Epidemiological Assessment of Risk Factors Associated with Bovine Ephemeral Fever Virus Exposure among Sheep and Goats in South Korea

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ABSTRACT

Background: Bovine ephemeral fever virus (BEFV) is an arthropod-borne virus classified as a type species of the genus *Ephemerovirus*, family *Rhabdoviridae*. BEFV is the causative agent of bovine ephemeral fever (BEF), a non-contagious disease of acute febrile clinical signs in cattle and water buffalo. Some species might act as reservoir hosts, and antibodies to BEFV have also been found in asymptomatic sheep, goats, pigs, and many wild animals. This study aimed to conduct a retrospective cross-sectional serological screening in South Korea to address BEFV seroprevalence and identify risk factors for becoming seropositive for the virus in sheep and goats.

Materials, Methods & Results: The apparent prevalence rates were considered to be the animal-level prevalence, defined as the proportion of serum neutralization test (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 was SNT-positive. At the national level in 2011, 28 of 177 flocks (15.8%; 95% CI, 11.2-21.9%) and 71 of 498 heads (14.3%, 95% CI: 11.5-17.6%) that were analyzed showed serum neutralizing antibodies against BEFV. Our results revealed that age class, vector control, and geographic location affected seroprevalence to differing extents. In the univariate analysis, older age was a significant risk factor (OR, 2.327; 95% CI, 1.147-4.721; $P = 0.017$ in adults). The management risk factor attributes showed that preventive measures, such as routine application of insecticides in farms, decreased the odds of seropositivity for BEFV (OR, 0.514; 95% CI, 0.267-0.991; $P = 0.044$). Vector control was a significant protective factor, while animal species, flock size, and flock structure were not significantly associated. Differences in seroprevalence between variations in the presence of ruminant farms, lakes, or rice paddies within a 1-km radius or type of land use were not statistically significant ($P > 0.05$). We observed a significant difference in the individual likelihood of being positive in the southern provinces with respect to that in the northern provinces (OR, 2.166; 95% CI, 1.228-3.824; $P = 0.007$). Differences in seroprevalence between variations in the eastern and western regions were not statistically significant ($P > 0.05$). The retrospective study results showed that the virus was widely distributed in sheep and goats in South Korea, with seropositive rates ranging from 7.8% to 19.7% between 2003 and 2008.

Discussion: This is the first report of circulating antibodies against BEFV in sheep and goats in South Korea. The serological prevalence of BEFV infection in sheep and goats was significantly different between different age cohorts, vector control, and geographical locations: it was higher in the older group and the southern and western regions of South Korea. Determination of seropositivity rates often leads to an understanding of virus circulation dynamics and is useful in the formulation of disease control measures. Our results demonstrated that vector control was a significant protective factor; therefore, the summer control of vectors could be better implemented in provinces with elevated seropositivity rates. The results of this seroprevalence study may serve as a basis for future epidemiological studies on BEFV infection in South Korea.

Keywords: bovine ephemeral fever virus, goat, seroprevalence, sheep, South Korea.

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INTRODUCTION

Bovine ephemeral fever virus (BEFV) is an arthropod-borne rhabdovirus classified as a type species of the genus *Ephemerovirus*, within the family *Rhabdoviridae*. In dairy cows, the economic costs of BEF are associated with reduced milk yields, loss of valuable cows, and/or associated supportive treatment, and in beef cattle, they are associated with the loss of condition and trade restrictions [29,35,41,43]. Some species might act as reservoir hosts, and antibodies to BEFV have also been found in asymptomatic sheep, goats, pigs, and many wild animals, including water buffalo and various species of deer [2,4]. Although several serological studies conducted in BEFV-enzootic regions have failed to find evidence of infection in sheep [4,7,10], BEFV-neutralizing antibodies have been reported in sheep and goats in Taiwan [31]. BEF occurs over a vast expanse of the globe [43], where seasonal outbreaks, particularly following periods of high rainfall, are indicative of vector-borne transmission [8,14,17,29,42]. The competent vectors responsible for transmitting BEFV may be mosquitoes and *Culicoides* [29].

Although it has been accepted that BEFV is endemically established in South Korea, the epidemiology of BEFV infection is poorly defined and remains unclear. This study aimed to conduct a retrospective cross-sectional serological screening in South Korea to address BEFV seroprevalence and identify risk factors for becoming seropositive for the virus. In addition, a retrospective survey to determine the presence of anti-BEFV antibodies in archived sheep and goat sera (2003-2008) was also conducted to improve the understanding of the epidemiological situation in South Korea.

