



## Short communication

## Evidence of bluetongue virus circulation in farmed and free-ranging cervids from the Republic of Korea: A retrospective cross-sectional study

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## ABSTRACT

Bluetongue, which is caused by bluetongue virus (BTV), is a vector-borne viral disease that affects wild and domestic ruminants. Trade restrictions can have a devastating impact in areas where BTV is endemic, regardless of the incidence of clinical disease. Currently, little is known about the prevalence of BTV infection in the Republic of Korea (ROK), and limited data on the BTV situation in the ROK are available. In this study, an epidemiological survey of BTV infection in farmed and free-ranging cervids from the ROK was conducted by a countrywide retrospective cross-sectional study. In total, BTV infection was widespread in the ROK, as 74 of 790 (9.4%, 95% confidence interval = 7.5–11.6%) cervid sera samples collected from 318 herds contained antibodies to BTV. Additionally, 42 herds evaluated in this study contained BTV seropositive cervids (13.2%). Serological evidence of bluetongue virus infection was observed in 17 of 158 free-ranging cervid animals, which accounts for the prevalence rate of approximately 10.8% (17/158; 95% CI = 6.8–16.6). Neutralizing antibodies to BTV-1, -2, -4, -7, and -15 serotypes were identified and RNAs of the BTV-1, -7, and -15 serotypes were detected, indicating that BTV was circulating in the cervids in ROK. These results suggest that cervids were actively exposed to BTV in the ROK and these species might serve as an important reservoir for the transmission of BTV. This is the first report on the evidence of circulating antibodies against BTV and serotype distribution in cervids in the ROK.

## 1. Introduction

Bluetongue virus (BTV) is the type species of genus *Orbivirus* within family *Reoviridae*. Bluetongue, which is caused by BTV and is transmitted by *Culicoides* spp. midges, is one of the most economically important transboundary animal diseases. The indirect losses associated with decreased body weight and condition, a drop in milk production, and poor subsequent reproductive performance are thought to have a greater economic effect than the occasional overt cases of disease (Maclachlan et al., 2015; MacLachlan and Osburn, 2006). Bluetongue is classified by the Office International des Epizooties (OIE) as a notifiable disease (former List A) because BTV has the potential for serious and rapid spread irrespective of national borders and is of major importance to the international trade of livestock and livestock products. Trade restrictions can have a devastating impact in areas where BTV is endemic, regardless of the incidence of clinical disease. BTV-affected countries or provinces are banned from trading livestock and livestock

products, and the banning of trade for any country may result in severe economic repercussions. Currently, little is known about the prevalence of BTV infection in the Republic of Korea (ROK). No BTV serotype has been detected in cervids in ROK yet and no information is available regarding the potential risk factors associated with cervids and BTV infection in the ROK. Especially in the ROK, a number of elks and deer have been raised as farmed animals because deer antlers have been sold at a high price as a traditional medicine. Therefore, the present study was conducted with the objective of determining the current distribution and seroprevalence of BTV infection of cervids in the ROK. This study was designed in light of previous studies of BTV surveillance that suggested that cervids (animals in the family *Cervidae*), ruminant artiodactyl mammals that have solid deciduous antlers borne only by males, can act as reservoirs for the virus, enabling transmission to highly susceptible sheep, and that cervids may play an important role in the epidemiology of BTV (Linden et al., 2010, 2008; Rodriguez-Sanchez et al., 2010).

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## 2. Methods

### 2.1. Samples

The target population of this study was farmed and free-ranging cervids and animals involved in this study were asymptomatic. Seroprevalence rates were estimated at the herd and animal levels in the ROK (33°06′ N - 39°25′ N, 124°36′ E - 131°52′ W). Serum samples from cervids were obtained from two separate sources: 1) from the serum bank of the National foot-and-mouth disease (FMD) Surveillance Program maintained by the Foreign Animal Diseases Division of the Animal, Plant and Fisheries Quarantine and Inspection Agency (Anyang, the ROK) and 2) from free-ranging cervid animals rescued by local rescue parties or captured wildlife rescue centers during a 2011 FMD epidemic in the ROK (Park et al., 2013). A total of 790 cervid blood samples were collected from 318 herds all over the South Korean territory for the purpose of active surveillance of foreign animal diseases including FMD in 2011 in the ROK. The samples from serum bank were collected from farmed cervids and the serum samples used in this study were initially tested for FMD according to the monitoring measures issued and conducted by the Ministry of Agriculture, Food and Rural Affairs in 2011. Only serum samples confirmed to be negative for FMD virus were analyzed for bluetongue-specific antibodies in this study. An additional 158 cervid blood samples from animals (Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*)) rescued by local rescue parties or from captured wildlife rescue centers were available for assay. The procedure to collect blood from the cervids was performed by qualified veterinarians following proper physical restraint of animals to ensure both personnel and animal safety. Blood samples from the caudal vein were collected using vacutainers and tubes containing the anticoagulant heparin sodium by local veterinary officers and/or local veterinary practitioners in 10 provinces of the ROK. Serum was separated from clotted blood by 10 min centrifugation at 2000 × g at 4 °C and stored at -20 °C until analysis. Sera were heat-inactivated at 60 °C for 20 min, followed by centrifugation. The blood and serum from the blood samples were stored at -20 °C until further analysis.

