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RESEARCH ARTICLE

Factors associated with seropositivity to Aino virus among sheep and goats in South Korea

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ABSTRACT

Aino virus infection is responsible for epizootic and/or sporadic outbreaks of abortions, stillbirths and premature delivery among pregnant ruminants. The epizootiology of Aino virus infection is poorly defined in South Korea, therefore our aim was to assess its seroprevalence among sheep and goats. We also wanted to evaluate management and regional risk factors that might influence the frequency of infection. Between 2012 and 2013, 26 of 331 flocks (7.9%) and 139 of 915 heads (15.2%) were found serologically positive. In 2018, when samples were again collected in the same regions, 35 of 308 flocks (11.4%) and 89 of 735 heads (12.1%) showed serum-neutralising antibodies against Aino virus. Our results revealed that the age class and history of reproductive problems in the flocks are connected to an increased risk of being positive. The management risk factor attributes showed that preventive measures, such as the routine application of insecticide in farms, decreased the odds for seropositivity to Aino virus (OR = 0.453, $P = 0.001$). We observed a significant difference in the individual likelihood of being positive in the southern and western provinces with respect to that in the northern and eastern provinces, respectively (OR = 2.199, $P < 0.001$ and OR = 2.177, $P < 0.001$). The results of this study may serve as a basis for future epizootic studies on Aino virus infection in South Korea.

KEYWORDS

Aino virus, goat, seroprevalence, sheep, South Korea

INTRODUCTION

Aino virus is an arthropod-borne virus belonging to the Simbu group of the genus *Orthobunyavirus* within the family *Bunyaviridae*. Orthobunyaviruses are widely distributed in the temperate and tropical areas of the world, and some of them induce subclinical or clinical illnesses in animals (Porterfield and Della-Porta, 1981; Parsonson and McPhee, 1985). Aino virus infection is responsible for epizootic and/or sporadic outbreaks of abortions, stillbirths, and premature delivery among pregnant ruminants (Coverdale et al., 1978; Yoshida et al., 2000). Aino virus has been considered a probable cause of congenital malformation characterised by arthrogryposis, hydranencephaly and cerebellar hypoplasia in cattle, sheep and goats (Coverdale et al., 1978; Brenner et al., 2004). It is transmitted by haematophagous arthropod vectors, such as *Culicoides* biting midges and mosquitoes, and is widely distributed in the temperate-to-tropical regions, including Asia and Australia (Doherty, 1972; Coverdale et al., 1978; Miura et al., 1982; Yoshida et al., 2000; Jun et al., 2018; Wang et al., 2019).

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By virus neutralisation assays, 11% of the thoracic fluids from aborted calves have been found positive for Aino virus in South Korea (Lim et al., 2007). It has also been reported that the seroprevalence of Aino virus in cattle and native goats (*Capra hircus*) were 4.5% and 13.3%, respectively (Yang et al., 2008a; Jun et al., 2018). In another study, the screening of sentinel calves has revealed the seropositivity to Aino virus to range from 14.1% to 33.2% (Kim et al., 2015). Although it has been accepted that Aino virus occurs enzootically in South Korea, many aspects are still poorly defined and remain unclear.

Because of the scarcity of epizootic information on Aino virus, we designed a study to evaluate the seroprevalence in the ovine and caprine species in South Korea. Blood samples, collected at different times at the same geographical regions, were tested and the seroprevalences from the different time periods were compared. In addition, a retrospective serosurvey to determine the presence of anti-Aino virus antibodies in archived sheep and goat sera (2003–2008) was also conducted to improve our understanding. The objectives of the present study also included the analyses of the association of individual, management and regional risk factors with the virus infection in sheep and goats.

