Seroprevalence and epidemiological risk factors for Kasba virus among sheep and goats in South Korea: a nationwide retrospective study

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Abstract

Introduction: Kasba virus belongs to the Palyam serogroup of the *Orbivirus* genus and *Reoviridae* family. Kasba virus is the causative agent of many reproductive disorders in infected animals, which result in considerable economic losses, mainly in the cattle industry. The epidemiology of Kasba virus infection is poorly defined and remains unclear in South Korea. **Material and Methods:** This study investigated the prevalence of antibodies against Kasba virus in sheep and goats in South Korea. Individual, management, and regional risk factors associated with seropositivity were also evaluated. In addition, a retrospective serosurvey was conducted. **Results:** Serum samples from 28 out of 441 sheep or goat flocks (6.3%, 95% confidence interval (CI): 4.4–9.0%) and 115 out of 1003 animals (11.5%, 95% CI 9.6–13.6%) were positive for antibodies against Kasba virus. According to our results, a history of reproductive problems increased the probability of Kasba virus positivity. Preventive measures such as routine insecticide application decreased this probability. We observed significant differences in the prevalence of seropositivity between southern provinces and northern provinces and between western provinces and eastern provinces at the individual level. **Conclusion:** The virus was widely distributed among sheep and goats in South Korea, with seropositivity ranging from 6.8% in 2004 to 13.7% in 2008. The current study represents the first assessment of factors associated with Kasba virus seroprevalence in sheep and goats in South Korea.

Keywords: Kasba virus, seroprevalence, sheep, goat, South Korea.

Introduction

A Palyam serogroup virus of the *Orbivirus* genus in the *Reoviridae* family, which was initially named Chuzan, was implicated as the causative agent of an epidemic of congenital abnormalities in cattle in Japan between November 1987 and April 1988 (4, 5, 16–18). Subsequently, Chuzan virus was determined to be the same as Kagoshima virus, which had previously been isolated from midges in Japan, and both viruses were subsequently found to be the same as Kasba virus, which had originally been isolated in India (8, 11). The three viruses were indistinguishable from each other, and Chuzan and Kagoshima viruses were subsequently recognised to be the same virus as Kasba, one of the original members of the Palyam serogroup that was originally isolated in India in 1957 (2). Kasba virus is the causative agent of many reproductive disorders in infected animals (16), resulting in considerable economic losses, mainly in the cattle industry (17, 18).

Kasba virus is transmitted by haematophagous arthropod vectors such as *Culicoides* biting midges and mosquitoes, and is widely distributed in temperate to tropical East Asian regions such as Japan, Korea, Taiwan, and China (25). The virus was isolated in Taiwan in 1993 and its reported seroprevalence was 91% among dairy cattle (12). Two isolates of Kasba virus were obtained from sentinel cattle in the southern part of mainland China in 2012 (23, 27). Kasba virus was also detected in yaks on the Qinghai-Tibetan Plateau in

2016–2017; however, no cases of disorders related to the virus were reported (24). In South Korea, the presence of Kasba virus has been recognised since 1993, but only a few sporadic detections in calves have been reported in recent years (3, 10, 13). A study conducted in South Korea in 2004 documented the detection of antibodies against Kasba virus in 22.8% of thoracic fluid specimens from aborted calves using virus neutralisation assays (13). Although Kasba virus is endemic in South Korea, the epidemiology of Kasba virus infection in the country is poorly defined and remains unclear.

The lack of baseline data on the seroprevalence of Kasba virus in small ruminants in South Korea has resulted in a poor overall understanding of the epidemiology of the diseases which it causes. The objective of this study was to estimate the seroprevalence of antibodies to Kasba virus in sheep and goats in South Korea. In addition, individual, management, and regional risk factors associated with seropositivity were evaluated, including flock size, history of reproductive problems, vector control, presence of ruminant farms, lakes or rice paddies within a 1 km radius, type of land use, and location. A retrospective serosurvey was also conducted to determine the presence of Kasba virus antibodies in archived sheep and goat sera and to improve our understanding of the epidemiological situation in South Korea.

