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Role of cervids in the epidemiology of bovine ephemeral fever virus infection in the Republic of Korea: A cross-sectional retrospective study

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Abstract

Background: Bovine ephemeral fever (BEF) is a viral disease in cattle and buffaloes, with subclinical involvement in various ruminant species.

Objectives: This study aimed to investigate bovine ephemeral fever virus (BEFV) transmission in deer in the Republic of Korea (ROK) and the potential risk factors associated with seropositivity.

Methods: We conducted a retrospective cross-sectional serological survey of neutralising antibodies against BEFV in cervid sera collected from the ROK.

Results: The seroprevalence of BEFV was estimated to be 10.8% (95% confidence interval [CI] = 8.5–14.1), demonstrating that exposure to this virus is prevalent among farmed and free-ranging cervids in the ROK. The results revealed that age class and geographic location affected seroprevalence. Older age and the presence of neighbouring ruminant farms were significant risk factors (odds ratio [OR] = 2.394, 95% CI = 1.195–4.796) and (OR = 1.533, 95% CI = 1.026–2.288), respectively. We also observed that the individual likelihood of positivity in the southern provinces was significantly higher than that in the northern provinces (OR = 1.744, 95% CI = 1.001–3.037). There were also significant differences in the seroprevalence of cervids between the western and eastern provinces (OR = 2.021, 95% CI = 1.047–3.900). Factors that were not significantly associated with BEFV antibody prevalence included herd size and species ($p > 0.05$).

Conclusions: These results suggest that cervid species may serve as important reservoirs for the transmission of BEFV, highlighting the need for closer monitoring of BEFV infections in cervids in the ROK.

KEYWORDS
bovine ephemeral fever, cervids, risk factor, seroprevalence, the Republic of Korea

1 | INTRODUCTION

Bovine ephemeral fever (BEF) is a viral disease in cattle and buffaloes, with subclinical involvement in various ruminant species. Outbreaks of BEF can cause severe economic consequences due to reduced milk production, poor cattle conditions, costs of supportive care, and trade restrictions (Lee, 2019; St George, 1988; Walker & Klement, 2015). The bovine ephemeral fever virus (BEFV) is an arthropod-borne rhabdovirus belonging to the genus *Ephemerovirus* within the family *Rhabdoviridae*. BEF occurs throughout a large proportion of the world (Walker & Klement, 2015), where seasonal outbreaks, particularly following periods of high rainfall, are indicative of vector-borne transmission (Davies et al., 1975; Finlaison et al., 2010; Geoghegan et al., 2014; Lee, 2019; Tonbak et al., 2013). Sheep, goats, pigs, and many wild animals, including water buffalo and various species of deer, are seropositive for BEFV, indicating that these animals may serve as reservoir hosts (Akakpo, 2015; Aziz-Boaron et al., 2015).

In the Republic of Korea (ROK), a live attenuated vaccine for BEFV has been used for the prevention of BEF in cattle since the 1980s because its epizootics occur periodically. However, rates of BEFV vaccination in Korean cattle are low and it is difficult to distinguish between vaccinated and unvaccinated animals in ROK . A follow-up study based on a serological survey after the BEFV outbreaks in ROK indicated that mass vaccination would be required to prevent BEF (Yang et al., 2015). The seropositive rates for BEFV in cattle in ROK were found to be 15.7% based on a virus neutralisation assay (Lim et al., 2007).

Although BEF is endemic to ROK, its epidemiology is poorly defined. BEFV is maintained in the environment by unknown means but presumably by cycling between insect vectors, such as *Culicoides* biting midges and mosquitoes and susceptible hosts. However, information on the reservoirs of BEFV remains unclear, and little is known about BEFV transmission among cervids. Therefore, it is important to understand the involvement of deers in the ecology and epidemiology of the BEFV. This study aimed to investigate BEFV transmission in deers in the ROK and the potential risk factors associated with seropositivity.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a cross-sectional retrospective study using serum bank samples collected in 2015 and provided by the National Veterinary Research and Quarantine Service (NVRQS, Anyang, ROK) concerning farmed deer sera, as well as serum sample deer rescued by local rescue parties or housed at wildlife rescue centres. The target population in this study was free-ranging and farmed cervids. The necessary sample size to estimate prevalence was calculated using EpiTools-Epidemiological Calculators (Ausvet, Canberra, Australia) based on the methods described by Humphry et al. (2004). A total of 73 herds were required to analyse the nationwide seroprevalence of BEFV based on a 5% desired precision, 95% confidence and 5% assumed true prevalence. A total of 385 animals were required to analyse the nationwide seroprevalence of BEFV based on a 5% desired precision, 95% confidence and 50% assumed true prevalence. The expected seroprevalence was determined based on bovine serological data previously reported in the ROK (Kim et al., 2015). Flocks and animals within each flock were selected using a simple random sampling method in each province based on national statistics (Livestock Management Division, 2012). Animals younger than 6 months were excluded from the sampling frame to avoid the detection of maternal antibodies. All the sera used in this study were collected from asymptomatic animals and were tested for FMD according to the monitoring measures issued and conducted by the Ministry of Agriculture, Food and Rural Affairs. Only serum samples confirmed to be negative for the FMD virus were evaluated for neutralising antibodies specific to BEFV in this study. Finally, 44 blood samples from Korean water deers (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*) rescued by local rescue parties or housed at wildlife rescue centres were included in the study.

