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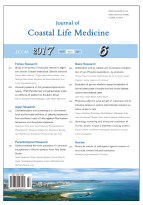

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Evaluation of gamma interferon assay for detection of bovine tuberculosis in inactive foot and mouth disease vaccine administered cattle

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ABSTRACT

Objective: To investigate the relationship between inactive foot and mouth disease virus (FMDV) vaccination and result of gamma interferon (IFN-γ) assay for tuberculosis in cattle negative for bovine tuberculosis.

Methods: A total of 300 tuberculosis-negative cattles were subcutaneously vaccinated with inactive FMD vaccine. Nine hundred blood samples were collected from the same 300 cattles after 7, 15, and 30 days of vaccination. Then, all samples were tested with IFN-γ assay for tuberculosis.

Results: A total of 900 blood samples were tested with IFN-γ assay for tuberculosis and all of the samples were found negative for bovine tuberculosis.

Conclusions: Our results indicate that inactive FMD vaccination in cattle do not affect results of IFN-γ assay for tuberculosis.

1. Introduction

Mycobacterium bovis (*M. bovis*) complex comprises *M. bovis*, *M. tuberculosis*, *M. africanum*, *M. microti*, *M. canetti*, and *M. caprae*[1]. Bovine tuberculosis, caused by the acid-fast bacillus *M. bovis*, is a chronic, zoonotic, and highly contagious disease for cattle, pigs, goats, and several wild species. The nodular granuloma and tubercle formation with calcification in the lungs, lymph nodes, and other vital organs are characteristic for this infection. Bovine tuberculosis affects public health, socioeconomic conditions, animal health, and animal products[2,3].

The early detection of bovine tuberculosis is critical to avoid an epidemic and useful for eradication of disease. For this purpose, many diagnostic methods, such as bacterial culture, histopathology, polymerase chain reaction, and ELISA have been developed and widely used to date[4]. The intradermal tuberculin skin test is also used for standard screening for the detection of bovine tuberculosis. As an alternative, gamma interferon release assay (IFN-γ assay) for detection of tuberculosis has been developed to measure cell-mediated immune response (CMI) under laboratory conditions. In this test, blood cells are stimulated by tuberculin and production of IFN-γ is quantified by ELISA[5].

The culture conditions and quality of sample and test reagents are important for accurate result of IFN-γ assay. The assay may also be negatively affected by corticosteroid application, stress

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condition, and first month period of lactation[6]. IFN-γ release in IFN-γ assay is affected by different conditions, such as the time and temperature of blood sample storage, the time of test culture, and the kind of anticoagulant used for blood collection[7,8]. However, the correlation between viral or bacterial vaccination and result of IFN-γ assay for tuberculosis in cattle negative for bovine tuberculosis has not been investigated.

The inactive foot and mouth disease virus (FMDV) vaccines are administered to healthy cattle for prevention of cattle against FMD[9]. Because of absence of studies describing the relationship between inactive FMD vaccination and result of IFN-γ assay for tuberculosis in bovine tuberculosis-negative cattle, the present study aimed to test the whole blood samples from cattles vaccinated with inactive FMD vaccine by using the INF-γ assay for determination of tuberculosis and evaluate the results of assay.

2. Materials and methods

2.1. Animals

A total of 300 cattles were selected from 4 different farms located in Ankara and Aksaray provinces for this study. The farms have different numbers of animals recorded between 200 and 1 100 cattles. The ages of the animals were between 6 months and 4 years old. All of the animals had been tested by IFN-γ assay for tuberculosis and tuberculosis skin test 1 and 7 months respectively before vaccination, and all of them were found negative for bovine tuberculosis. The cattles were subcutaneously vaccinated at neck skin with 2 mL of inactive FMD vaccine (Turvac-oil, FMD Institute, Ankara, Turkey). After one month, all of the vaccinated animals were secondly vaccinated with the same vaccine at the same dose. Whole blood samples were taken into lithium heparin tubes from the animals before first vaccination and 7, 15, and 30 days after second vaccination. The samples were transported to laboratory in 8 h at 18–25 ˚C. The study protocol was approved by the Ethical Committee of Veterinary Faculty, Selcuk University.

2.2. Antigens

Bovine purified protein derivative (PPD B) and avian purified protein derivative (PPD A) were provided by BionoteInc, Gyeonggi-do, Republic of Korea. To stimulate whole blood samples, PPD A and PPDB (0.3 mg/mL each) were used.