Material and Methods

Study design. A cross-sectional serosurvey was conducted to determine the prevalence of neutralising antibodies against Kasba virus in sheep and goats and possible factors associated with infection. The necessary sample size to estimate the true prevalence was calculated using Epitools Epidemiological Calculators (22), based on methods described by Humphry *et al.* (6). A total of 861 animals were required to investigate the nationwide prevalence of antibodies to Kasba virus based on 5% desired precision, 95% confidence, 30% expected prevalence, 70% assumed sensitivity, and 90% assumed specificity. The expected prevalence was determined from bovine serological data previously reported in South Korea (10). Flocks and animals within these flocks were selected by a simple random sampling method in each province based on the government's national statistics (15). In South Korea, a trivalent vaccine against Aino virus, Akabane virus and Kasba virus was developed in 2011 (9). However, this trivalent vaccine has only been administered occasionally in the field. Therefore, we were unable to distinguish between vaccinated and unvaccinated animals because a diagnostic method to differentiate vaccinated and naturally infected animals has not been developed. In the present study, farms with history of vaccination were excluded from the sampling frame to avoid the detection of antibodies produced as vaccine-induced immunity.

In addition, sheep or goats younger than 6 months were excluded from the sampling frame to avoid the detection of antibodies present as conferred maternal immunity (7).

Samples. Based on the sample size calculated in this study, serum samples were obtained mainly from the blood and serum bank of the National Surveillance Program maintained by the Foreign Animal Diseases Division (FADD) of the National Veterinary Research and Quarantine Service (NVRQS, Anyang, South Korea). Samples were also obtained in close collaboration with local veterinary practitioners and/or local government veterinary officers. The study protocol was assessed and approved by the FADD of the NVRQS. The samples for this study were chosen from among the sera collected from October 2011 to February 2012. Blood samples were collected by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare. The number of samples collected 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. Seroprevalence was estimated at the flock and animal levels for sheep and goats. Additionally, for the retrospective serosurvey, serum samples collected from sheep and goats between 2003 and 2008 were analysed for the presence of Kasba virus–specific antibodies.

Determination of antibodies against Kasba virus. The Kasba virus YoungAm strain (VR66; Korea Veterinary Culture Collection, Anyang, South Korea) was used for serum neutralisation tests. Briefly, Vero cells (CCL-81; American Type Culture Collection, Manassas, VA, USA) were maintained in minimum essential medium alpha (MEM α; Gibco, Grand Island, NY, USA) containing 5% foetal bovine serum and antimycotics/antibiotics. Sera were heat inactivated at 56°C for 30 min before testing. Subsequently, the samples were serially diluted two-fold with MEM α from an initial dilution of 1:4 to a final dilution of 1:256. Then an equal volume of 50 μL containing a 100–300 50% tissue culture infectious dose ($TCID_{50}$) titre of Kasba virus was added to each well (9, 13, 14). After a 1 h incubation at 37°C with 5% CO_2, approximately 10^4 Vero cells were added to each well in a volume of 100 μL of MEM α containing antibiotics, and the plates were incubated. Positive and negative control sera, virus control wells and cell control wells were included in each neutralisation experiment. After incubation for 3–5 days, the plates were scored for the degree of cytopathic effect (CPE) observed in the virus control wells (back titration from 100 to 300 $TCID_{50}$) and for the titre of the reference positive (expected titre ± 1 dilution factor) and negative sera (no neutralisation). A sample was considered positive when it showed more than 75% CPE neutralisation at the lowest dilution (1:4). The serum titre was defined as the highest serum dilution capable of neutralising more than 75% of the CPE in the tissue culture.

Questionnaire. The sampling frame was established using sheep and goat farm IDs and flock sizes obtained from the Korea Animal Health Integrated System (KAHIS) of the Animal and Plant Quarantine Agency (Anyang, South Korea). This study involved a questionnaire-based survey of farmers and an analysis of blood samples from their animals. A structured questionnaire was drafted with the primary objective of elucidating the multifactorial background of the diseases with Kasba virus as their aetiological agent. It was conducted in an interactive manner with the owners of all the animals included in this study at all selected flock sites. The questionnaire included questions about individual risk factors, *e.g.* animal species (goat or sheep). Management-related risk factors, *e.g.* the size of the flocks, the presence of other animal species on the farm (particularly the presence of other ruminant animals such as cattle), a history of reproductive problems including abortion, and implementation of vector control (use or no use of insecticides) were also investigated. Regional risk factors, *e.g.* the presence of neighbouring ruminant farms, lakes, or rice paddies; land use (agricultural, woodland or seminatural, or urban areas, according to the KAHIS) and geographic factors (the specific location of the farm in South Korea) were also studied. Because the most common *Culicoides* flight range is <1 km (21), the radius for the evaluation of regional risk factors in this study was 1 km around the sampling farm. Questionnaires to obtain additional information about the farm or animals were subsequently conducted *via* telephone interview.

