Evaluating the efficiency of an immunochromatographic test strip in detecting *Mycobacterium bovis* infection in Korean cattle

Soyoon Ryoo¹, Yun-Ho Jang¹, Narae Kim¹, Yoonra Jang¹, Shin-seok Kang², Hyun Sub Byun², Suk Chan Jung¹, Bong Kwan Choi¹, Jung-Ho Kim³, Dong-hyuk Kim³, Jae Myung Kim¹,‡

¹Bacterial Disease Division, Animal and Plant Quarantine Agency, Anyang 430-757, Republic of Korea
²Chungbuk Institute and Veterinary Research, Cheongwon 363-931, Republic of Korea
³Research Unit, BIONOTE, Hwaseong 445-170, Republic of Korea

**Abstract:** The aim of the present study was to evaluate the use of an immunochromatographic test (ICT) strip using recombinant MPB70 (rMPB70) protein as a complementary tool for the diagnosis of naturally occurring tuberculosis in cattle. The study was performed on 249 cattle from populations known to be free from *Mycobacterium bovis* (M. bovis) and 119 cattle with *M. bovis* infection, confirmed postmortem. Compared to reference standards (culture isolation and/or visible lesion), the sensitivity of ICT was 94.12% (95% CI: 89.89 – 98.35%) while the specificity was 96.80% (95% CI: 94.62 – 96.82%). The findings indicate that the ICT strip is efficient for diagnosing bovine tuberculosis in cattle from Korea.

**Key words:** *Mycobacterium bovis*, immunochromatographic test, MPB70

**INTRODUCTION**

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* remains an important zoonotic disease with a significant impact on the economy of many countries [5, 19]. *M. bovis* has a wide host range and is the species most often isolated from cattle with tuberculosis. Wildlife reservoir hosts for *M. bovis* infection in cattle include Eurasian badgers (*Meles meles*) in Great Britain and Ireland [4, 9], white-tailed deer (*Odocoileus virginianus*) in the United States [16], brushtail possums (*Trichosurus vulpecula*) in New Zealand, and the wild boar (*Sus scrofa*) in Spain [15].

Early infection with *M. bovis* produces a predominantly cell-mediated immune (CMI) response, with little or no antibody production. As the disease progresses, there is an increase in antibody production, with heavily infected animals sometimes becoming anergic to the skin test [18]. However, when animals are exposed to relatively high numbers of microorganisms, antibodies may be produced as early as two weeks post-infection [10]. Considering the inverse relationship between cell-mediated and humoral immune responses to *M. bovis*, complementing the skin test with *M. bovis*-specific serological tests could potentially increase the sensitivity of detecting *M. bovis* and thus help control the spread of the disease. Several serological tests that exhibit promising accuracy have been developed recently [8, 24]. Moreover, antibody response to *M. bovis* correlates positively with *M. bovis*-induced pathology and *M. bovis* antigen burden [13, 21, 22].

The most commonly used tests to detect tuberculosis in cattle include a skin test with purified protein derivative (PPD) and/or an in vitro assay for interferon gamma (IFN-γ) produced in response to PPD stimulation [28]. These tests depend on early CMI responses. In addition, PPD is a crude water-soluble protein extract from a heat-treated culture of *M. bovis* and shows cross-reactivity with environmental mycobacteria such as *M. avium* [6]. Therefore, there is a need to develop sensitive and easy-to-use *M. bovis*-specific serological tests that can distinguish between animals exposed to *M. bovis* and environmental mycobacteria. Considering the ease of sample collection and test procedure, serologic (antibody-based) tests may be used in a wide range of applications and provide additional testing opportunities not afforded by CMI response-based tests. MPB70 is highly expressed in *M. bovis* and minimally in *M. tuberculosis* in vitro and probably in vivo. Therefore,
this protein is an important target antigen for humoral and cellular immune responses in case of infection with *M. bovis* [25].