2.2 | Serologic testing: detection of antibodies to BEFV

The presence of specific antibodies against BEFV in the serum samples was analysed using serum neutralisation tests (SNTs) to estimate exposure to BEFV. The BEFV strain TongRae (VR41, Korea Veterinary Culture Collection, Anyang, ROK) was used for SNTs. Vero cells (C-1586; American Type Culture Collection, Manassas, VA, USA) were maintained in alpha-minimum essential medium (Gibco, Grand Island, NY, USA) containing 5% foetal bovine serum and antimycotic antibiotics (Gibco). The SNTs against BEFV were performed in flat-bottomed 96-well plates. Briefly, approximately hundred 50% tissue culture infective doses ($TCID_{50}$) of the standard or untyped virus were added to a volume of 50 μl in the test wells of a flat-bottomed microtitre plate and mixed with an equal volume of standard antiserum that had been serially diluted in the tissue culture medium (Kim et al., 2011; Lim et al., 2007; Lim et al., 2005). Approximately 10^4 Vero cells were added to each well at a volume of 100 μl and assessed after incubation for 3–5 days using an inverted microscope (Olympus, Tokyo, Japan). The wells were scored based on virus-specific cytopathic effects (CPEs). Antibody titres were expressed as the reciprocal of the highest serum dilution at which CPEs was inhibited. A titre of 1:4 or higher was considered positive.

The apparent prevalence (AP) rate was considered the animal-level prevalence, defined as the proportion of SNT-positive animals out of the total number of animals tested in the study area, and flock prevalence was defined as the proportion of SNT-positive flocks out of the total number of tested flocks in the area. A flock was classified as positive if at least one animal showed a positive SNT result.

2.3 | Risk factor analysis

Apparent clinical signs of BEF were not observed in any of the animals analysed. Risk factor information was obtained from (1) the Korea

TABLE 1 Seroprevalence of bovine ephemeral fever virus infection in cervids in the Republic of Korea

			Herds				Heads			
Province	Latitude (N)	Longitude (E)	Positive	Tested	AP	TP ± 95% CI	Positive	Tested	AP	TP ± 95% CI
Incheon	36°55'–37°58'	124°36'–126°47'	3	12	25.0	6.3–47.2	3	13	23.1	8.1–55.3
Gyeonggi	36°53'–38°17'	126°22'–127°51'	8	52	15.4	8.2–27.4	12	155	7.7	3.9–13.5
Gangwon	38°09'–39°25'	126°46'–128°22'	2	16	12.5	9.0–23.6	3	63	4.8	0.7–13.6
Chungbuk	37°15'–36°00'	127°16'–128°38'	1	20	5.0	0.0–20.1	3	37	8.1	2.0–22.8
Chungnam	35°58'–37°03'	125°32'–127°38'	4	33	12.1	4.7–27.0	7	119	5.9	2.1–12.0
Jeonbuk	35°18'–36°09'	125°58'–127°54'	5	19	26.3	11.8–47.7	7	54	13.0	6.1–26.3
Jeonnam	33°54'–35°30'	125°04'–127°54'	5	31	16.1	9.0–35.6	14	40	35.0	23.8–55.6
Gyeongbuk	35°34'–37°33'	127°48'–131°52'	3	21	14.3	5.5–35.4	5	31	16.1	6.9–35.6
Gyeongnam	34°39'–35°54'	127°35'–129°28'	3	10	30.0	8.1–55.3	3	32	9.4	2.5–26.1
Jeju	33°06'–34°00'	126°08'–126°58'	3	13	23.1	5.8–45.0	5	14	35.7	17.2–67.7
Total	33°06'–39°25'	124°36'–131°52'	37	227	16.3	11.4–20.5	62	558	11.1	8.7–14.6

Note: A number of free-ranging animals, such as the Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*), were excluded because they were rescued by local rescue parties or housed at wildlife rescue centres, and their herds could not be identified.
AP, apparent (estimated) prevalence; TP, true prevalence; CI, confidence interval.

