Evaluation of the Performance of Interferon Gamma Assays for the Diagnosis of Bovine Tuberculosis in Hanwoo beef cattle in Korea

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Abstract: *Bovine tuberculosis (TB) is a contagious disease, and leads to major economic losses in agriculture; therefore, implementation of efficient diagnosis is essential for the control of bovine TB. The performance of a newly licensed commercial interferon gamma assay, ID Screen Ruminant IFN-γ ELISA (ID Screen ELISA, ID-Vet) was evaluated against the single caudal fold tuberculin (SCFT) test, an official test for detecting bovine TB, compared to the TB feron ELISA (Bionote), for the detection of bovine TB in Hanwoo beef cattle in Korea. In a total of 543 Hanwoo beef cattle, 75 (13.8%) and 73 (13.4%) were found to be positive for bovine TB using the ID Screen ELISA and TB feron ELISA, respectively. These results were slightly higher than those obtained with the SCFT test (10.1%, 55 of 543); however, the differences were not significant. Using the SCFT test as a reference, the sensitivity of both ID Screen ELISA and TB feron ELISA was 67.3% (37 of 55) and their specificities were 96.2% (450 of 488) and 92.6% (470 of 488), respectively. Additionally, there was a significant correlation between ID Screen ELISA and TB feron ELISA for the detection of bovine TB in Hanwoo beef cattle (r = 0.98). In conclusion, the interferon gamma assays, ID Screen ELISA and TB feron ELISA, correlated well with the SCFT test, and both assays showed excellent correlation between two gamma interferon assay for detecting bovine TB in Hanwoo beef cattle.*

Keywords — *Bovine tuberculosis (TB), interferon gamma assay, single caudal fold tuberculin (SCFT) test, Hanwoo beef cattle.*

I. INTRODUCTION

Bovine tuberculosis (TB) is a contagious disease, which represents a major problem for both animal and human health [1]. Furthermore, bovine TB is responsible for economic losses in agriculture. Accordingly, many countries have adopted the control and eradication program for bovine TB, which are carried out using testing and culling [2]. Bovine TB was first reported in 1913 in Korea, and its prevalence was approximately 15% in the 1940s [3]. Consequently, Korea initiated a National Control Program on bovine TB in 1964 to eradicate the disease [4]. The incidence of bovine TB was reduced to 0.15% by 2005 through implementation of the bovine TB eradication program, which used tuberculin skin test as the official diagnostic assay for bovine TB in Korea [5, 6].

Tuberculin skin test has been used as a diagnostic assay for bovine TB in cattle in many countries; this test measures the immune response of animals to *Mycobacterium bovis* (*M. bovis*) using an intradermal injection of Purified Protein Derivative (PPD) produced from *M. bovis* (bovine PPD) [7]. The procedure of the tuberculin skin test is simple; bovine PPD or avium PPD is injected intradermally into the cervical or caudal fold region of cattle, and the degree of swelling and induration of the injected site is examined after 72 hours. The single caudal fold tuberculin (SCFT) test, one of the tuberculin skin test, has been used for the detection of bovine TB in dairy cattle. This has been a major target of bovine TB control program in Korea due to the importance to public health. However, this test is laborious and time-consuming because it requires two visits: one for the tuberculin injection and one for the tuberculin reaction measurement. Moreover, it is more difficult and dangerous to perform the tuberculin test to beef cattle compared to dairy cattle due to their wildness. For these reasons, the use of the tuberculin test in Hanwoo beef cattle, Korean traditional beef cattle,

are limited even though approximately 3 million heads of Hanwoo beef cattle are being raised (eight times more than dairy cattle) in Korea [8]. In recent years, bovine TB in Hanwoo beef cattle has become a problem [9,10,11].

An interferon gamma assay, a cell-mediated immune-based diagnostic assay, has been used for the detection of bovine TB in several countries and was adopted as an official diagnostic assay for bovine TB in Korea in 2013 [12]. The performance of these commercial interferon gamma assays differed between dairy cattle and beef cattle, and there was a low agreement between the interferon assays and the tuberculin skin test on the diagnosis of bovine TB [13,14]. The efficacy and performance of a diagnostic assay can be affected by livestock breeds, breeding environment, and epidemiological status; therefore, it is necessary to evaluate them in the field situation with the target livestock.

In the present study, the efficacy of the newly licensed ID Screen Ruminant IFN-γ ELISA (ID Screen ELISA) was evaluated for the diagnosis of bovine TB in Hanwoo beef cattle. Additionally, the assay was compared to both the SCFT test and the TB feron ELISA.

