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SEROPREVALENCE AND RISK FACTORS OF *BESNOITIA BESNOITI* INFECTION IN KOREAN CATTLE – SHORT COMMUNICATION

Seung-Hun LEE[1,2], Kyung-Yeon EO[3], Byeong Yeal JUNG[4], Dongmi KWAK[1] and Oh-Deog KWON[1*]

[1]College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Korea; [2]Foreign Animal Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Korea; [3]Conservation and Health Center, Seoul Zoo, Gwacheon, Korea; [4]Bacterial Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Korea

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Besnoitia besnoiti is an obligate intracellular parasite that is transmitted by direct contact or via mechanical transmission by flies as vectors. Besnoitiosis causes economic losses in the cattle industry and is regarded as a re-emerging disease in Europe. This study evaluated the seroprevalence of *B. besnoiti* in Korean cattle using a commercial ELISA kit. Among 558 serum samples, 19 (3.4%) tested seropositive for *B. besnoiti*. The statistically significant risk factors included age (≥ 2 years), sex (castrated males), and region (lower latitudes) ($P < 0.05$). The overall seroprevalence suggested a wide distribution of *B. besnoiti* infection in cattle reared in Korea. Thus, the practice of intensive cattle husbandry and the regionally different seroprevalence of *B. besnoiti* infection in cattle in Korea warrant routine monitoring and vector control to reduce economical losses due to bovine besnoitiosis in the country.

Key words: *Besnoitia besnoiti*, cattle, re-emerging disease, seroprevalence, vector-borne disease

Besnoitia besnoiti (Apicomplexa: Sarcocystidae) is an obligate intracellular parasite that causes bovine besnoitiosis (Cortes et al., 2014). In contrast to other coccidian parasites, the main route of spreading of *B. besnoiti* between animals is mechanical transmission by flies as vectors (Hornok et al., 2015). The clinical signs of besnoitiosis in cattle differ according to the stage of infection. Pyrexia, nasal and ocular discharge, salivation, stiff gait, and subcutaneous oedema are observed in the acute stage (Schares et al., 2013). In the chronic stage, severely lichenified and alopecic skin is observed, and the bulls can develop orchitis, resulting in infertility (Schares et al., 2013). Generally, while bovine

[*]Corresponding author; E-mail: odkwon@knu.ac.kr; Phone: 0082 (53) 950-5960; Fax: 0082 (53) 950-5955

besnoitiosis is associated with a high morbidity and low mortality, not all infected animals develop clinical signs (Jacquiet et al., 2010; Hornok et al., 2015).

Bovine besnoitiosis has been reported worldwide, including countries in Africa, Asia, America, and Europe (Lee et al., 1970; EFSA, 2010; Olias et al., 2011; Rinaldi et al., 2013; Cortes et al., 2014; Álvarez-García et al., 2014; Ashmawy and Abu-Akkada, 2014; Talafha et al., 2015). Many studies were published before 1992, and the disease apparently receded between 1992 and 2001 (Olias et al., 2011). However, since 2002, besnoitiosis has been reported frequently in Europe and has been recognised as a re-emerging and endemic disease by the European Food and Safety Authority (EFSA, 2010).

Cattle are among the most economically significant livestock in Korea, where the estimated cattle population approaches three million (Oh et al., 2016). Only few studies have examined the prevalence of besnoitiosis in Korea, and most of them were published several decades ago (Lee et al., 1970; Lee, 1975). The purpose of this study was to evaluate the current serologic distribution of *B. besnoiti* in cattle reared in Korea and to analyse its epidemiologic aspects.

Materials and methods

Sample collection and study area

Whole jugular venous blood samples (n = 558) collected from cattle throughout Korea from 2010 to 2015 were tested (Fig. 1). Serum samples were separated from the whole blood by centrifugation and stored at −20 °C until use. Age (≤ 1 year, young; ≥ 2 years, adults), sex (male, female, and castrated animals), type of cattle (beef and dairy), and region (northern, central, southern, and Jeju Island) were recorded. The geographic location of Korea ranged from 33°06' to 38°27' northern latitude and 125°04' to 131°52' eastern longitude. The mean annual temperature was 12.7 °C (range: 8.2–17.9 °C) and the annual precipitation was 1500 mm (Lee et al., 2016). In Korea, lower latitude regions are warmer and have more precipitation (Lee et al., 2015).

Sample size determination

The sample size was determined by a simple random sampling method, with 95% confidence, 10% expected prevalence, and 5% desired absolute precision (Thrusfield, 2005). The expected prevalence was estimated based on previous reports of prevalence in Korea (Lee et al., 1970).