Statistical analysis. Prevalence and Wilson's 95% confidence intervals (CIs) (20) were calculated using Epitools Epidemiological Calculators (22). The intraclass correlation coefficient μ was calculated to measure the agreement in serological status between animals sampled within the same flock. This coefficient (minimum 0, maximum 1) was estimated using analysis of variance, with the flock serving as the independent variable and the serological status of individual animals (seropositive or seronegative) serving as the dependent variable (1).

In this study, the following individual exposure variables were evaluated in the univariate analyses: animal species; population size of the flock; flock structure; history of reproductive problems; vector control; presence of neighbouring ruminant farms, lakes, or rice paddies; land use; and geography (the region of the country in which the farm was located). For the study of the risk factors associated with Kasba virus infection, the epidemiological questionnaire variables were first submitted to an exploratory data analysis using the chi-square test (X^2, univariate). These factors were considered variables with a significance level greater than or equal to 95% ($P \leq 0.05$). The SPSS Statistics package in version 25 (IBM Corp., Armonk, NY, USA) was used for all data analyses.

Results

At the national level between 2011 and 2012, 28 out of 441 sheep and goat flocks (6.3%, 95% CI: 4.4–9.0%) and 115 out of 1003 animals (11.5%, 95% CI: 9.6–13.6%) were positive for neutralising antibodies against Kasba virus, as shown in Table 1 and Fig. 1. The agreement in serological status between animals sampled within the same flock as measured by the intraclass correlation coefficient was high at 0.65. This finding indicates a tendency toward most animals in any particular flock being seropositive or most being seronegative.

In the univariate analysis presented in Table 2, the management risk factor attributes showed that a history of reproductive problems on the farm was associated with an increase in the probability of seropositivity (odds ratio (OR) = 1.708, 95% CI :1.116–2.615, P value = 0.013). Preventive measures, such as the routine application of insecticide on the farm, decreased the odds of seropositivity for Kasba virus (OR = 0.611, 95% CI : 0.388–0.961, P = 0.031). No significant differences in risk were observed for the presence of ruminant farms, lakes or rice paddies within a 1 km radius. The regional risk factor attributes showed that the location of the farm being near an agricultural area was a significant risk factor (OR = 2.021, 95% CI : 1.098–3.718, P = 0.022). Additionally, associations between the probability of positivity and the region of the country where the farm was located were identified. We observed significantly increased odds of positivity in southern provinces compared with northern provinces (OR = 1.773, 95% CI : 1.184–2.654, P = 0.005) and in western provinces compared with eastern provinces (OR = 1.884, 95% CI : 1.211–2.931, P = 0.004) at the individual level. The retrospective serosurvey results showed that the virus was widely distributed in sheep and goats in South Korea, with seroprevalences ranging from 6.8% in 2004 to 13.7% in 2008 at the animal level (Table 3). Information on the distribution of neutralising antibody titres against Kasba virus in sera from sheep and goats in South Korea is presented in Table 3.

Discussion

To the best of our knowledge, no national epidemiological study of Kasba virus in sheep has been performed in South Korea; however, Yang *et al.* (26) assessed the seroprevalence of Kasba virus in native goats. Our seroprevalence estimates in sheep and goats were higher than those reported in native Korean goats in 2008 (2.0%) (26) and lower than those reported in Korean cattle (22.8%) in 2007 (13). This study constituted a nationwide serosurvey of Kasba virus in Korean small ruminants and an initial attempt to identify the factors that drive the epidemiology of Kasba virus in sheep and goats.

Table 1. Seroprevalence of Kasba virus in sheep and goats in South Korea (2011–2012)

Province latitude–longitude	Flock-level seroprevalence			Animal-level seroprevalence		
	Positive*	Tested	%	Positive*	Tested	%
Incheon (N) 36°55′–37°58′ (E) 124°36′–126°47′	1	10	10.0	2	25	8.0
Ulsan (N) 35°19′–35°43′ (E) 128°58′–129°27′	1	12	8.3	3	27	11.1
Gyeonggi (N) 36°53′–38°17′ (E) 126°22′–127°51′	10	105	9.5	31	281	11.0
Gangwon (N) 38°09′–39°25′ (E) 126°46′–128°22′	1	38	2.6	2	84	2.4
Chungbuk (N) 37°15′–36°00′ (E) 127°16′–128°38′	2	34	5.9	6	91	6.6
Chungnam (N) 35°58′–37°03′ (E) 125°32′–127°38′	1	60	1.7	2	87	2.3
Jeonbuk (N) 35°18′–36°09′ (E) 125°58′–127°54′	3	33	9.1	8	93	8.6
Jeonnam (N) 33°54′–35°30′ (E) 125°04′–127°54′	2	44	4.5	30	105	28.6
Gyeongbuk (N) 35°34′–37°33′ (E) 127°48′–131°52′	2	43	4.7	8	85	9.4
Gyeongnam (N) 34°39′–35°54′ (E) 127°35′–129°28′	3	44	6.8	10	87	11.5
Jeju (N) 33°06′–34°00′ (E) 126°08′–126°58′	2	18	11.1	13	38	34.2
Total (N) 33°06′–39°25′ (E) 124°36′–131°52′	28	441	6.3	115	1003	11.5