II. MATERIALS AND METHODS

A. Study herds and animals

In this study, a total of 543 Hanwoo beef cattle from seven herds located in the Chungbuk province of Korea were included (Table 1). These herds were selected from those tested with the tuberculin test during the National Control Program on bovine TB from May 2017 to September 2018, based on the high predicted prevalence of the tuberculin reactor in the SCFT test.

B. Tuberculin skin test

Hanwoo beef cattle over 6 months old were subjected to the SCFT test, an official tuberculin skin test in Korea. The SCFT test was performed by official veterinarians at the Chungbuk Veterinary Service Institute in accordance with regulations of the National Control Program on bovine TB [12]. Briefly, 0.1 mL of bovine PPD was injected intradermally into the caudal fold region of cattle, and the skin thickness of the injected site was measured after 72 h. The result was interpreted as positive if skin thickness increased by 5 mm or more, inconclusive if skin thickness increased between 1 and 4 mm, and negative when the skin thickness increased less than 1 mm [12].

C. Interferon gamma assay

Heparinized blood samples were collected from all Hanwoo beef cattle (n = 543) over 6 months old prior to intradermal injection of bovine PPD and delivered to the Chungbuk Veterinary Service Institute within 4 h of collection at room temperature. Bovine and avium PPD stimulation was performed according to manufacturer's instructions.

For the ID Screen ELISA (ID-Vet, Grabels, France), 200 µL of blood samples was added to each well of a 96-well plate (Thermo Fisher Scientific, Waltham, MA, USA) and incubated overnight with PBS (negative control), bovine PPD, avium PPD, or mitogen (positive control). Culture supernatants were collected by centrifugation and stored at –70˚C until use. Interferon gamma was measured using an interferon gamma sandwich ELISA supplied by ID-Vet. The results were then converted to sample to positive ratios (S/P) according to the manufacturer's user manual as follows:

S/P = (optical density (OD) of a sample stimulated with bovine PPD – OD of the same sample stimulated with avium PPD) / (mean OD of positive control - mean OD of negative control) × 100

According to the manufacturer's instructions, if the S/P ratio was equal to or greater than 35, the sample was considered positive and if the S/P ratio was less than 35, the sample was considered negative. Furthermore, if the mean OD of the positive control was not greater than 0.5, or if the ratio of the mean OD of positive control to the mean OD of negative control was not larger than 3, the result was considered invalid.

For the TB feron ELISA (Bionote, Yongin, Korea), 1.5 mL of the blood samples were added in each well of a 24–well plate (ThermoFisher Scientific) and incubated overnight with PBS, bovine PPD, or avium PPD. Interferon gamma was measured using an interferon gamma sandwich ELISA kit supplied by the manufacturer, together with positive and negative controls. The results were interpreted according to the manufacturer's instructions. If (1) the difference between the OD of a bovine PPD-stimulated sample and the OD of the negative control and (2) the difference between the OD of a sample stimulated with bovine PPD and the OD of the same sample stimulated with avium PPD were equal to or greater than 0.1, the sample was considered positive. The test was determined to be invalid if the OD of the positive control was less than 1.0 or if the OD of the negative control was equal to or greater than 0.2.

D. Statistical analysis

For statistical analysis, the SPSS statistical software package (IBM® SPSS Statistics for Windows v24, SPSS Inc., Chicago, IL, USA) was used. Analysis of the estimated parameter was performed by means of an ANOVA test and the correlation analysis was performed using the Pearson's product moment correlation. The sensitivity and specificity of the assay were calculated as the proportions of positive and negative cases, which was determined using the SCFT test as reference test. $P<0.05$ indicates statistical significance.

III. RESULTS

A. Results of tuberculin skin test and interferon gamma assays

To investigate the performance of commercial interferon gamma assays for bovine TB in Hanwoo beef cattle, 543 Hanwoo beef cattle from seven farms were tested using the SCFT test, ID Screen ELISA and TB feron ELISA (Table 1). The positive rate obtained for the SCFT test of herds ranged from 0.0% (0 of 81) to 78.1% (32 of 41) (95% Confidence interval (CI): 0.00 - 88.0), and the mean positive rate for the SCFT test was 10.1% (55 of 543). The positive rate obtained for the interferon gamma assays ranged from 5.4% (9 of 168) - 51.2% (21 of 41) (95% CI: 1.0 - 65.7) and 2.5% (2 of 81) - 56.1% (23 of 41) (95% CI: 0.4 - 70.1) for the ID Screen ELISA and TB feron ELISA, respectively. For the entire herds, the positive rates of the interferon gamma assays, ID Screen ELISA and TB feron ELISA, were slightly higher than those of the SCFT test, but the difference was not significant (ID Screen ELISA vs SCFT test, $P = 0.68$; TB feron ELISA vs SCFT test, $P = 0.77$). In some