### 2.2. Enzyme-linked immunosorbent assay (ELISA)

It has previously been reported that specific serodiagnostic techniques, such as the competitive enzyme-linked immunosorbent assay (cELISA) or BTV neutralization tests, should be used for bluetongue surveillance in Ibaraki virus (IBV)-endemic areas because IBV-positive serum samples may result in false-positive bluetongue agar gel immunodiffusion (AGID) test reactions (Shimizu et al., 2004). Because the ROK is an IBV-endemic region, two commercially available commercial cELISA kits based on a VP7-specific monoclonal antibody, namely, the BTV Antibody Test Kit (VMRD cELISA; Veterinary Medical Research and Development Inc., Pullman, WA, USA) and the BTV VP7 Antibody Test Kit (IDEXX cELISA; IDEXX Laboratories, Inc., Institute Pourquier, Montpellier, France), were used to identify the presence of antibodies against BTV in the serum samples. The assays were performed according to the manufacturer's instructions. The specificity and sensitivity of the VMRD cELISA kit are 99.3% and 69.5%, respectively, as stated by the manufacturer, and those of the IDEXX cELISA kit are 100% and 82.8%, respectively (Niedbalski, 2011). To maximize the test specificity of this study, all samples were tested using both cELISA kits, and a serum sample was defined as "positive" only when it yielded positive results from both tests. A herd was defined as "positive" when at least one seropositive sample was present. The seroprevalence rates and the 95% confidence intervals were calculated using the program 'Survey Toolbox for Livestock Diseases' (Ausvet, Australia).

### 2.3. Serum neutralization test (SNT)

The serotype-specific SNT was used to confirm the positive ELISA results. Positive and negative controls for the SNT were obtained from the Institute for Animal Health, Pirbright, United Kingdom. The microtiter neutralization method was used in this study according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE-World Organisation for Animal Health, 2008). Briefly, approximately 100 TCID<sub>50</sub> (50% tissue culture infective dose) of the standard or untyped virus was added in 50 µl volumes to 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. Approximately 10<sup>4</sup> cells were added per well in a volume of 100 µl and assessed after incubation for 4–6 days using an inverted microscope. Wells were scored for the degree of cytopathic effects observed.

### 2.4. Risk factors analysis

Signs typical of bluetongue disease were not described in any of these animals. The risk factor information was obtained from the animal owners through a questionnaire form, which permitted use of the blood samples for diagnostic and research purposes under the national active surveillance programs for foreign animal diseases including FMD in 2011. We also investigated associations between the seroprevalence and local seropositivity risk factors (herd population size, whether the herd was mixed with other animal species (e.g., sheep, goats, or cattle), and availability of a vector habitat in the environment [whether the herd was located in a rural area or near an urban area]). Statistical significance was set at the 5% level, and two-sided *p*-values were calculated for the correlation analysis. The statistical analysis was performed via the paired *t*-test, and a *p*-value < 0.05 was accepted as significant. All data were analyzed using GraphPad PRISM software (version 6.07 for Windows; GraphPad Software Inc.).

## 3. Results

The number of samples from each province is shown in Table 1. Herd sizes and species of farmed animals were classified into three groups based on the questionnaire, as shown in Table 2. The number of samples from free-ranging cervids rescued by local rescue parties or from captured wildlife rescue centers have been presented in Table 2. Overall, BTV infection was widespread in the province, as 74 of 790 (9.4%, 95% confidence interval [95% CI] = 7.5–11.6) farmed cervid sera samples collected from 318 herds contained antibodies to BTV as determined by competitive ELISA. Additionally, 42 herds evaluated in this study included BTV-seropositive cervids (13.2%, 95% CI = 1.0–17.4) as shown in Table 1. Seropositive animals occurred in most districts surveyed except the Gangwon and Chungbuk Provinces. The highest prevalence values for bluetongue were observed in the Jeonbuk Province, with 22.2% (95% CI = 10.6–40.8) of herds affected (6/27), and the Jeonnam Province, with 35.7% (95% CI = 24.5–48.8) of heads affected (20/56).