MATERIALS AND METHODS

Sampling design and samples for disease monitoring

The necessary sample size to estimate prevalence was calculated using Epitools-Epidemiological Calculators, based on methods described by Humphry *et al*. [23]. A total of 483 animals were required to analyze the nationwide prevalence of antibodies to BEFV based on 5% desired precision, 95% confidence, and 5% expected prevalence. The expected prevalence was determined according to bovine serological data (2009-2010) previously reported in South Korea [26]. Flocks, and animals within the flocks, were selected using a simple random sampling method in each province based on the government's national statistics. In South Korea, live vaccines for BEFV have been com-

mercially available since the 1980s. However, the rates of inoculation with the BEFV vaccine are low in Korean native cattle because the vaccine has not been used extensively in the field. Therefore, it is difficult to distinguish between vaccinated and unvaccinated Korean cattle. In this study, farms with vaccination experience were excluded from the sampling frame to avoid the detection of antibodies due to vaccine-induced immunity. In addition, animals younger than 6 months were excluded from the sampling frame to avoid detection of antibodies due to maternal immunity.

Based on the sampling size designed in this study, serum samples were mainly obtained from the blood and serum bank of the Emerging Infectious Diseases - National Surveillance Program maintained by the Foreign Animal Diseases Division of the National Veterinary Research and Quarantine Service (Anyang, South Korea). In addition, samples were obtained in close collaboration with local veterinary practitioners and/or local government veterinary officers. The blood collection procedure was performed by qualified veterinarians following proper physical restraint of the animals to ensure both personnel and animal safety. The number of samples from each province in South Korea (33°06' N–39°25' N, 124°36' E–131°52' W) is shown in Table 1. The serum was separated from the blood samples and stored at 20°C until further analysis. The seroprevalence rates were estimated at the flock and animal levels in sheep and goats. For the retrospective study, serum samples collected from sheep and goats between 2003 and 2008 were analyzed for the presence of BEFV-specific antibodies. The study using samples collected in 2011 involved a questionnaire-based survey of farmers, and the effect of the exposure variables on individual seropositivity was analyzed.

Serologic testing

The BEFV strain TongRae[1] (VR41) was used for the serum neutralization tests (SNTs). Vero cells[2] (C-1586) were maintained in alpha-minimum essential medium[3] containing 5% fetal bovine serum and antimycotic-antibiotics[3]. The SNT against BEFV was performed in flat-bottomed 96-well plates. Briefly, approximately 100 TCID50 (50% tissue culture infective dose) of the standard or untyped virus was added to a volume of 50 μL in the test wells of a flat-bottomed microtiter plate and mixed with an equal volume of standard antiserum that had been serially diluted in tissue culture medium [25,32,33]. Approximately 10^4 Vero cells[2] (CCL-81) were added per well in a volume of 100 μL and were assessed after incubation for 3-5 days using an inverted microscope[4]. The wells were scored based on the

virus-specific cytopathic effect (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 positive.

The apparent prevalence rates were 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 the 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 was SNT-positive.

Questionnaire

The sampling frame was established using sheep or goat farm ID and flock size obtained from the Korea Animal Health Integrated System (KAHIS)[5]. The study using samples collected in 2011 involved a questionnaire-based survey of farmers as well as blood samples from their animals. A pretested structured questionnaire with the primary objective of elucidating the multifactorial background of the disease was conducted interactively for all selected flocks. All animals included in this study were subjected to a questionnaire that was completed by the animal owners. The questionnaire included individual risk factors, such as animal species (goats or sheep). The animals were also classified into 3 age groups based on tooth replacement and livestock owner questionnaires: juveniles (between 6 months and 1 year old), subadults (between 1 and 2 years old), and adults (>2 years old). The management risk factors, e.g., the population size of the flocks; presence of other animal species in the farm (the presence of other ruminant animals, such as cattle, in the farm); and vector control (use of insecticides or not) - were also investigated. Regional risk factors, e.g., the presence of neighboring ruminant farms, lakes, or rice paddies; land use (agricultural, woodland and seminatural and urban areas, according to KAHIS 5); and geographic factors (localization of the farm in South Korea) were also studied. Considering that the Culicoides flying range is most likely <1 km [40], the radius size of the regional risk factor in this study was chosen inside a 1 km buffer around the sampling farm. Questionnaires for additional information on the farm or animal were completed while following up via telephone interviews.

Statistical analysis

The prevalence and Wilson's 95% confidence interval (CI) [39] were calculated using Epitools-Epidemiological Calculators[6]. In this study, the following individual exposure variables were considered for the univariable analyses of samples collected in 2011: animal species; age class; population size of the flock; flock structure; vector control; presence of neighboring ruminant farms; lakes or rice paddies; land use; and geographic factors (localization of the farm in South Korea). A logistic regression model was used to assess the association of the animal seropositivity outcome with potential risk factors. The effect of the exposure variables on individual seropositivity was analyzed 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 the chi-squared test for continuous or categorical variables, respectively. The strength of the association was calculated using odds ratios (ORs) at 95% CIs. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using the SPSS Statistics version 25[7].