Animal Health Integrated System (Animal and Plant Quarantine Agency, Anyang, ROK), (2) veterinarians in wildlife rescue centres, and (3) animal owners who completed a questionnaire form and permitted the use of blood samples for diagnostic and research purposes under the national active surveillance programs for foreign animal diseases, including FMD. The AP and 95% confidence interval (CI) (Reiczigel et al., 2010) of true prevalence (TP) were calculated using Epitools-Epidemiological Calculators (Ausvet). Logistic regression models were used to analyse the associations between animal seropositivity outcomes and potential risk factors. The effects of the exposure variables on individual seropositivity were analysed using univariable logistic regression models, and the variables in the univariable analysis were screened for pairwise collinearity or associations using Pearson's correlation coefficient or chi-squared test for continuous or categorical variables, respectively. The strength of the association was calculated using the odds ratio (OR) with 95% CI. The p value <0.05 was considered statistically significant. All statistical analyses were performed using the statistical software SPSS version 25 (IBM Corp., Armonk, NY, USA).

3 | RESULTS

Overall, 602 serum samples were analysed: 558 (92.7%) from farmed cervid populations (n = 227) and 44 (7.3%) from free-ranging cervid populations. BEFV infection was widespread, as the 37 herds evaluated in this study included seropositive cervids (AP 16.3%, TP 95% CI = 11.4–20.5), as shown in Table 1. Additionally, 62 of 558 (AP 11.1%, TP 95% CI = 8.7–14.6) farmed cervid serum samples collected from 227 herds contained antibodies to BEFV, as determined by SNTs.

Seropositive animals were detected in most of the surveyed provinces. The highest prevalence values for BEFV were observed in Gyeongnam Province, with an AP of 30.0% (TP 95% CI = 8.1–55.3) (3 out of 10 herds), and Jeonnam Province, with an AP of 35.0% (TP 95% CI = 23.8–55.6) (14 out of 40 heads) as seen in Figure 1 and Table 1.

Serological evidence of BEFV infection was observed in three of the 44 free-ranging cervids that were rescued by local rescue parties or from wildlife rescue centres (Table 2), as determined by SNTs. Free-ranging animals, such as the Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*), were excluded from Table 1 because the regionalities of these animals could not be determined in many cases.

Our analysis of cervid serum samples revealed that age and geographic location were associated with seroprevalence. The factors associated with a positive BEFV infection status are shown in Table 2. In univariate analysis, older age and the presence of neighbouring ruminant farms were significant risk factors (OR = 2.394, 95% CI = 1.195–4.796, p = 0.012 in adults) and (OR = 1.533, 95% CI = 1.026–2.288, p = 0.036)), respectively. Additionally, there were substantial regional differences in seroprevalence within the ROK. We observed that the likelihood of seropositivity was significantly higher in the southern provinces than in the northern provinces (OR = 1.744, 95% CI = 1.001–3.037, p = 0.047). There were also statistically significant differences in the seroprevalence of cervids between the western and eastern provinces (OR = 2.021, 95% CI = 1.047–3.900, p = 0.033). In contrast, no significant association was observed between seroprevalence and herd size or cervid species (p > 0.05).

4 | DISCUSSION

This is the first report on circulating antibodies against BEFV among cervids in the ROK. The wide geographical distribution of BEFV in the Korean cervid populations is noteworthy. To the best of our knowledge, no exhaustive national study of BEFV in wild ruminants has been conducted worldwide.