herds, there was a difference in the positive rates between tests. A higher positive rate was detected at 'C' and 'G' herds using the commercial interferon gamma assays when compared to the SCFT test ($P < 0.05$ for herd 'C', $P < 0.01$ for herd 'G'). On the other hand, a higher proportion of tuberculin-positive cattle was detected in the herd 'E', when compared to the ID Screen ELISA ($P < 0.05$). There were no significant differences in the amount of positive test from any herd when the commercial interferon gamma assays were used. In addition, there were significant correlations among the tested assays for bovine TB (SCFT test vs ID Screen ELISA, correlation coefficient (r) = 0.87, $P < 0.01$; SCFT test vs TB feron ELISA, r = 0.91, $P < 0.01$; ID Screen ELISA vs TB feron ELISA, r = 0.98, $P < 0.001$).

These results suggest that there are no significant differences of bovine TB in Hanwoo beef cattle between the SCFT test and the commercial interferon gamma assays, even though there were some differences in the test results of some herds.

TABLE 1. Results of SCFT test and the interferon gamma assays, ID Screen ELISA and TB feron ELISA in Hanwoo beef cattle from herds

Herd	No. of cattle	No. of positive cattle (%)		
		SCFT test	ID Screen ELISA	TB feron ELISA
A	81	0 (0.0)	3 (3.7)	2 (2.5)
B	168	10 (6.0)	9 (5.4)	6 (3.6)
C	64	8 (12.5)	21 (32.8)*,a	21 (32.8)*,a
D	3	1 (33.3)	1 (33.3)	1 (33.3)
E	41	32 (78.1)	21 (51.2)*,a	23 (56.1)
F	122	1 (0.8)	6 (4.9)	6 (4.9)
G	64	3 (4.7)	14 (21.9)**,a	14 (21.9)
Total	543	55 (10.1)	75 (13.8)	73 (13.4)

SCFT test: single caudal fold tuberculin test; ID Screen ELISA: ID Screen® Ruminant IFN-γ ELISA (ID-Vet) TB feron ELISA: TB feron ELISA (Bionote); [a] The analytical result was compared against the SCFT test. *, $P<0.05$; **, $P < 0.01$

TABLE 2. Results of gamma interferon assays, ID Screen ELISA and TB feron ELISA, compared against the SCFT test in Hanwoo beef cattle

SCFT test[a]	Gamma interferon assay				
	ID Screen ELISA[b]		TB feron ELISA		
	No. of positive (%)	No. of negative (%)	No. of positive (%)	No. of negative (%)	
Positive (n=55)	37 (67.3)	18 (32.7)	37 (67.3)	18 (32.7)	
Negative (n=488)	38 (7.8)	450 (92.2)	36 (7.4)	452 (92.6)	
Total (n=543)	75 (13.8)	468 (86.2)	73 (13.4)	470 (86.6)	
Sensitivity	67.3% (53.3% - 79.3%)[d]		67.3% (53.3% - 79.3%)		ns
Specificity	92.2% (89.5% - 94.4%)		92.6% (89.9% - 94.8%)		ns
Positive predictive value	49.3 (37.6% - 61.1%)		50.7% (38.7% - 62.6%)		ns
Negative predictive value	96.2% (94.0% - 97.7%)		96.2% (94.0% - 97.7%)		ns
Agreement rate (%)	87.7%		90.1%		

[a] Criterion for tuberculin-positive: skin thickness ≥ 5 mm; [b] ID Screen ELISA: ID Screen® Ruminant IFN-γ ELISA (ID-Vet); [c] TB feron ELISA: TB feron ELISA (Bionote); [d] 95% Confidence Interval (CI); ns: not significant

B. Performance of interferon gamma assays based on the tuberculin skin test

The performance of two commercial interferon gamma assays, ID Screen ELISA and TB feron ELISA, was compared against those obtained with the SCFT test, a tuberculin skin test used as a reference test for the determination of bovine TB in Hanwoo beef cattle. The sensitivity of both interferon gamma assays was 67.3% (37 of 55, 95% CI: 53.3 - 79.3%) (Table 2) and the specificities of ID Screen ELISA and TB feron ELISA were 96.2% (450/488, 95% CI: 89.5 - 94.4%) and 92.6% (470/488, 95% CI: 89.9–94.8%), respectively. The positive predictive value (PPV) of ID Screen ELISA and TB feron ELISA was 49.3% (95% CI: 37.6 - 61.1%) and 50.7% (95% CI: 38.7 - 62.6%), respectively. Moreover, the negative predictive value (NPV) of both commercial interferon gamma assays was 96.2% (95% CI: 94.0 - 97.7%). The agreement rates between the ID Screen ELISA and TB feron ELISA, compared to the SCFT test were 89.7% and 90.1%, respectively. There were no significant differences in the sensitivity, specificity, PPV, and NPV between the ID Screen ELISA and TB feron ELISA. When comparing the two commercial interferon gamma assays, of the 73 TB feron ELISA-positive cattle, 51 (69.9%) were positive with the ID Screen ELISA; conversely, 446 of the 470 (94.9%) TB feron ELISA-negative cattle were negative with the ID Screen ELISA (agreement rate 91.5%) (Table 3). These results suggest that there is a significant correlation between the ID Screen ELISA and TB feron ELISA.