Serological examination

A commercial enzyme-linked immunosorbent assay (ELISA) kit (ID Screen® *Besnoitia* Indirect, IDvet, France) was used according to the manufacturer’s instructions to detect anti-*B. besnoiti* antibodies. Positive and negative controls provided by the manufacturer were included in each experiment. Doubtful results were considered negative for statistical analysis. The sensitivity and specificity of the ELISA kit have been reported as 97.2% and 100%, respectively (García-Lunar et al., 2013).

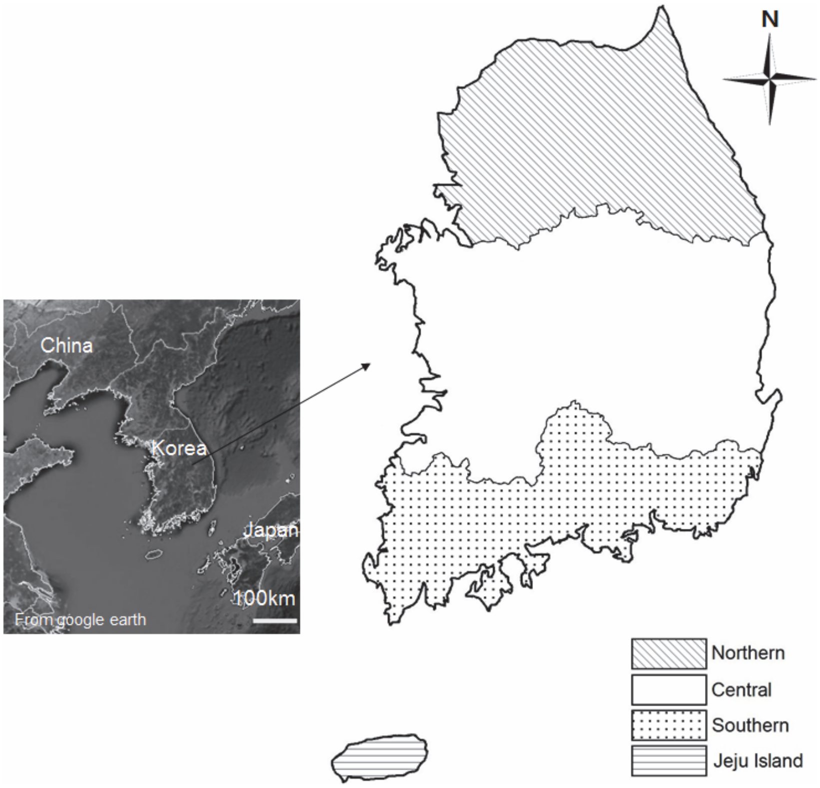

Fig. 1. The study regions in Korea. Seropositivity for anti-*Besnoitia besnoiti* antibodies among cattle was analysed by region. The regions are grouped according to administrative boundaries

Due to imperfection of the diagnostic method, the observed prevalence (OP; OP = No. positives/No. of total samples) and true prevalence (TP; TP = OP + Sensitivity – 1/Sensitivity + Specificity – 1) were calculated. The 95% confidence intervals were calculated for all estimates using Blaker's method (Reiczigel et al., 2010).

Statistical analysis

Seroprevalence was analysed to evaluate association with age, sex, type of cattle, and region using the chi-squared test or Fisher's exact test. P values < 0.05 were considered statistically significant.

Results

Among 558 bovine serum samples, 19 (3.4%; TP 3.5%) and 2 (0.4%) tested positive and doubtful, respectively, for anti-*B. besnoiti* antibodies (Table 1). Seropositivity was 1.0% (1/103; TP 1.0%) among young cattle and 4.0% (18/455; TP 4.1%) in adult cattle; seropositivity was 0% (0/59; TP 0%) among male cattle, 1.9% (6/319; TP 2.0%) among female cattle, and 7.2% (13/180; TP 7.4%) among castrated cattle. Beef cattle had 4.1% (14/340; TP 4.2%) seropositivity and dairy cattle had 2.3% (5/218; TP 2.4%) seropositivity. With regard to analysis based on the region, the seropositivity rates were 0.5% (1/188; TP 0.5%), 4.0% (6/151; TP 4.1%), 7.1% (8/112; TP 7.3%), and 3.7% (4/107; TP 3.8%) for northern, central, and southern regions, and the Jeju Island, respectively. Age, sex, and region showed statistically significant ($P < 0.05$) correlation with seroprevalence (Table 1).