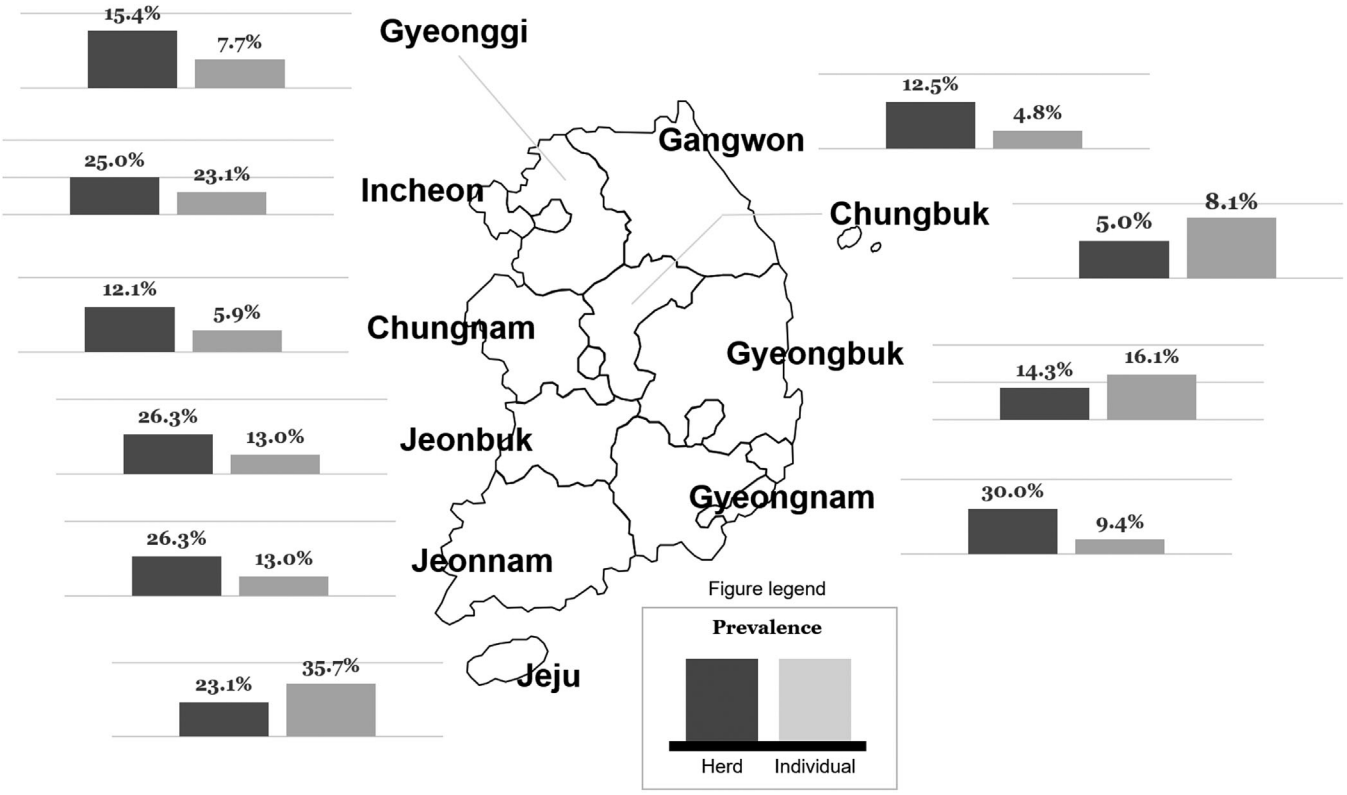

FIGURE 1 Geographical location of the provinces in the Republic of Korea. Geographical distribution patterns of the seroprevalence to bovine ephemeral fever virus. Percentages of tested herds and animals seropositive for bovine ephemeral fever virus in 10 provinces in the Republic of Korea.

This retrospective study demonstrated the presence of BEFV antibodies in cervid samples collected from the ROK. The AP of BEFV seropositivity was estimated to be 10.8% (TP 95% CI = 8.5–14.1), indicating that exposure to this virus is prevalent among farmed and free-ranging cervids in the ROK despite the lack of reported outbreaks, probably because of the often unapparent clinical signs of BEFV. These results support the involvement of cervids in the epidemiology of BEFV.

Evidence from the serological survey conducted in this retrospective study indicates that BEFV exposure is common among cervids in the ROK, as approximately 1 in 10 animals were infected. Thus, this study showed that BEFV exists in the ROK and that susceptible cervids are at risk of becoming infected with BEFV, although an outbreak of BEFV among native cervids in the ROK has not yet been reported. For reference, in previous studies conducted in the ROK, seroprevalences in pigs and cattle were found to be 86.7% (Lim et al., 2007) and 0%–64% (Yang et al., 2018), respectively, suggesting that pigs may also serve as a reservoir for the transmission of BEFV. We acknowledge a limitation that proximity to pig farms was not considered as one of BEFV exposure variables relative to seropositivity outcomes in cervids because in the study design phase, it was only taken into account that BEFV had been well known to be a viral disease mainly in cattle.