2.3. IFN-γ assay

Heparinized blood samples were gently mixed before aliquoting. Sample cultures were performed with 24-well tissue culture tray. Three 1.5 mL aliquots of heparinized blood for each animal were dispensed into 3 wells of a tissue culture tray under aseptic condition. Then 100 μL of phosphate buffered saline (PBS), PPD A and PPD B were added to the 3 wells containing the heparinized blood. After 24 h of culture at 37 ˚C with 5% CO_2 in a humidified incubator, the supernatants were gently harvested. INF-γ concentrations were determined by commercial ELISA test kit (Anigen TB-feron, BionoteInc, Gyeonggi-do, Republic of Korea). ELISA was performed according to the instruction recommended by manufacturer. The absorbance at 450 nm (OD450) was determined by a spectrophotometer (Lambda scan 200, Bio-TekInstInc, USA). A sample was accepted as positive when PPD B OD450 minus PBS OD450 and PPD B OD450 minus PPD A OD450 were equal or higher than 0.1.

2.4. Statistical analysis

The statistical differences among groups were determined by *Chi*-square test. SPSS software version 12 was used for the statistical analysis. $P < 0.05$ was accepted as statistically significant.

3. Results

A total of 300 whole blood samples collected from cattles before first vaccination were found negative for IFN-γ assay. All of the animals were subcutaneously vaccinated with inactive FMD vaccine twice at 1-month interval. Heparinized whole blood samples were collected from the same 300 cattles after 7, 15, and 30 days of second vaccination (Table 1). Then, a total of 900 blood samples were tested with IFN-γ assay for tuberculosis and all of the samples were found negative for bovine tuberculosis. The results did not show differences between age groups (1, 2, 3, and 4 years) of animals. Also, the results obtained from 4 different farms were the same.

Table 1
The result of IFN-γ assay before and after FMD vaccination.

Result of IFN-γ assay	Before vaccination	After vaccination		
		Day 7	Day 15	Day 30
Negative [*n* (%)]	300 (100)	300 (100)	300 (100)	300 (100)
Positive animals [*n* (%)]	0 (0)	0 (0)	0 (0)	0 (0)
Total No. of animals [*n* (%)]	300 (100)	300 (100)	300 (100)	300 (100)

4. Discussion

FMD is highly contagious and an acute disease affecting all ruminants. FMDV belongs to the Picornaviridae family and is the main member of the genus *Aphthovirus*[9]. The outbreaks of this disease have been a major cause of economic losses to livestock

industries worldwide[10]. The inactive FMD vaccines are widely used in the region of outbreaks for prevention of cattle against FMD. *M. bovis* and FMDV are intracellular microorganisms. The relationship between viral vaccination and result of IFN-γ assay for tuberculosis in cattle has not been investigated. The aim of this study was to investigate the correlation between inactive FMD vaccination and result of IFN-γ assay for tuberculosis in cattle.

IFN-γ assay for tuberculosis is based on measurement of bovine IFN-γ and CMI after incubating heparin-treated whole blood with simulating antigen. The different parameters or conditions of IFN-γ assay for tuberculosis, such as the cell culture temperature, geometry of the test plate, the need for carbon dioxide, the animal holding conditions and the influence of time to culture initiation have been reported[7,8,11,12]. Schiller *et al*.[8] reported that the time and temperature of culture will affect the result of assay, and the stimulation temperature needs to be 33 ˚C or higher. The same researchers have stated that carbon dioxide level during stimulation and geometry of the test plate did not affect the assay. Ryan *et al*.[11] obtained similar result from blood samples which were tested on the day of sampling and in 24 h of sampling by IFN-γ assay for tuberculosis. In contrast to this finding, Gormley *et al*.[12] found that the delay in blood culture decreased sensitivity of assay and similar result was reported by Schiller *et al*.[8]. However, the correlation between viral vaccination and result of IFN-γ assay for tuberculosis in bovine tuberculosis-negative cattle has not been evaluated until now. In our study, 300 bovine tuberculosis-negative cattles were subcutaneously vaccinated with inactive FMD vaccine twice. A total of 900 blood samples collected from 300 cattles after 7, 15, and 30 days of second vaccination were tested with IFN-γ assay for tuberculosis and all of the samples were found negative for bovine tuberculosis. Our data suggest that inactive FMD vaccination do not affect result of IFN-γ assay for tuberculosis in bovine tuberculosis-negative cattle.

In conclusion, our results indicate that inactive FMD vaccination in tuberculosis-negative cattles do not affect results of IFN-γ assay for tuberculosis and do not cause a false positivity for bovine tuberculosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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