MATERIALS AND METHODS

Sampling design and samples for disease monitoring

The necessary sample size to estimate prevalence was calculated using the Epitools-Epidemiological Calculators (Sergeant, 2018) based on the methods described previously (Humphry et al., 2004). According to the calculations, 861 animals were required to analyse the nationwide prevalence of antibody to Aino virus based on 5% desired precision, 95% confidence and 30% expected prevalence. The expected prevalence was determined according to the bovine serological data reported in South Korea previously (Kim et al., 2015; Jun et al., 2018). The flocks and the animals within the flocks were selected by a simple random sampling method in each province based on the government's national statistics (Livestock Management Division, 2012). In South Korea, a trivalent vaccine against Aino virus, Akabane virus and Chuzan virus was developed in 2011 (Kim et al., 2011). However, this trivalent vaccine has received little use in the field. Therefore, it is difficult to distinguish between vaccinated and unvaccinated Korean animals. 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 of age were also excluded from the sampling to avoid the detection of antibodies due to maternal immunity (Iwamoto and Furukawa, 2020).

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 also 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, separated from the blood samples was stored at −20 °C until further analysis. The seroprevalence rates were estimated at the flock and the individual animal levels among sheep and goats. For the retrospective study, serum samples collected from sheep and goats between 2003 and 2008 were analysed for the presence of Aino virus-specific antibodies. Thus, the seroprevalence values from different time periods, namely from 2003–2008, 2012–2013 and 2018 from the same regions could be compared.

Table 1. Comparison of seroprevalences of Aino virus infection in sheep and goats in South Korea between 2012–2013 and 2018

Province	2012–2013						2018					
	Flock-level			Animal-level			Flock-level			Animal-level		
	Pos*	Tested	%	Pos*	Tested	%	Pos*	Tested	%	Pos*	Tested	%
Chungbuk	2	24	8.3	5	69	7.2	4	26	15.4	5	60	8.3
Chungnam	4	63	6.3	29	218	13.3	7	58	12.1	8	83	9.6
Gangwon	1	27	3.7	2	64	3.1	3	26	11.5	5	56	8.9
Gyeongbuk	2	30	6.7	7	65	10.8	3	29	10.3	4	62	6.5
Gyeonggi	4	73	5.5	26	213	12.2	7	67	10.4	9	125	7.2
Gyeongnam	2	31	6.5	9	66	13.6	2	24	8.3	6	65	9.2
Incheon	1	7	14.3	2	19	10.5	1	6	16.7	5	63	7.9
Jeju	3	13	23.1	11	29	37.9	3	9	33.3	6	24	25.0
Jeonbuk	3	23	13.0	20	71	28.2	1	24	4.2	14	67	20.9
Jeonnam	3	31	9.7	25	80	31.3	2	31	6.5	25	108	23.1
Ulsan	1	9	11.1	3	21	14.3	2	8	25.0	2	22	9.1
Total	26	331	7.9	139	915	15.2	35	308	11.4	89	735	12.1

*Number of seropositive flocks or animals (heads); Pos = positive

Serologic testing

The Aino virus strain KSA9910 (VR64, Korea Veterinary Culture Collection, Anyang, South Korea) was used for serum neutralisation tests (SNTs). Vero cells [CCL-81, American Type Culture Collection (ATCC), Manassas, VA, USA] were maintained in alpha-minimum essential medium (Gibco, Grand Island, NY, USA) containing 5% fetal bovine serum and antimycotic-antibiotics (Gibco). The SNT against Aino virus was performed in flat-bottomed 96-well plates. Briefly, approximately 100 $TCID_{50}$ (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 microtitre plate and was mixed with an equal volume of standard antiserum that had been serially diluted in tissue culture medium (Lim et al., 2005, 2007; Kim et al., 2011). Approximately 10^4 Vero cells were added per well in a volume of 100 µL and were assessed after incubation for 3–5 days using an inverted microscope (Olympus, Tokyo, Japan). The wells were scored based on the observation of virus-specific cytopathic effect (CPE). Antibody titres were expressed as the reciprocal of the highest serum dilution at which the CPE was inhibited. A titre 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 the sheep or goat farm ID and flock size obtained from the Korea Animal Health Integrated System (KAHIS, Animal and Plant Quarantine Agency, Anyang, South Korea). A pretested structured questionnaire with the primary objective of elucidating the multifactorial background of the disease was conducted in an interactive manner at all selected flocks. All animals included in this study were subjected to the questionnaire, which was completed by the animal owners. The questionnaire included individual risk factors, e.g., animal species (goat or sheep). The animals were also classified into three age groups based on tooth replacement and the 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 sizes of the flocks; presence of other animal species in the farm (the presence of other ruminant animals, such as cattle, in the farm); history of reproductive problems, including abortion; and vector control (use or non-use of insecticide) – were also investigated. Regional risk factors – e.g., the presence of neighbouring ruminant farms, lakes or rice paddies; land use (agricultural, woodland and semi-natural and urban areas, according to KAHIS); and geographic factors (localisation of the farm in South Korea) – were also studied. Considering that the *Culicoides* flying range is most likely <1 km (Sanders et al., 2017), the radius size of the regional risk factor in this study was chosen inside a 1-km buffer around the sampled farm. Questionnaires for additional information on the farm or animal were followed up via telephone interview.