RESULTS

The retrospective study results at the national level showed that the virus was widely distributed in sheep and goats in South Korea, with seropositive rates ranging from 7.8% to 19.7% between 2003 and 2008. The highest seropositivity rate was observed in 2004, while the seropositivity rate was observed in 2005. In 2011, 28 of 177 flocks (15.8%; 95% CI, 11.2-21.9%) and 71 of 498 heads (14.3%; 95% CI, 11.5-17.6%) that were analyzed showed serum neutralizing antibodies against BEFV, as shown in Table 1. Our analysis of samples collected in 2011 revealed that age class, vector control, and geographic location affected seroprevalence to differing extents. In the univariable analysis (Table 2), older age was a significant risk factor (OR, 2.327; 95% CI, 1.147-4.721; $P = 0.017$ in adults). The management risk factor attributes showed that preventive measures, such as routine application of insecticides in farms, decreased the odds of seropositivity for BEFV (OR, 0.514; 95% CI, 0.267-0.991; $P = 0.044$). Vector control was a significant protective factor, while animal species, flock size, and flock structure were not significantly associated. Differences in seroprevalence between variations in the presence of ruminant farms, lakes, or rice paddies within a 1-km radius or type of land use were not statistically significant ($P > 0.05$). Additionally, there were substantial regional differences in seroprevalence in South Korea. We observed a significant difference in the individual likelihood of being positive in the southern provinces with respect to that in the northern provinces, respectively (OR, 2.166; 95% CI, 1.228-3.824); $P = 0.007$). Differences in seroprevalence between variations in the eastern and western regions were not statistically significant ($P > 0.05$).

Table 1. Seroprevalence of bovine ephemeral fever virus infection in sheep and goats in South Korea.

Province	Latitude (N)	Longitude (E)	2003 #*	2003 Pos	2003 %	2004 #	2004 Pos	2004 %	2005 #	2005 Pos	2005 %	2006 #	2006 Pos	2006 %	2007 #	2007 Pos	2007 %	2008 #	2008 Pos	2008 %	2011† #	2011† Pos	2011† %
Incheon	36°55'-37°58'	124°36'-126°47'																			10	1	10.0
Ulsan	35°19'-35°43'	128°58'-129°27'																			11	1	9.1
Gyeonggi	36°53'-38°17'	126°22'-127°51'	21	3	14.3	16	1	6.3	56	2	3.6	28	2	7.1	42	2	4.8	10	0	0.0	117	14	12.0
Gangwon	38°09'-39°25'	126°46'-128°22'	35	2	5.7	31	4	12.9	6	1	16.7	48	6	12.5	20	1	5.0	36	0	0.0	35	11	31.4
Chungbuk	37°15'-36°00'	127°16'-128°38'	42	6	14.3	33	4	12.1	36	1	2.8	76	3	3.9	45	1	2.2	52	1	1.9	37	2	5.4
Chungnam	35°58'-37°03'	125°32'-127°38'	57	7	12.3	82	6	7.3	42	3	7.1	63	4	6.3	62	1	1.6	80	4	5.0	119	5	4.2
Jeonbuk	35°18'-36°09'	125°58'-127°54'	120	4	3.3	149	33	22.1	137	5	3.6	65	5	7.7	54	7	13.0	48	11	22.9	39	11	28.2
Jeonnam	33°54'-35°30'	125°04'-127°54'	88	16	18.2	103	21	20.4	102	12	11.8	121	21	17.4	132	19	14.4	141	27	19.1	44	10	22.7
Gyeongbuk	35°34'-37°33'	127°48'-131°52'	56	4	7.1	48	8	16.7	78	3	3.8	59	16	27.1	68	3	4.4	77	2	2.6	35	6	17.1
Gyeongnam	34°39'-35°54'	127°35'-129°28'	90	9	10.0	61	22	36.1	20	6	30.0	104	1	1.0	82	7	8.5	93	6	6.5	36	4	11.1
Jeju	33°06'-34°00'	126°08'-126°58'	12	7	58.3	10	6	60.0	135	15	11.1	63	23	36.5	3	2	66.7	4	1	25.0	15	6	40.0
Total	33°06'-39°25'	124°36'-131°52'	521	58	11.1	533	105	19.7	612	48	7.8	627	81	12.9	508	43	8.5	541	52	9.6	498	71	14.3

*Number of tested samples; †The study conducted in 2011 involved a questionnaire-based survey of farmers and the effect of the exposure variables on individual seropositivity was analyzed.

Table 2. Univariable analysis of bovine ephemeral fever virus exposure variables relative to seropositivity outcomes in samples collected fom sheep and goats in 2011.