Table 1

Seroprevalence of *Besnoitia besnoiti* among cattle in Korea

Variable	Group	No. tested	No. positive	No. doubtful[*]	Observed prevalence (%)	True prevalence (%)	95% C.I.[†]	P value
Age	≤ 1 year	103	1	0	1.0	1.0	0.0–5.1	< 0.001
	≥ 2 years	455	18	2	4.0	4.1	2.5–6.4	
Sex	Male	59	0	0	0.0	0.0	0.0–6.2	0.001
	Female	319	6	0	1.9	2.0	0.8–4.1	
	Castrated	180	13	2	7.2	7.4	4.0–12.4	
Type of cattle	Beef	340	14	2	4.1	4.2	2.5–7.0	0.175
	Dairy	218	5	0	2.3	2.4	0.9–5.3	
Region	Northern	188	1	0	0.5	0.5	0.0–2.8	0.009
	Central	151	6	1	4.0	4.1	1.8–8.6	
	Southern	112	8	1	7.1	7.3	3.3–13.9	
	Jeju Island	107	4	0	3.7	3.8	1.3–9.3	
Total		558	19	2	3.4	3.5	2.1–5.4	

[*]Doubtful results were regarded as negative for statistical analysis; [†]C. I.: Confidence interval

Discussion

Although a few studies (Lee et al., 1970; Lee, 1975) had been reported on *B. besnoiti* in Korean cattle with 3.8% and 6.7% prevalence rates which were slightly higher or similar to those found in the present study, a direct comparison among these data cannot be made as different diagnostic methods were used (ELISA vs. clinical signs). Evaluation of the seroprevalence of *B. besnoiti* according to age showed that adult cattle (age ≥ 2 years) had a significantly higher seroprevalence compared to those aged ≤ 1 year (P < 0.001). The age dependence of *B. besnoiti* infection has been suggested in other studies (Lee et al., 1970; Lee, 1975; Álvarez-García et al., 2014; Gutiérrez-Expósito et al., 2014; Talafha et al., 2015). Talafha et al. (2015) stated that because *B. besnoiti* is transmitted via direct contact or by vectors, older individuals are more likely to get infected than younger ones. This may be a factor in the higher seroprevalence in cattle aged ≥ 2 years.

Seroprevalence was also significantly greater (P = 0.001) among castrated male cattle compared to females and non-castrated males. The association between sex and seropositivity has been controversial. Some authors found no association (Álvarez-García et al., 2014; Waap et al., 2014), others have reported higher seroprevalence in females (Ashmawy and Abu-Akkada, 2014), while others have reported greater susceptibility to besnoitiosis among males (Jacquiet et al., 2010). In this study, when castrated males and non-castrated males were counted together, seropositivity was greater in males than in females, and the difference was significant (P = 0.032). Further investigations will be necessary to clarify the association between sex and *B. besnoiti* infection.

In terms of the regional prevalence of *B. besnoiti* infection in Korea, with the exception of Jeju Island, there was higher seroprevalence among cattle in regions at lower latitudes. The lower latitudes of Korea have higher temperatures and total precipitation, which have been correlated to the abundance of flies, thereby suggesting that higher seropositivity among cattle in southern regions may be attributed to vector transmission. This tendency has also been observed in Europe, where the distribution of *B. besnoiti* is moving northward and spreading is due to the availability of vectors (Hornok et al., 2014). Jeju Island has a peculiar climate, and because of its geographical separation from other regions of the Korean peninsula, the seroprevalence in Jeju may have no relationship with the seroprevalence in other regions.

While beef cattle had higher seroprevalence than dairy cattle in the present study, the difference was not significant (P = 0.175). Higher seroprevalence among beef cattle has been observed by some authors (Rinaldi et al., 2013; Álvarez-García et al., 2014), but not by others. Jacquiet et al. (2010) found that beef and dairy cattle were affected equally by besnoitiosis, while Álvarez-García et al. (2013, 2014) suggested that higher seroprevalence among beef cattle was related to the sampling region or husbandry conditions, but not to the breed.

This is the first study to report the seroprevalence of *B. besnoiti* in cattle in Korea using ELISA. The results suggest that *B. besnoiti* is widely distributed in Korea. Considering the intensive cattle husbandry systems being practiced and the regionally different seroprevalence of *B. besnoiti* in cattle in Korea, continuous monitoring and vector control are needed to reduce economic losses due to bovine besnoitiosis in the country.

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