Statistical analysis

The prevalence and Wilson's 95% confidence interval (CI) (Reiczigel et al., 2010) were calculated using the Epitools-Epidemiological Calculators (Sergeant, 2018). The intraclass correlation coefficient µ was calculated to measure the serologic status agreement between animals sampled within the same flock. The intraclass correlation coefficient (minimum 0, maximum 1) was estimated by using analysis of variance, with the flock as the independent variable and the serologic status of individual animals (seropositive or seronegative) as the dependent variable (Donald and Donner, 1987).

In this study, the following individual exposure variables were considered for the univariable analyses: animal species, age class, population size of the flock, flock structure, history of reproductive problems, vector control, presence of neighbouring ruminant farms, lakes or rice paddies, land use, geographic factor (localisation of the farm in South Korea). A logistic regression model was used to check the association of the animal's serologic status outcome with the potential risk factors. The effect of the exposure variables on individual seropositivity was 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 the chi-squared test for continuous or categorical variables, respectively. The strength of association was calculated using odds ratios at 95% CIs. A P value <0.05 was considered statistically significant. All statistical analyses were performed using the statistical software SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA).

RESULTS

The overall prevalences were not found significantly different in the periods investigated in this study (Fig. 1). No considerable changes were observed in the prevalence values of the regions among the periods. At the national level in 2018, 35 of 308 flocks (11.4%, 95% CI: 8.3–15.4%) and 89 of 735 heads (12.1%, 95% CI: 10.0–14.7%) were found to have serum neutralising antibodies against Aino virus. Between 2012 and 2013, 26 of 331 flocks (7.9%, 95% CI: 5.4–11.3%) and 139 of 915 heads (15.2%, 95% CI: 13.0–17.7%) were serologically positive, as shown in Table 1. The agreement in the serologic status among animals, sampled within the same herd in our prevalence study, as measured by the intraclass correlation coefficient, was high (0.68). This finding indicates that in any particular flock, a tendency exists that either most animals in that flock will be seropositive or most will be seronegative.

