A PRELIMINARY TRIAL TO EVALUATE THE GAMMA-INTERFERON ASSAY FOR THE DETECTION OF TUBERCULOSIS IN CATTLE UNDER LOCAL CONDITIONS IN SERBIA

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Summary

The early preclinical stages of bovine tuberculosis (BTB) can be detected in live animals by the use of tests of cellular mediated immunity, such as tuberculin skin test and gamma-interferon assay. None of the tests currently available for routine diagnosis of tuberculosis, in cattle provides a perfectly accurate determination of infectious status. In Serbia, like the most of other countries, primary diagnostic tool for detection of bovine tuberculosis is skin testing, although various factors can influence the sensitivity and specificity of these. When γ-interferon (γ-IFN) assay complements the skin tests it is capable to improve the overall probability of detecting infected cattle in regions and herds accounting for a high incidence of BTB. The paper presents and compares results of tuberculin skin tests and γ-interferon test in ante mortem diagnosis of BTB. Single intradermal cervical test (SICT) was used as routine screening test. All the animals (60) that yielded positive or suspicious reactions were retested 50 days later and single intradermal comparative cervical test (SICCT) was performed with addition of blood based γ-interferon test for confirmation of late hypersensitivity. The obtained results showed that when the SICCT alone was used positive reactions were detected in 40 (66.66%), negative reactions in 18 (30%) and inconclusive in 2 (3.33%). When SICCT and γ-IFN were used in parallel 46 (76.66%) animals were diagnosed as BTB positive.

Key words: bovine tuberculosis, diagnosis, SICCT, gamma-interferon

Bovine tuberculosis caused by Mycobacterium bovis is a serious and deteriorating disease of cattle and wide range of other mammalian species including men. As the relevant zoonosis, in the past M. bovis has been associated with extrapulmonary child tuberculosis (10) before the obligatory milk pasteurization was introduced. Tuberculosis can also be spread to humans by the inhalation of infectious droplet nuclei and certain occupations such as farmers, veterinarians and abattoir workers are at special risk (8).

In Serbia the program for the control and eradication of BTB in cattle was initiated by the government in early fifties of the 20th century. The program was based on systematic and regular skin testing of cattle herds, supported by compulsory slaughter of positive reactors and surveillance for undetected infection in public abattoirs conducted by state veterinary inspectors. The scheme based on test and slaughter policy made a significant progress towards eradication of bovine
tuberculosis. Although epidemiological situation considering BTB in cattle is favourable in country as a whole, in some endemic areas of Vojvodina province there is still a low-level, but persistent tuberculosis problem (6) that is proving to be difficult to eliminate. The accurate detection and prompt removal of animals infected with *M. bovis*, is of crucial importance for BTB control in cattle (9). In order to improve the overall probability of detecting tuberculous cattle in endemic regions and herds with a history of positive reactors, in the year 2006 we started to use γ-IFN assay as an ancillary test to the tuberculin skin test. In the European Union (EU), the assay has been recognised under Directive 64/432/EEC as an adjunct to the tuberculin skin test.

**Materials and methods**

For performing skin tests we used protein purified (PPD) bovine tuberculin, produced by Veterinarski zavod Zemun, Serbia, that contains 30000 IU of purified tuberculoprotein in 1 ml and avian tuberculin that contains 20000 IU of tuberculoprotein in 1 ml. The skin fold thickness was measured with callipers immediately before the injection of the tuberculin in addition to subjective palpation and visual observation of the injection site. The SICT was performed by injecting 0.1 ml of bovine PPD tuberculin into the skin of the left side of animals neck. Approximately 72 h after the injection, the site of injection was examined for the signs of inflammatory reaction and increase in skin thickness was measured. The total number of 60 animals has shown positive or suspicious reactions. All the reactors were from herds chronically infected, or herds with a high probability of containing infected animals. Those animals were retested by SICCT after a period of 50 days. The SICCT consisted of simultaneous injection of both bovine and avian tuberculin side-by-side into the skin of the neck. Bovine tuberculin was injected on the left side of the neck, and avian on the right. We applied a severe interpretation of the SICCT results. The SICCT severe interpretation is positive when the bovine reaction is 3mm or more and is greater than the avian reaction by 2mm or more. Before intradermal application of tuberculin a whole blood heparinised samples were collected for *in vitro* γ-IFN testing. The samples were transported to the laboratory 2-8 hours after sampling. Small duplicate aliquotes were incubated within 12 hours after collection, in the presence of test antigens bovine PPD tuberculin, avian PPD tuberculin and a PBS as a negative control. After 24-36h of incubation at 37 °C, the plasma supernatants were harvested and the amount of produced γ-IFN was quantified by a sandwich enzyme-linked immunosorbant assay (ELISA). For γ-IFN testing we used *Mycobacterium bovis* Gamma interferon Test Kit for Cattle Bovigam® manufactured by Prionics, Switzerland.