This study represents the first assessment of the factors associated with BEFV seropositivity in cervids in the ROK. Potential factors associated with seropositivity were analysed to further understand BEFV infection among cervid populations. The seroprevalence of BEFV in cervids was significantly different among age cohorts and the presence of neighbouring ruminant farms. BEFV seroprevalence was the highest in the older cervid groups, which may suggest continuous transmission of the virus. The reason for the association with neighbouring ruminant farms might be due to a higher chance of being exposed to BEFV from infected ruminants, such as cattle. Our results indicate that farmed and free-ranging cervids, perhaps in combination with bovine populations or other wild and domestic hosts, are potential reservoirs for BEFV circulation among cervids in the ROK and perhaps elsewhere in BEFV-affected areas. Further clarification of particular cervid-bovine interaction rates at different geographic scales should improve the chance of preventing BEFV transmission. In the present study, BEFV serological status was significantly associated with geographic location variables; it was higher in the southern and western regions of the ROK. The reasons for the region-specific differences in BEFV transmission are unclear. One possibility is that ecological and climatic factors may promote increased exposure to infected reservoirs in tropical rainforests. Alternatively, viral persistence or transmissibility may be higher in areas with elevated temperature and rainfall.

A major limitation of this study was the lack of virus detection tests, such as virus isolation or RT-PCR, for the serologically analysed samples because the samples used in this study were obtained from a serum bank and unsuitable for researching the virus or its genetic material. If blood samples or tissues collected during the febrile-viraemic period (generally short, up to 48 h after the onset of clinical

TABLE 2 Univariable analysis results of bovine ephemeral fever virus exposure variables relative to seropositivity outcomes in cervids in the Republic of Korea

Variable	Positive	Negative	OR (95% CI)[†]	*P* Value
Herd size				
>50	12	147	0.571 (0.276–1.183)	0.128
10–50	26	181	1.006 (0.556–1.820)	0.985
<10	24	168	Reference	
Age class				
Juvenile	11	158	Reference	
Subadult	10	92	1.561 (0.639–3.818)	0.326
Adult	41	246	2.394 (1.195–4.796)	0.012
Presence of ruminant farms within a 1-km radius				
No	47	184	Reference	
Yes	92	235	1.533 (1.026–2.288)	0.036
Species				
Farmed				
Elk (*Cervus canadensis*)	30	210	Reference	
Sika deer (*Cervus nippon*)	19	169	0.787 (0.428–1.447)	0.440
Red deer (*Cervus elaphus*)	13	117	0.778 (0.391–1.549)	0.474
Free-ranging[‡]				
Siberian roe deer (*Capreolus pygargus*)	1	18	0.389 (0.050–3.020)	0.350
Korean water deer (*Hydropotes inermis argyropus*)	2	23	0.609 (0.137–2.714)	0.511
Area[§]				
Northern	21	234	Reference	
Southern	41	262	1.744 (1.001–3.037)	0.047
Eastern	12	162	Reference	
Western	50	334	2.021 (1.047–3.900)	0.033

[†]CI, confidence interval.

[‡]Some blood samples in this study were collected from animals rescued by local rescue parties or housed at wildlife rescue centres because of car accidents or because their presence in the downtown area necessitated their removal. A number of free-ranging animals, such as the Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*), are included in the free-ranging category.

[§]In this study, the northern area of the Republic of Korea includes Incheon, Gyeonggi, Gangwon, Chungbuk and Chungnam provinces on the basis of latitude 36 °N, whereas the southern area includes Ulsan, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam and Jeju. The western area includes Incheon, Gyeonggi, Chungnam, Jeonbuk, Jeonnam and Jeju on the basis of longitude 127 °east, whereas the eastern area includes Ulsan, Gangwon, Gyeongbuk and Gyeongongnam.

signs) were targeted and included in this study, BEFV circulating in the field in the ROK could be detected and identified.

Based on the study results, cervids are considered a reservoir host candidate in the ROK. At the very least, farmed and free-ranging cervids may play a role in the circulation of BEFV in the natural habitats around BEFV-affected ruminant farms in the Far East, including the ROK. These results suggest that cervid species may serve as important reservoirs for the transmission of BEFV, highlighting the need for closer monitoring of BEFV infections in cervids in the ROK. Moreover, the results of this seroprevalence study may serve as a basis for future epidemiological studies on BEFV infections in the ROK. Further studies are necessary to precisely identify the reservoir of BEFV and the economic impact of BEFV transmission among cervids in ROK.

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CONFLICT OF INTEREST

No conflict of interest to declare.

DATA AVAILABILITY STATEMENT

None.

ETHICAL STATEMENT

This study did not involve purposeful killing of the animals. The collection of blood samples from farmed and free-ranging cervids was part of the official Foreign Animal Diseases Monitoring Program of the National Veterinary Research and Quarantine Service of the Republic of Korea. Sera from farmed animals were obtained from serum banks or specimens subjected to health programs, medical checkups or surgical interventions at local wildlife rescue centres. Qualified veterinarians collected blood from the cervids following proper physical restraint to ensure both personnel and animal safety. Therefore, no ethical approval was necessary.

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