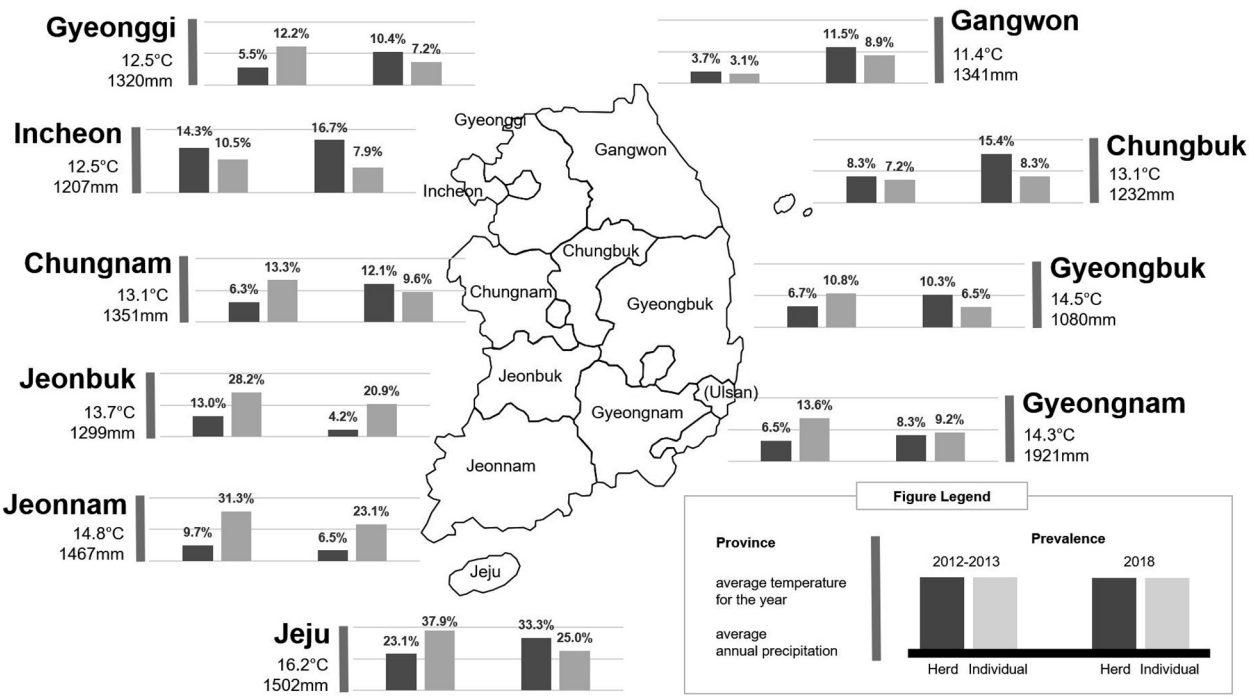

Fig. 1. A map of the Republic of South Korea showing the provinces and geographical regions with the relevant climatic conditions such as the average temperature for the year and average annual precipitation. The changes in seroprevalence of Aino virus infection are also presented. Tables 1 and 3 provide detailed information regarding each province

Our results revealed that the age class and history of reproductive problems in the flocks are associated with an increased risk of testing positive. In the univariable analysis (Table 2), older age was shown to be a significant risk factor (OR = 2.748, 95% CI = 1.617–4.668, $P < 0.001$ in adults), and the occurrence of reproductive problems in the farm was also linked to a higher risk of being positive (OR = 1.903, 95% CI = 1.257–2.881, $P = 0.002$).

The management risk factor attributes showed that preventive measures, such as routine application of insecticide in the farms, decreased the odds for seropositivity for the Aino virus (OR = 0.453, 95% CI = 0.277–0.739, $P = 0.001$). Vector control was a significant protective factor, while animal species, flock size, and flock structure were not significantly associated with protection. No significant risk differences based on the presence of other ruminant farms, lakes or rice paddies within a 1-km radius, or the type of land use were found. On the other hand, there were substantial regional differences in the seroprevalence within South Korea. We observed a significant difference in the individual likelihood of being positive in the southern and western provinces compared to the northern and eastern provinces (OR = 2.199, 95% CI = 1.461–3.308, $P < 0.001$ and OR = 2.177, 95% CI = 1.386–3.420, $P < 0.001$, respectively).

The results of the retrospective study showed that the virus had been widely distributed in sheep and goats in South Korea. The rates of seropositivity fluctuated from 5.7% to 19.2% during the time between 2003 and 2008 (Table 3). The highest rate (19.2%) was demonstrated in 2004 while the lowest one (5.7%) in 2008.

DISCUSSION

The current study represents the first assessment of factors associated with Aino virus seropositivity in sheep and goats among the small ruminants of South Korea. Aino virus seroprevalence was estimated to be 15.2%, demonstrating that exposure to Aino virus is prevalent among sheep and goats in South Korea despite the paucity of reported Aino virus outbreaks, probably due to the often unapparent clinical signs and under-ascertainment of Aino virus when abortions and other reproductive problems occur in cattle or small ruminant farms (Kim et al., 2015). In this study, meteorological factors such as average temperature for the year and average annual precipitation in South Korea did not tend to be factors positively associated with seroconversion to Aino virus (Fig. 1).