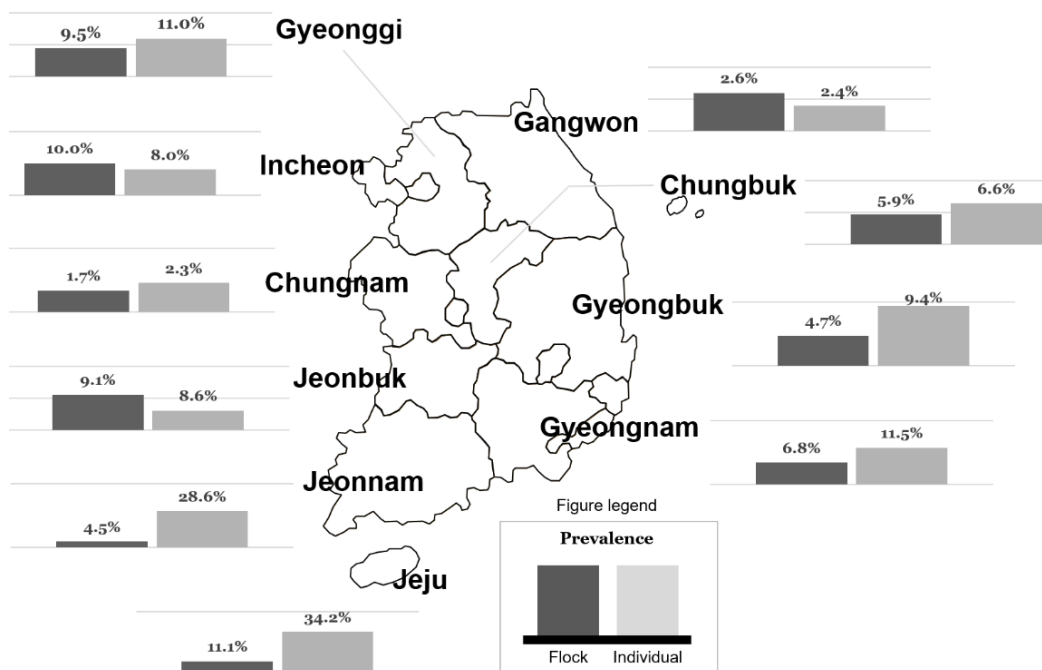* – number of seropositive flocks or animals (individuals)

Fig. 1. Geographical regions and the seroprevalence of Kasba virus in South Korea

Table 2. Univariate analysis of Kasba virus exposure variables relative to seropositivity outcomes in sheep and goats

Variable	Positive (n=139)	Negative (n=776)	OR (95% CI)	P value	OR (95% CI)	P value
Animal species						
Goat	60	450	Reference			
Sheep	55	438	0.942 (0.638–1.389)	0.762		
Age class						
Juvenile	20	242	Reference		0.575 (0.334–0.989)	0.044
Subadult	44	291	1.830 (1.050–3.188)	0.031	1.052 (0.683–1.621)	0.816
Adult	51	355	1.738 (1.011–2.990)	0.044	Reference	
Population sizes of the flocks						
<6 head	28	280	Reference		0.707 (0.428–1.166)	0.173
6-10 head	43	297	1.448 (0.875–2.395)	0.148	1.023 (0.653–1.604)	0.920
>10 head	44	311	1.415 (0.858–2.334)	0.173	Reference	
Flock structure						
Goats and/or sheep alone	66	502	Reference			
With other ruminants	49	386	0.966 (0.652–1.430)	0.861		
Reproductive problems						
No	79	701	Reference			
Yes	36	187	1.708 (1.116–2.615)	0.013		
Vector control						
No	88	591	Reference			
Yes	27	297	0.611 (0.388–0.961)	0.031		
Presence of ruminant farms within a 1-km radius						
No	60	461	Reference			
Yes	55	427	0.990 (0.671–1.460)	0.958		
Presence of lakes or rice paddies within a 1-km radius						
No	63	512	Reference			
Yes	52	376	1.124 (0.761–1.661)	0.558		
Land use						
Urban	15	199	Reference		0.540 (0.297–0.981)	0.041
Agricultural	54	387	2.021 (1.098–3.718)	0.022	Reference	
Woodland and seminatural	46	302	1.851 (1.019–3.363)	0.041	1.092 (0.716–1.663)	0.683
Location of the farm in South Korea						
Northern	41	440	Reference			
Southern	74	448	1.773 (1.184–2.654)	0.005		
Location of the farm in South Korea						
Eastern	86	543	Reference			
Western	29	345	1.884 (1.211–2.931)	0.004		