Among farmed animals evaluated in this study, 16 serum samples positive by ELISA could not be analyzed by SNT due to cytotoxicity, and 9 samples were positive by ELISA but negative by SNT. Therefore, a total of 49 out of 790 farmed cervids analyzed showed antibodies against BTV by both ELISA and SNT. 10 serum samples showed cross-neutralizing activity to two serotypes. In total, neutralizing antibodies to BTV-1, -2, -4, -7, and -15 serotypes were identified (Table 1) and seroprevalences to BTV serotypes were 3.9% to BTV-1 (31/790; 95% CI = 2.8–5.5), 1.1% to BTV-2 (9/790; 95% CI = 0.6–2.2), 1.4% to BTV-4 (11/790; 95% CI = 0.8–2.5), 0.5% to BTV-7 (4/790; 95% CI = 0.2–1.3), and 0.5% to BTV-15 (4/790; 95% CI = 0.2–1.3). RNAs of the BTV-1, -7, and -15 serotypes were detected in three blood samples. None of the 74 seropositive samples was positive by BTV RT-PCR. In contrast, BTV RNA was detected in 3 out of 716 samples that tested

**Table 1**  
Seroprevalence of bluetongue virus infection among cervids in the Republic of Korea (2011).

Province	Latitude (N)	Longitude (E)	Herds				Heads				Serotype	
			Positive <sup>a</sup>	Tested	AP <sup>b</sup>	TP ± 95% CI <sup>c</sup>	Positive	Tested	AP	TP ± 95% CI <sup>c</sup>	SNT <sup>d</sup>	RNA (number) <sup>e</sup>
Incheon	36°55'-37°58'	124°36'-126°47'	3	17	17.6	6.2-41.0	3	18	16.7	5.8-39.2	1, 4	ND <sup>f</sup>
Gyeonggi	36°53'-38°17'	126°22'-127°51'	11	74	14.9	8.5-24.7	16	221	7.2	4.5-14.4	1, 2, 4, 7, 15	1(1)
Gangwon	38°09'-39°25'	126°46'-128°22'	0	22	0	0-14.9	0	89	0	0-4.1	–	ND
Chungbuk	37°15'-36°00'	127°16'-128°38'	0	28	0	0-12.1	0	52	0	0-6.9	–	ND
Chungnam	35°58'-37°03'	125°32'-127°38'	5	46	10.9	4.7-23.0	9	169	5.3	2.8-9.8	2, 4, 7, 15	15(1)
Jeonbuk	35°18'-36°09'	125°58'-127°54'	6	27	22.2	10.6-40.8	9	76	11.8	6.4-21.0	1, 2, 4, 15	ND
Jeonnam	33°54'-35°30'	125°04'-127°54'	7	43	16.3	8.1-30.0	20	56	35.7	24.5-48.8	1, 2, 4, 7, 15	ND
Gyeongbuk	35°34'-37°33'	127°48'-131°52'	4	29	13.8	0.6-30.6	6	44	13.6	6.4-26.7	4, 7, 15	7(1)
Gyeongnam	34°39'-35°54'	127°35'-129°28'	3	14	21.4	7.6-47.6	4	45	8.9	3.5-20.7	1, 2	ND
Jeju	33°06'-34°00'	126°08'-126°58'	3	18	16.7	5.8-39.2	7	20	35.0	18.1-56.7	2, 7, 15	ND
Total	33°06'-39°25'	124°36'-131°52'	42	318	13.2	1.0-17.4	74	790	9.4	7.5-11.6		

Free-ranging animals, such as Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*) were excluded in Table 1 because regionalities of the animals could not be determined in many cases.

- <sup>a</sup> Number of seropositive herds or animals (heads).
- <sup>b</sup> AP, apparent (estimated) prevalence.
- <sup>c</sup> TP ± 95% CI, 95% confidence interval for the true proportion.
- <sup>d</sup> Serotypes identified by serum neutralization tests.
- <sup>e</sup> BTV serotype identified by RT-PCR for blood sample.
- <sup>f</sup> ND, not detected.

negative in the ELISA screening and these three samples were from two adult elks and one sika deer. Virus isolation from these three samples was not successful.