TABLE 3. Comparison of ID Screen ELISA and TB feron ELISA in Hanwoo beef cattle

TB feron ELISA[a]	ID Screen ELISA[b]	
	Positive (n=75)	**Negative (n=468)**
Positive (n=73)	51	22
Negative (n=470)	24	446
Agreement rate (%)	91.5%	

[a] TB feron ELISA: TB feron ELISA (Bionote); [b] ID Screen ELISA: ID Screen® Ruminant IFN-γ ELISA (ID-Vet)

IV. DISSCUSSION

In the present study, we evaluated the performance of two licensed interferon gamma assays for the detection of bovine TB in Hanwoo beef cattle and compared it to the tuberculin skin test, a reference test of bovine TB. The commercial interferon gamma assays showed a slightly higher positive rate than that from the SCFT test (Table 1). In some herds, there was a difference between the positive rate obtained from the SCFT test and the one obtained with the interferon gamma assays, but overall, there was no significant difference between the tests. There were no significant differences between the two commercial interferon gamma assays in the detection of bovine TB in Hanwoo beef cattle. Furthermore, there was a highly significant agreement between the SCFT test and commercial interferon gamma assays, and the agreement between the two commercial interferon assays was significantly higher than that with the SCFT test (SCFT test vs ID Screen ELISA, 87%; SCFT test vs TB feron ELISA, 91%; ID Screen ELISA vs TB feron ELISA, 98%). This result was concordant to the result presented in Amei et al's report where the tuberculin skin test and ID Screen ELISA showed a similar performance with respect to the detection of bovine TB in zebu oxen in Ethiopia [15]. van der Heijden et al. also reported that a commercial interferon gamma assay, BOVIGAM® 1G (Prionics AG, Schieren, Switzerland), which uses bovine PPD and *M. fortuitum* PPD (PPD-F), showed a high sensitivity and good agreement with the single intradermal comparative tuberculin test (SICTT) in African buffalo [16]. Moreover, another new interferon gamma assay, BOVIGAM® 2G (Prionics AG), which uses a peptide cocktail as a stimulating antigen, showed less sensitivity and lower agreement with SICT, when compared to BOVIGAM® 1G [16]. Moreover, the IDEXX TB ELISA showed very low sensitivity compared to SICTT and interferon gamma assays [17]. A similar performance between the tuberculin skin test and interferon gamma assays appears to be the result of cell-mediated immune responses using the same bovine PPD as a stimulator. On the contrary, the lower sensitivity of BOVIGAM® 2G compared to BOVIGAM® 1G may be due to different stimulatory antigens because BOVIGAM® 2G uses a *M. bovis*-specific peptide cocktail instead of bovine PPD [16]. However, the performance of ID Screen ELISA was relatively low compared to that of Bovigam IFN-γ (Thermo Fisher Scientific), and the agreement between these interferon gamma assays was poor according to de la Cruz's et al.'s study [13]. In addition, the performance of ID Screen ELISA was higher in beef cattle than dairy cattle [13]. This indicates that the interferon gamma assays showed a better performance in Hanwoo beef cattle and good accordance with the tuberculin skin test; however, the validity for other animals, including dairy cattle, needs to be tested because diagnostic assays can vary according to the animal, breeding circumstance, and epidemiological status.

V. CONCLUSIONS

The interferon gamma assays evaluated in this study – ID Screen ELISA and TB feron ELISA – showed a high diagnostic performance for bovine TB in Hanwoo beef cattle. The positive rates of ID Screen ELISA and TB feron ELISA were not statistically different than those from the SCFT test, and there was a good agreement between the results of these interferon assays and the SCFT test.

ACKNOWLEDGMENT

This work was supported by a grant from the Bio-industry Technology Development Program, the Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (grant no. 314025–03), and in part by a grant from Basic Science Co., Republic of Korea.

COMPLETING INTEREST

The authors declare that they have no competing interests.

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