**Results and discussions**
A total of 12 small farms and individual cattle breeders in tuberculosis hot spots throughout South Bačka region, were surveyed over past two years in effort to eliminate the disease from the herds. The tuberculosis in these herds has been confirmed in post-mortem examination of slaughtered cattle by the presence of lesions, histopathology and/or culture for *M. bovis*. During the period of surveillance a total number of 247 bovine animals were tested by using the SICCT, and a total number of animals showing positive or suspicious reaction were estimated at 60. Positive reaction was defined as increase in skin fold thickness for 4mm or more, while suspicious reaction was declared when increase in skin fold thickness was more than 2 and less than 4 mm. All of these animals were retested after a period of 50-58 days by applying the SICCT. By applying a severe interpretation on the SICCT a total number of 40 (66.66%) test- positive reactions were detected, 18 (30.00%) negative, while in 2 animals (3.33%) reaction was inconclusive. The interpretation of reaction is inconclusive when the bovine reaction is equal or 1-2 mm greater than avian reaction. Results of the γ- IFN assay, has shown positive reaction in 33 (55.00%) animals, negative in 21 (35.00%) and inconclusive in 6 (10.00%) of tested animals. As an inconclusive reactors that should be retested to reduce the possibility of a false-negative result we considered animals that have had a significantly high OD values for both bovine and avian PPD but when compared, the result was slightly below 0,1. When the results of severe SICCT and the γ- IFN assay were combined, the sensitivity of the tests in parallel was superior to either test alone and detected 46 (76.66%) positive test reactors. The results are presented in table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Test result</th>
<th>Number of tested cattle (No=60)</th>
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</thead>
<tbody>
<tr>
<td>SICCT positive</td>
<td>40 (66.66%)</td>
</tr>
<tr>
<td>γ- IFN positive</td>
<td>33 (55.00%)</td>
</tr>
<tr>
<td>SICCT and/or γ- IFN positive</td>
<td>46 (76.66%)</td>
</tr>
</tbody>
</table>

In spite of intensive test and slaughter campaign, there remains a low-level, but long lasting tuberculosis problem in some parts of South Bačka region in Serbia. Although in the last two years we merely had registered new infected herds, in some chronically infected farms the overall number of culled tuberculous cows was more than 60%, and one farm in which test- positive animals were detected on 5 consecutive tests was completely eliminated. Our aim is to stop the spread of the disease in other parts of the country, currently cattle tuberculosis free and to achieve sustainable reduction in BTB incidence in tuberculosis high incidence endemic regions.
To control bovine tuberculosis the accurate detection of infected cattle and early disposal of such animals is of primary significance (1). Infection of cattle with \textit{M. bovis} is usually chronic and such animals rarely express any clinical signs until late stages of the disease. However, infected cattle can shed the pathogen long before they exhibit any obvious clinical signs which even if present are vague and not pathognomonic (9). With the tuberculosis in cattle, as in humans it has been shown that the pro-inflammatory cell mediated immune (CMI) response tends to dominate in the early stages of the infection (4, 7). Therefore ante mortem tests of cellular immunity are of major importance in early identifying BTB infected animals. The two tests that follow this principle are currently approved in EU for BTB diagnosis in living animals: in vivo intradermal tuberculin test and in vitro blood based \textgamma-IFN assay. The skin tests represent the gold standard in ante mortem BTB diagnosis and are prescribed by the Office des Epizooties (OIE) in the international cattle trading. After the 2000\textsuperscript{th} the \textgamma-IFN assay has been widely adapted as in vitro test for BTB in large number of countries, in most been an ancillary test to skin testing (5).

There are numerous field studies that evaluated the diagnostic performance of conventional \textgamma-IFN assay under local conditions in different countries. The test sensitivities are reported to lie between 55\% and 97\% depending on the interpretation used (3, 11). Some of the recognized limitations in the performance of the SICCT led to the establishing of the \textgamma-IFN Testing Laboratory at the Scientific Veterinary Institute in Novi Sad. The assay is used as an ancillary test to SICCT and is targeted to the chronically infected herds or to in contact herds with a high probability of containing infected cattle. The aim is to improve the overall sensitivity in endemic region sustaining a high incidence of BTB by parallel testing and removing any positive reactor on either test. We have compared the sensitivity of SICCT and the \textgamma-IFN assay in the field conditions of South Bačka region in 12 herds with confirmed BTB.

The obtained results suggested that the SICCT tests assessed under so-called “severe” interpretation, which is lowering the cut off points for an animal to be declared a positive reactor appeared to be more sensitive when compared with single use of \textgamma-IFN assay. But if the results of the \textgamma-IFN test that contained high OD ratios for both avian and bovine tuberculin should be interpreted as positive in the context of all available historical and epidemiological information relevant to the animal under test then the overall sensitivity of \textgamma-IFN assay would be 65,00\% which is close to the results obtained by the SICCT. All six animals that were marked as inconclusive reactors on \textgamma-IFN test, would under standard interpretation of SICCT be declared as non-specific or inconclusive reactors. When using the standard interpretation of the SICCT, the recognition of few \textit{M. bovis} infected cattle can be masked by prior or concomitant exposure to mycobacteria of the \textit{Mycobacterium avium} intracellular complex, if the skin reaction to avian tuberculin exceeds that to bovine tuberculin (2). It is common practice in lot of farms in South Bačka region of keeping hen or pigeon in cohabitation with cattle and there is a high probability that
some cattle could be coinfected with both pathogens, thus making reaction interpretation difficult even to experienced clinician. The gamma-interferon test can be applied in different ways and a particular advantage of the test in infected herds is that the interpretation criteria for defining a positive γ-IFN reactor can be readily adjusted so as to change both the sensitivity and specificity of the test (3). When the herd is heavily infected the specificity of test is of less importance, and the sensitivity can be set to maximum in order to remove all infected animals in a shortest time (1). The use of gamma-interferon test and SICCT in parallel proved to meet these requirements best.

Conclusions

The results of our investigation suggests that the γ-IFN assay should not be used as an screening test in routine surveillance, but its strategic use should be targeted in herds and regions with high BTB incidence, in parallel with the SICCT to lower the possibility of BTB infected cattle to be misdiagnosed as being clear.

References