Serological evidence of bluetongue virus infection was observed in 17 of 158 free-ranging cervid animals that were rescued by local rescue parties or from captured wildlife rescue centers, which accounts for the prevalence rate of approximately 10.8% (17/158; 95% CI = 6.8–16.6) (Table 2) as determined by competitive ELISA. Free-ranging animals, such as Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*) were excluded in Table 1 because regionalities of the animals could not be determined in many cases.

Samples collected from free-ranging cervid animals could not be used for SNT and RT-PCR due to an insufficient volume and quality.

Cervids from larger (> 51) and smaller (< 10) sized herds had 3.0 and 2.6 times the odds of being infected with BTV, respectively, compared with cervid animals from medium (10–50) sized herds, as shown in Table 2. Herds from the southern area of the ROK were 3.7 times more likely to be infected with BTV (19.1%, 95% CI = 14.6–24.5) compared with those from the northern area. In addition, herds from the western area of the ROK were 2.0 times more likely to be infected with BTV (11.4%, 95% CI = 9.1–14.3) compared with those from the eastern area. When assessing herd size and area as risk factors, there

**Table 2**  
Seroprevalence of bluetongue virus among cervids by herd size, species, and study area in the Republic of Korea (ROK) (2011).

Characteristic	Herds				Heads			
	Positive <sup>a</sup>	Tested	AP <sup>b</sup>	TP ± 95% CI <sup>c</sup>	Positive <sup>a</sup>	Tested	AP <sup>b</sup>	TP ± 95% CI <sup>c</sup>
<b>Herd size<sup>d</sup></b>								
> 51	3	14	21.4	7.6 - 47.6	20	142	14.1	9.3 - 20.8
10-50	11	79	13.9	8.0 - 23.2	16	339	4.7	2.9 - 7.5
< 10	28	225	12.4	8.8 - 17.4	38	309	12.3	9.1 - 16.4
<b>Species<sup>e</sup></b>								
Elk ( <i>Cervus canadensis</i> )	29	127	22.8	16.4 - 30.9	59	415	14.2	11.2 - 17.9
Sika deer ( <i>Cervus nippon</i> )	6	87	6.9	3.2 - 14.2	7	199	3.5	1.7 - 7.1
Red deer ( <i>Cervus elaphus</i> )	7	104	6.7	3.3 - 13.2	8	176	4.5	2.3 - 8.7
Siberian roe deer ( <i>Capreolus pygargus</i> )	–	–	–	–	8	72	11.1	5.7-20.4
Korean water deer ( <i>Hydropotes inermis argyropus</i> )	–	–	–	–	9	86	10.5	5.6 - 18.7
<b>Area<sup>f</sup></b>								
Northern	20	187	10.7	7.0 - 15.9	28	549	5.1	3.6 - 7.3
Southern	22	131	16.8	11.4 - 24.1	46	241	19.1	14.6 - 24.5
Eastern	7	65	10.8	5.3 - 20.6	10	178	5.6	3.1 - 10.0
Western	35	225	15.6	11.4 - 20.9	64	560	11.4	9.1 - 14.3

- <sup>a</sup> Number of seropositive herds or animals (heads).
- <sup>b</sup> AP, apparent (estimated) prevalence.
- <sup>c</sup> TP ± 95% CI, 95% confidence interval for the true proportion.
- <sup>d</sup> Free-ranging animals, such as Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*), were excluded.
- <sup>e</sup> Some blood samples in this study were collected from animals rescued by local rescue parties or found in captured wildlife rescue centers because of road traffic accidents or because their presence in the urban area necessitated their removal. The numbers of these free-ranging animals, such as Korean water deer (*Hydropotes inermis argyropus*) and Siberian roe deer (*Capreolus pygargus*), are included.
- <sup>f</sup> In this study, the northern area of the ROK includes Incheon, Gyeonggi, Gangwon, Chungbuk, and Chungnam Provinces on the basis of latitude 36° north, whereas the southern area of the ROK includes Ulsan, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju. The western area of the ROK includes Incheon, Gyeonggi, Chungnam, Jeonbuk, Jeonnam, and Jeju on the basis of longitude 127° east, whereas the eastern area of the ROK includes Ulsan, Gangwon, Gyeongbuk, and Gyeongnam. Chungbuk is located in central area in the Republic of Korea and excluded in both eastern and western areas.

was a significant association between the BTV infection rate and the herd size ( $p < 0.05$ ) as well as the areas where the animals were distributed ( $p < 0.05$ ). In our study, seroprevalence was higher in elks (*Cervus canadensis*) than other cervid species (Table 2). Attempts to establish a correlation between seropositive rate and whether the herd was mixed with other animal species (e.g., sheep, goats, or cattle) or the availability of a vector habitat in the environment (whether the herd was located in a rural area or near an urban area) did not yield statistically significant results (data not shown).

