994. The distribution of Akabane virus in the Middle East. *Epidemiol. Infect.* **113**: 175–185. [Medline] [CrossRef]
  19. Uchinuno, Y., Noda, Y., Ishibashi, K., Nagasue, S., Shirakawa, H., Nagano, M. and Ohe, R. 1998. Isolation of Aino virus from an aborted bovine fetus. *J. Vet. Med. Sci.* **60**: 1139–1140. [Medline] [CrossRef]
  20. Uren, M. F., St George, T. D. and Murphy, G. M. 1992. Studies on the pathogenesis of bovine ephemeral fever in experimental cattle. III. Virological and biochemical data. *Vet. Microbiol.* **30**: 297–307. [Medline] [CrossRef]
  21. Yanase, T., Kato, T., Kubo, T., Yoshida, K., Ohashi, S., Yamakawa, M., Miura, Y. and Tsuda, T. 2005. Isolation of bovine arboviruses from *Culicoides* biting midges (Diptera: Ceratopogonidae) in southern Japan: 1985–2002. *J. Med. Entomol.* **42**: 63–67. [Medline] [CrossRef]
  22. Yang, D. K., Hwang, I. J., Kim, B. H., Kweon, C. H., Lee, K. W., Kang, M. I., Lee, C. S. and Cho, K. O. 2008. Serosurveillance of viral diseases in Korean native goats (*Capra hircus*). *J. Vet. Med. Sci.* **70**: 977–979. [Medline] [CrossRef]
  23. Yeruham, I., Van Ham, M., Stram, Y., Friedgut, O., Yadin, H., Mumcuoglu, K. Y. and Braverman, Y. 2010. Epidemiological investigation of bovine ephemeral Fever outbreaks in Israel. *Vet. Med. Int.* **2010**: 1–5. [Medline] [CrossRef]