The present study was conducted to evaluate the diagnostic performance of an immunochromatographic test (ICT) strip using recombinant MPB70 (rMPB70) protein as a complementary tool for the diagnosis of natural infection by *M. bovis.*

**MATERIALS AND METHODS**

**Animals and serum samples**

The study included 368 cattle (Table 1), sampled in 2012 and 2013 during the BTB Management Survey in Korea. These cattle were not experimental animals. Handling of animals, sampling, and euthanasia were performed by veterinarians in accordance with Korean regulations.

For all animals included in this study, the ICT was performed between 8 and 10 days after a skin test (caudal fold test, CFT). For CFT, 0.1 mL of bovine PPD (PPD-T; CA vac, Daejeon, Korea) was injected in the caudal fold. The injection site was inspected 48–72 h later to determine pathogen exposure status.

Serum samples were collected from 119 *M. bovis*-infected animals, where infection was confirmed by culture or visible lesions and from 249 animals from populations considered to be BTB-free. In the infected group, infection was confirmed by culture in 94 animals and by histopathology in 105 animals (in combination with gross pathological examination). In addition, 249 serum samples were collected from over 14 herds from different regions across Korea. These herds were assumed to be free from BTB based on a history of absence of BTB-like lesions at slaughter, negative antemortem test results conducted for the purpose of animal movement, and routine BTB surveillance testing. In addition, these animals were confirmed as TB-negative by bacterial culture.

Blood samples were taken from the caudal or jugular vein of each animal and collected in serum-separator tubes. Serum was obtained by centrifugation (2,000 × g; 10 min) within 12 h, and samples were stored at −20°C until used.

**Bacteriology**

Lymph nodes and lungs of the 368 necropsied cattle were collected. Samples were decontaminated with 0.75% hexadecylpyridinium chloride (HPC) or 5% oxalic acid, inoculated on Lowenstein-Jensen (LJ) medium, and incubated at 37°C for 12 weeks. Growth on the LJ slants resembling *Mycobacterium* colonies was subjected to multiplex polymerase chain reaction (PCR) to confirm the presence of *M. bovis* and rule out contamination [26].

**Principle and procedure of the ICT**

The ICT strip (BIONOTE, Hwasung Korea) consisted of a sample pad, a reagent pad, a nitrocellulose membrane that allowed independent delivery of the test sample and the antibody-detecting reagent (MPB70 hybrid conjugated to colloidal gold particles), and an absorbent pad. The flow assay was performed in the following manner: 10 μL serum was added in the sample slot marked “S” on the test device using a capillary tube. After 1 min, 3 drops of the developing buffer were added into the developer slot and results were read after 20 min. Any visible band in the test (T) and control (C) area of the test strip was considered as a positive result for antibody reaction, whereas no band in the test area with a visible control band was considered as a negative result.

**Data analysis**

The ICT strip was evaluated against the reference standards by calculating test sensitivity, specificity, and accuracy using VassarStats software (http://vassarstats.net) and reported using 95% confidence interval (CI). Sensitivity and specificity in the test strip were estimated from dichotomized data for infected and uninfected cattle.

Likelihood ratios (LRs) were calculated to facilitate estimation of the post-test probability of BTB infection given a positive (LRP) or negative (LRN) ICT result for BTB-infected and uninfected animals. Test results with LRP > 10 or LRN < 0.1 produced substantial changes in post-test probability estimates as compared to pretest values, whereas LRP between 5 and 10 or between 0.1 and 0.2 produced moderate changes in these probabilities. An LR of 1 indicates no change in post-test probability of disease and signals a lack of test accuracy [7].

**RESULTS**

**Sensitivity and specificity of the ICT strip**

To evaluate the accuracy of the ICT strip, serum samples were obtained from cattle naturally infected with *M. bovis* as well as from uninfected cattle from several regions within
Korea (Table 1).