This is the first report on evidence of circulating antibodies against Aino virus among sheep in South Korea. Our estimate of the seroprevalence for sheep and goats was similar to that found among Korean native goats in 2008 (13.3%) (Yang et al., 2008a), higher than that found in Korean cattle (11.4%) in 2007 (Lim et al., 2007), and lower than that found in Thoroughbred horses in Korea in 2008 (19.5%) (Yang et al., 2008b). The data in this study were also similar to those demonstrated in other countries where

Table 2. Univariable analysis of the Aino virus exposure variables relative to the seropositivity outcome in sheep and goats

Variable	Positive (n = 139)	Negative (n = 776)	OR (95% CI)	P value	OR (95% CI)	P value
Animal species						
Goat	76	417	–			
Sheep	63	359	0.963 (0.670–1.384)	0.838		
Age class						
Juvenile	19	198	–		0.364 (0.214–0.618)	<0.001
Subadult	38	267	1.483 (0.830–2.650)	0.181	0.540 (0.355–0.820)	0.004
Adult	82	311	2.748 (1.617–4.668)	<0.001	–	
Population sizes of the flocks						
<6 heads	35	239	–		0.777 (0.492–1.228)	0.280
6–10 heads	49	245	1.366 (0.854–2.183)	0.192	1.062 (0.697–1.617)	0.780
>10 heads	55	292	1.286 (0.814–2.031)	0.280	–	
Flock structure						
Goat (and/or) sheep alone	78	461	–			
With other ruminants	61	315	1.145 (0.795–1.648)	0.468		
Reproductive problems						
No	100	644	–			
Yes	39	132	1.903 (1.257–2.881)	0.002		
Vector control						
No	118	557	–			
Yes	21	219	0.453 (0.277–0.739)	0.001		
Presence of ruminant farms within a 1-km radius						
No	65	423	–			
Yes	74	353	1.364 (0.950–1.959)	0.092		
Presence of lakes or rice paddies within a 1-km radius						
No	77	470	–			
Yes	62	306	1.237 (0.859–1.780)	0.252		
Land use						
Urban	28	170	–		0.958 (0.589–1.557)	0.861
Agricultural	59	343	1.044 (0.642–1.698)	0.861	–	
Woodland and semi-natural	52	263	1.200 (0.729–1.976)	0.472	1.149 (0.766–1.725)	0.501
Localisation of the farm in South Korea						
Northern	35	330	–			
Southern	104	446	2.199 (1.461–3.308)	<0.001		
Localisation of the farm in South Korea						
Eastern	26	259	–			
Western	113	517	2.177 (1.386–3.420)	<0.001		

Table 3. Retrospective study on the sheep and goat sera, sampled between 2003 and 2008, for anti-Aino virus antibodies

Year	2003		2004		2005		2006		2007		2008	
Province	POS	TEST	POS	TEST	POS	TEST	POS	TEST	POS	TEST	POS	TEST
Chungbuk	9	84	20	84	8	96	4	116	4	128	2	128
Chungnam	7	60	15	86	4	56	8	120	11	176	4	91
Gangwon	10	136	11	82	10	120	2	96	6	56	2	88
Gyeongbuk	7	58	27	94	18	104	12	98	8	92	4	92
Gyeonggi	21	160	15	126	10	74	6	80	10	130	7	154
Gyeongnam	11	94	15	64	16	76	6	96	8	72	4	132
Jeju	20	112	20	90	10	75	10	80	4	80	2	80
Jeonbuk	9	86	28	156	12	182	12	84	2	152	26	120
Jeonnam	16	92	20	108	20	136	9	144	18	76	6	112
Subtotal	110	882	171	890	108	919	69	914	71	962	57	997
Positivity rate	12.5%		19.2%		11.8%		7.3%		7.4%		5.7%	

POS: positivity, TEST: testeds

surveys have been performed, such as Japan (10.0% of wild boars) (Sugiyama et al., 2009) and Ethiopia (13.4% of camels) (Melaku et al., 2016). For reference, the prevalence of anti-Aino virus antibody in sera collected from herds whose annual abortion rates ranged from 3% to 21% was 62.1% (64/103) (Norton et al., 1989).

The serological prevalence of Aino virus infection in sheep and goats was significantly different between age cohorts, vector controls, and geographical locations: it was higher in the older group and in the southern and western regions in South Korea. Our results demonstrated that vector control was a significant protective factor, and therefore, the summer control of vectors should be better implemented in provinces with elevated 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. The results of this seroprevalence study may serve as a basis for future epizootic studies of Aino virus infection in South Korea.

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