#### 4. Discussion

BTV has worldwide distribution and can cause large economic losses and significant effects on the international trade of livestock and livestock products. Although the authors previously reported the first identification of BTV serotype 1 in the ROK (Seo et al., 2015) and the seroprevalence of BTV antibodies in the domestic goat population (Yeh et al., 2018), no published report exists regarding BTV epidemiology in cervids from the ROK. To advance beyond the current knowledge regarding the epidemiology of this disease, we conducted this study to determine the prevalence of BTV infection in cervids and the associated risk factors among cervids in the ROK. We report the results of the first survey for antibodies against BTV, which was conducted retrospectively in the ROK using cervid sera samples in the year 2011. This is the first report on the evidence of circulating antibodies against BTV and serotype distribution in cervids in the ROK.

Our study provides evidence for the serological prevalence of circulating antibodies against BTV and the presence of BTV antibodies in approximately one out of every ten cervid animals included in the survey. The results of this study also demonstrated evidence for RNA of the BTV-1, -7, and -15 in the cervids, indicating that BTV was circulating in ROK. To understand the results of the present study, it is important to know that the ROK has no vaccination program for bluetongue. Thus, the seroprevalence of BTV infection in the ROK can be assumed to reflect natural infection of the cervids evaluated and the results indicate that cervids seem to be implicated in the dissemination and persistence of BTV in the ROK. Our data support the usefulness of this species as indicators of the activity of BTV (Linden et al., 2010, 2008; Rodriguez-Sanchez et al., 2010).

In our study, three samples determined as seronegative were positive for BTV RNA by RT-PCR and this could be related to a recent infection before seroconversion. BTV may be occasionally found in seronegative animals, as it has been reported in red deer from Belgium (Linden et al., 2010) although viremia can be detected up to a couple of months after infection in domestic and wild species, usually in seropositive animals in the case of BTV infection (Lopez-Olvera et al., 2010). Further studies might include serological and virological investigations of BTV-2, and -4 circulating in the ROK. Although neutralizing antibodies against BTV-2, and -4 have been detected in cervid serum samples used in this study, none of the blood samples in the present study showed virological evidence of BTV-2, and -4. Although over the past few years, three novel “atypical” serotypes (BTV-25, -26, and -27) were characterized by sequencing studies (Belbis et al., 2017; Savini et al., 2017; Schulz et al., 2016; Zientara et al., 2014), this study investigated more established BTV serotypes 1 to 24 as a first step in BTV epidemiological research in ROK. Future surveillance programs for BTV should be extended to include the novel “atypical” serotypes as well as other susceptible animals, such as sheep, goats and cattle. An improved serosurveillance system for BTV that is suitable for endemic areas will be of great help in understanding the epidemiology of BTV and formulating effective control and prevention strategies in the ROK. The distribution of *Culicoides* vectors in the ROK should also be considered in future attempts to better predict and respond to a possible BTV outbreak in the ROK. Because BTV infection is normally spread through the bites of *Culicoides* species midges, but the particular vectors responsible for transmission of the disease in the ROK have not yet been

identified. Entomological surveillance for biting *Culicoides* midges have been consistently conducted in ROK since 2010 (Bellis et al., 2013; Kim et al., 2012, 2014; Kim et al., 2015; Oem et al., 2013) and epidemiological analysis is being assessed based on vector species abundance, diversity, and competence of *Culicoides* midges distributed in ROK.

#### 5. Conclusion

Collectively, the present study demonstrated that BTV infection was prevalent among the farmed and free-ranging cervids analyzed, in which approximately one in ten animals were infected. The results of this study indicated that BTV exists in the ROK and that susceptible cervids are at risk of becoming infected with BTV, although an outbreak of BTV among native cervids or other ruminants in the ROK has yet to be reported. These results suggest that cervid species might serve as an important reservoir for the transmission of BTV, highlighting the need for closer monitoring of BTV infections in cervids in the ROK.

#### Conflict of interest statement

The authors declare no conflict of interest. None of the authors of this paper had a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the institutions with which the authors are affiliated.

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