The results show that the test strip was able to detect the presence of anti-\textit{M. bovis} antibodies in BTB-positive cattle (as assessed by visible lesions or \textit{M. bovis} growth detected on LJ medium) with an efficiency of 94.12% (95% CI: 89.89~98.35%) from the five regions investigated. While the overall sensitivity of the test strip was 94.12%, the detection rate in cattle with visible lesions was higher (95.24%, 95% CI: 91.16~99.31%) than that in animals without visible lesions (85.71%, 95% CI: 67.38~100%). Combining the skin and serological test results produced a small improvement in sensitivity (Table 2). Moreover, 65 animals that were confirmed as infected with by reference standards but CFT negative were included in the study. Of these, 61 (93.85%, 95% CI; 88.00~94.02%) animals were positive with ICT (Table 3).

The specificity of the test strip was determined using 249 uninfected cattle. Among these animals, 241 (96.79%, 95% CI; 94.60~98.81%) were found to be antibody-negative based on ICT.

**Association between ICT results and BTB status**

The LRP for ICT was 52.71 (95% CI: 22.08~125.78) while the LRN was 0.06 (95% CI: 0.03~0.12). The LRP indicates that the risk of disease is nearly 53 times the pre-test risk of disease if the ICT result is positive. The LRN indicates that the risk of disease is 6% of the pre-test risk of disease if the ICT result is negative.

**DISCUSSION**

In many countries, control and eradication of BTB are

<table>
<thead>
<tr>
<th>Regions</th>
<th>n</th>
<th>Characteristics</th>
<th>TB status*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gyeonggi</td>
<td>78</td>
<td>No visible lesion, no isolation of \textit{M. bovis}, IFN-γ test negative</td>
<td>Uninfected</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>Visible lesion (n=22), \textit{M. bovis} isolation (n=18)</td>
<td>Confirmed infected</td>
</tr>
<tr>
<td>Chungbuk</td>
<td>48</td>
<td>Visible lesion (n=46), \textit{M. bovis} isolation (n=41)</td>
<td>Confirmed infected</td>
</tr>
<tr>
<td>Chungnam</td>
<td>11</td>
<td>Visible lesion (n=11), \textit{M. bovis} isolation (n=9)</td>
<td>Confirmed infected</td>
</tr>
<tr>
<td>Gyengnam</td>
<td>22</td>
<td>Visible lesion (n=16), \textit{M. bovis} isolation (n=20)</td>
<td>Confirmed infected</td>
</tr>
<tr>
<td>Ulsan</td>
<td>12</td>
<td>Visible lesion (n=10), \textit{M. bovis} isolation (n=6)</td>
<td>Confirmed infected</td>
</tr>
<tr>
<td>Jeju</td>
<td>171</td>
<td>No visible lesion, no isolation of \textit{M. bovis}, IFN-γ test negative</td>
<td>Uninfected</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>368</strong></td>
<td></td>
<td><strong>249 uninfected, 119 confirmed infected</strong></td>
</tr>
</tbody>
</table>

* TB status were determined by \textit{M. bovis} culture or presence of gross lesions.

<table>
<thead>
<tr>
<th>Test results*</th>
<th>n</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICT+</td>
<td>112</td>
<td>94.12% (89.89~98.35%)</td>
</tr>
<tr>
<td>ICT+ or CFT+</td>
<td>115</td>
<td>96.64% (93.40~99.88%)</td>
</tr>
</tbody>
</table>

*CFT = caudal fold test, ICT = immunochromatographic test.

<table>
<thead>
<tr>
<th>Reference standards</th>
<th>n</th>
<th>T+</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{M. bovis} isolation</td>
<td>52</td>
<td>48</td>
<td>92.31% (85.06~92.58%)</td>
</tr>
<tr>
<td>Visible lesion</td>
<td>54</td>
<td>52</td>
<td>96.30% (91.26~96.43%)</td>
</tr>
<tr>
<td>Visible lesion or \textit{M. bovis} isolation</td>
<td>65</td>
<td>61</td>
<td>93.85% (88.00~94.02%)</td>
</tr>
</tbody>
</table>
based tests to detect BTB in cattle [2]. Studies have reported 88.3% sensitivity for a multiplex ELISA test in cattle naturally infected with *M. bovis* [23]. The efficiency of using serum MPB70 ELISA for the diagnosis of caprine tuberculosis has also been described [14].