Table 3. Retrospective serosurvey of sheep and goats sampled between 2003 and 2008 for antibodies against Kasba virus

Province	2003 +	2003 Tested	2004 +	2004 Tested	2005 +	2005 Tested	2006 +	2006 Tested	2007 +	2007 Tested	2008 +	2008 Tested
Gyeonggi	7	159	14	124	9	73	5	79	13	129	8	148
Gangwon	10	130	9	79	8	129	2	95	8	55	3	87
Chungbuk	8	83	15	84	8	94	5	114	9	126	2	120
Chungnam	6	57	10	86	3	51	9	120	5	175	6	88
Jeonbuk	8	79	14	156	3	179	16	81	16	150	10	119
Jeonnam	14	91	15	108	9	135	10	139	24	73	9	108
Gyeongbuk	6	58	12	94	16	103	13	96	9	89	16	89
Gyeongnam	10	94	13	64	19	71	5	94	18	72	6	134
Jeju	19	112	19	90	9	72	9	79	16	79	6	79
Subtotal	88	863	121	885	84	907	74	897	118	948	66	972

We found that neutralising antibodies against Kasba virus were present in 11.5% of the sheep and goat populations and on 6.3% of sheep and goat farms. Kasba virus seroprevalence was estimated to be 15.2%, indicating that exposure to Kasba virus is prevalent among sheep and goats in South Korea, despite the paucity of reported Kasba virus outbreaks. This finding is probably due to the often unapparent clinical signs and underdiagnosis of Kasba virus when abortions and other reproductive problems occur in cattle (10).

The serological prevalence of Kasba virus infection in sheep and goats was significantly different between flocks with history of reproductive problems and those without, flocks farmed where vector control was used and where it was not, animals reared on land of different use types, and animals from different geographical locations. The reasons for these differences are unclear. Similar studies or publications that would be useful for the interpretation of these results are limited. One possibility is that specific ecological and climatic factors might 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. In addition, where one of the aetiological agents that had caused reproductive problems on farms in the past could have been Kasba virus, some difference may derive from it not having been recognised there as the cause of the disorder. Based on our results, vector control was a significant protective factor; therefore, control of vectors during the summer would seem to need to be better implemented in provinces with elevated seroprevalences.

D'Aguilar virus (DAGV), a member of the serogroup of Palyam viruses, is antigenically related to and serologically cross-reacts with Kasba virus. This virus has been repeatedly isolated in eastern Asia (25), and a previous study also reported that Kasba virus showed a cross-reaction with DAGV in cross-neutralisation tests (19). Unfortunately, because the distribution of DAGV was not studied and DAGV has not been isolated in South Korea to date, differentiation between DAGV and Kasba virus based on cross-reactivity could not be evaluated in this study. This lack of differentiation is a limitation of the present study because the DAGV seroprevalence in this region remains unclear.

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 epidemiological studies on Kasba virus infection in South Korea. For seroepidemiological studies to investigate virus exposure, immune responses to vaccination and those to infection must be distinguished. In the present study, we were unable to distinguish between vaccinated and unvaccinated animals because no diagnostic method is available to differentiate vaccinated and naturally infected animals. Therefore, in this study, farms with history of vaccination were excluded from the sampling frame to avoid the detection of antibodies due to vaccine-induced immunity. If a diagnostic test to differentiate these animals is developed and available, more detailed epidemiological information will be obtained by conducting serological surveys that include samples from vaccinated animals. The continuation of this research is crucial for deeper understanding of the epidemiology of this disease in the country and progress toward a full epidemiological assessment.

Animal Rights Statement: All animal handling, trapping, euthanasia, and blood collection procedures were carried out in compliance with the regulations of the "Animal Care and Use Manual" of the Animal, Plant, and Fisheries Quarantine and Inspection Agency (No. 75/2011) and the "Animal Protection Law" of the Ministry of Agriculture, Food and Rural Affairs of South Korea (No. 10310/2010).

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