Variable	Positive (n = 139)	Negative (n = 776)	Univariable analysis			
			OR (95% CI)	*P* value	OR (95% CI)	*P* value
Animal species						
Goat	37	229	Reference			
Sheep	34	198	1.063 (0.643-1.757)	0.812		
Age class						
Juvenile	11	108	Reference		0.430 (0.212-0.872)	0.017
Subadult	19	146	1.278 (0.584-2.796)	0.539	0.549 (0.305-0.987)	0.043
Adult	41	173	2.327 (1.147-4.721)	0.017	Reference	
Population sizes of the flocks						
<6 heads	19	131	Reference		0.783 (0.422-1.455)	0.438
6-10 heads	21	134	1.081 (0.555-2.102)	0.820	0.846 (0.463-1.546)	0.587
>10 heads	30	162	1.277 (0.687-2.371)	0.438	Reference	
Flock structure						
Goat (and/or) sheep alone	42	264	Reference			
With other ruminants	29	173	1.054 (0.632-1.756)	0.841		
Vector control						
No	59	306	Reference			
Yes	12	121	0.514 (0.267-0.991)	0.044		
Presence of ruminant farms within a 1-km radius						
No	35	232	Reference			
Yes	36	195	1.224 (0.740-2.023)	0.431		
Presence of lakes or rice paddies within a 1-km radius						
No	42	258	Reference			
Yes	29	169	1.054 (0.632-1.758)	0.840		
Land use						
Urban	15	93	Reference		0.872 (0.413-1.595)	0.545
Agricultural	27	188	0.890 (0.452-1.755)	0.737	Reference	
Woodland and semi-natural	29	146	1.232 (0.627-2.420)	0.545	0.723 (0.410-1.275)	0.261
Localization of the farm in South Korea						
Northern	18	181	Reference			
Southern	53	246	2.166 (1.228-3.824)	0.007		
Localization of the farm in South Korea						
Eastern	24	130	Reference			
Western	47	297	0.857 (0.503-1.461)	0.571		

DISCUSSION

BEFV is considered endemic and known to cause an epizootic disease in many regions, such as East Asia [45], Japan [19,24], China [16,27], the Philippines [28], Thailand [6], Taiwan [21,31,44], Australia [15,17], India [38], Israel [11,47], Jordan [20], Iran [3,5], Kingdom of Saudi Arabia [50], South Africa [37], and Turkey [9,36]. For example, previous studies performed in Australian deer reported serological evidence for BEFV in red deer [12] and another study conducted in Australia demonstrated that all samples screened in wild deer were PCR-negative for BEFV, although BEFV is endemic in Australia [22]. Serological evidence of BEFV has also been reported in a variety of species, including cattle [1,13,30,48,49], water buffalo, gazelle, Persian fallow deer [4], and yak [34]. In addition, Cybinski *et al*. showed that deer in Australia had antibodies specific to BEFV [7].

Although Hall *et al*. [18] demonstrated that BEF could be reproduced in sheep infected with BEFV, to our knowledge, this is the first national-scale serological study of BEFV in sheep and goats, in the world. BEFV seroprevalence was estimated to be 14.3%, demonstrating that exposure to BEFV is prevalent among sheep and goats in South Korea despite the paucity of reported BEFV outbreaks, probably due to the often unapparent clinical signs and under-ascertainment of BEFV. In South Korea, epizootics also occur periodically, and BEFV seroconversions were detected during 2009-2012 in sentinel cattle from all provinces except the heavily populated Incheon and Busan [26]. Our estimate of seroprevalence for sheep and goats was higher than that found in Korean sentinel cattle in 2009 (2.5%), 2010 (1.7%), 2011 (6.4%), and 2012 (6.4%) [26] and similar to that found among Korean pigs in 2007 (15.7%) [46].

This study represents the first assessment of factors associated with BEFV seropositivity in sheep and goats among small ruminants in South Korea. The serological prevalence of BEFV infection in sheep and goats was significantly different between age cohorts, vector control, and geographical locations. BEFV prevalence in sheep and goats in this study was higher in the older group, and this result may represent continuous episodes of transmission. In addition, the prevalence of BEFV was also higher in the southern and western regions of South Korea. Our results also demonstrated that the prevalence of BEFV antibodies varied widely in South Korea. The reasons for the region-specific differences in BEFV transmission are unclear. One possibility is that there may be ecological and climatic factors that promote increased exposure to infected reservoirs in tropical rainforest regions. Alternatively, viral persistence or transmissibility may be greater in regions with elevated temperatures and rainfall.

CONCLUSION

Determination of seropositivity rates often leads to an understanding of virus circulation dynamics and is useful in the formulation of disease control measures. Our results demonstrated that vector control was a significant protective factor, and therefore, the summer control of vectors could be better implemented in provinces with elevated seropositivity rates. The results of this seroprevalence study may serve as a basis for future epidemiological studies on BEFV infection in South Korea.

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