The current study indicates that the ICT strip using recombinant MPB70 antigen provides a test sensitivity of 94.12% (95% CI: 89.89–98.35%) and specificity of 96.69% (95% CI: 94.60–98.82%) with samples from cattle naturally infected with *M. bovis*. Other studies suggesting the use of an *M. bovis*-specific antigen (MPB70) in an ELISA to test the serological response to tuberculosis infection showed 96.4% specificity and 18.1% sensitivity [27]. The IDEXX *M. bovis* ELISA (IDEXX ELISA uses a mixture of MPB70 and MPB83 proteins) provides 63% test sensitivity and 98% specificity with samples from cattle naturally infected with *M. bovis* [20].

The sensitivity of the ICT strip was higher and the specificity was lower than what has been reported previously. Although the test specificity was slightly lower, a small number of cattle exposed to *M. avium* or *M. intracellularare* infection did not react to the test strip (data not shown). It is noteworthy that ICT identified 61 infected cattle (based on visible lesion or culture) which were negative with CFT. The results show that the level of detection of infected animals can be improved by the combined use of CFT and ICT. Furthermore, the value of the LR provided in this study suggest that a positive result adds substantial evidence that an animal is infected (LRP=52.71).

The ICT strip examined in this study is an assay similar to the ELISA. It offers advantages over other serological assays that have been described for detection of antibodies against *M. bovis* such as ELISA. Unlike ELISA, the test strip method is simple to perform since it requires a single reagent and does not require separation or washing steps. Moreover, the ICT strip provides results within minutes.

Several wild animal hosts are susceptible to *M. bovis* and have emerged as reservoirs of *M. bovis* infection in different countries, which are a threat to domestic livestock. Total eradication of any disease would be impossible if wildlife maintains a reservoir for infection [17]. However, in Korea, methods for TB diagnosis for species other than cattle are not fully validated. The ICT strip uses a direct sandwich method for antibody capture using rMPB70 protein. Therefore, the anti-MPB70 antibody present in the serum sample can be captured by the rMPB70 antigen on the test strip, regardless of animal species from which the sample is taken. Results from a prior study have demonstrated assays to detect specific antibodies during TB infection by a lateral-flow rapid test in Eurasian badgers, white-tailed deer, brush tail possums, and wild boars. Agreement of the results from the rapid test with that from cultures varied from 74% for possums to 97% for white-tailed deer [12]. These results highlight the potential of using MPB70 antigen-based, rapid testing kits to detect TB in wildlife in Korea.

To summarize, ICT utilizes rMPB70 antigen for BTB detection and can be used along with the skin test to determine the TB status of cattle in Korea. Additionally, immunochromatographic detection of anti-*M. bovis* antibodies in the serum of infected animals provides a technically simple diagnostic approach that requires limited manipulation of wild animals and zero need for species-specific secondary antibodies. Therefore, the ICT strip may provide a useful screening tool for wild animals that may be involved in the maintenance and transmission of *M. bovis* infection to domestic animals. Further investigations should focus on the detection of *M. bovis* in wild animals.

**ACKNOWLEDGMENTS**

This study was funded by the Veterinary Science Technical Development Research Project of Animal and Plant Quarantine Agency, South Korea (Project No. B-1541778-2012-13-02).

**REFERENCES**


23. Whelan C, Shuralev E, Kwok HF, Kenny K, Duignan A, Good M, Davis WC, Clarke J. Use of a multiplex enzyme-linked immunosorbent assay to detect a subpopulation


