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SHORT COMMUNICATION

In Vitro Diagnosis of Bovine Tuberculosis by γ-Interferon Assay

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Summary

The purpose of this study was to determine the sensitivity and specificity of the γ-IFN assay and to compare the γ-IFN assay and intradermal tuberculin test results for detection of bovine tuberculosis. Defibrined blood samples were collected from 126 cattle in 6 herds suspected with tuberculosis and tested by *in vitro* γ-IFN assay. Intradermal tuberculin test was also performed on the same animals. While 62 cattle (49.2%) were found positive by γ-IFN assay, 67 cattle (53.2%) were detected positive by intradermal tuberculin test. The sensitivity and specificity of γ-IFN assay was estimated to be 90% and 97%, respectively. In conclusion, it is thought that γ-IFN assay is easier to perform and it shortens the time for diagnosis of bovine tuberculosis and it can be an alternative test to intradermal tuberculin test in bovine tuberculosis eradication programs.

Keywords: *Bovine tuberculosis, γ-interferon assay, Intradermal tuberculin test, Diagnosis*

γ-Interferon Testi ile Sığır Tüberkülozunun *İn Vitro* Teşhisi

Özet

Bu çalışmanın amacı, sığır tüberkülozunun teşhisinde γ-IFN testinin sensitivite ve spesifitesini belirlemek ve γ-IFN testi ile intradermal tüberkülin testinin sonuçlarını karşılaştırmaktır. Tüberküloz şüpheli 6 sürüden toplam 126 sığırdan defibrine kan örnekleri toplandı ve *in vitro* γ-IFN testi uygulandı. Aynı zamanda, aynı hayvanlara intradermal tüberkülin testi yapıldı. γ-IFN testi ile sığırların 62'si (%49.2) pozitif bulunurken, intradermal tüberkülin testi ile 67 (%53.2) sığırın pozitif olduğu belirlendi. γ-IFN testinin sensitivite ve spesifitesi, sırasıyla %90 ve %97 bulundu. Sonuç olarak, sığır tüberkülozunun teşhisinde γ-IFN testinin, uygulaması kolay, kısa sürede sonuç veren bir test olduğu ve sığır tüberkülozunun eradikasyon proğramlarında intradermal tüberkülin testine alternatif olabileceği düşünüldü.

Anahtar sözcükler: *Sığır tübekülozu, γ-interferon testi, İntradermal tüberkülin testi, Teşhis*

INTRODUCTION

Despite the fact that eradication programs, bovine tuberculosis is still an important zoonoses in many countries [1-3]. In spite of lacking the sensitivity and specificity, *in vivo* intradermal tuberculin test is used as standard test for diagnosis of bovine tuberculosis in cattle in worldwide [1,4,5]. There are several factors influencing the sensitivity and specificity of intradermal tuberculin test [2,6,7]. Because many of purified protein derivate (PPD) used to detect the bovine tuberculosis are also found in nonpathogenic mycobacterial species, false positive results can occur due to cross reactions [6-8]. However the early stages of the infection and, anergy and weak immune system of animals can be responsible from low sensitivity and specificity of intradermal tuberculin test [7,8].

In recent years, gamma interferon (γ-IFN) assay has been used for detection of bovine tuberculosis in that it detects the cytokine γ-interferon which is predominantly

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released by T-cells after *in vitro* stimulation with bovine PPD and avian PPD [9-11]. The sensitivity and specificity of γ-IFN assay have been shown to be higher than intradermal tuberculin test in several studies [1,9,12,13]. γ-IFN is approved as an official test for diagnosis of bovine tuberculosis in New Zealand and Australia, and also has been approved that it can be used together with intradermal tuberculin test in eradication programs of bovine tuberculosis in many countries [3,6-9]. It has been reported that the use of two tests together could assist to the early detection of bovine tuberculosis in infected cattle [3,6,10,12,13]. While bovine tuberculosis could be diagnosed by intradermal tuberculin test in 3-6 weeks post infection, γ-IFN assay detects the infection as early as in 14 day post infection [9]. γ-IFN assay provides the result in 24 h after collection of blood and also removes the operators' errors [7,9]. It is a test easy to perform; it does not require farm visits to read the test result [6,7,9]. Also, there is no time period requirement to wait to repeat the test, but in case of the intradermal tuberculin test there should be 60 days interval to repeat the test. High cost of kits and incubation of heparinised blood with antigen within a few hours of collection are only disadvantages of γ-IFN assay [1,9,10].

In this study, we aimed to determine the sensitivity and specificity of the γ-IFN assay and to compare the γ-IFN assay and intradermal tuberculin test for detection of bovine tuberculosis in Burdur province of Turkey.

MATERIAL and METHODS

Animals

One hundred twenty six cows were selected from 6 herds suspected with tuberculosis in Burdur province of Turkey. Defibrined blood samples (5 ml) were collected from these animals and tested by *in vitro* γ-IFN assay. On the same animals intradermal tuberculin test was also performed.

Intradermal Tuberculin Test

A single comparative intradermal tuberculin test was performed by injecting 0.1 ml mammalian PPD (100.000 IU/ml) and avian PPD (25.000 IU/ml) (Etlik Veterinary Central Control and Research Institute, Turkey) to the skin of the animals after the clipping of hair at sites 12x6 cm in the mid-neck region of cattle. The changes in skin thickness due to swelling or indurations in injection sites were evaluated by skin thickness measurements in mm, before the intradermal injection and after 72 h.

γ-IFN Assay

A lithium heparinized blood sample (5 ml) was collected from each animal before application of the intradermal tuberculin test and brought to the laboratory within 8 h of collection. Blood samples collected from each animal were dispensed in 3x1.5 ml into a 24 well tissue culture plate. Then, 100 µl nil antigen (Phosphate Buffered Solution) as non stimulating control to the first well, 100 µl bovine PPD to the second well and 100 µl avian PPD to the third well of each sample were added, and the plates were incubated in humidified atmosphere at 37°C for 16 h. Then, plasma samples were harvested from the cultures and tested with the Bovigam ELISA test kit (Prionics, Switzerland) according to the instructions supplied with the kit.

The samples in the ELISA were run in duplicate. Positive and negative controls were used in each plate. The absorbance within 5 min of terminating the reaction was recorded using a 450 nm filter. The mean absorbance values of positive and negative controls were determined for the test validation and compared with positive and negative values provided by the kit for validation of the test (negative bovine γ-IFN control<0.130; positive bovine γ-IFN control>0.700). The mean nil antigen, avian and bovine PPD optical density (OD) for each sample were calculated and compared with the mean absorbance values of the nil antigens, avian and bovine PPD controls. A sample was considered as positive when the difference between OD value of a sample stimulated with bovine PPD and OD value of the same sample stimulated with avian PPD and nil antigen is equal or higher than 0.100. A sample was considered as negative when this difference is less than 0.100.

RESULTS

In this study, a total of 126 animals were tested by using intradermal tuberculin test and γ-IFN assay. Out of 126 animals, 67(53.2%) were found positive by the intradermal tuberculin test. Sixty-two (49.2%) of the animals were found reactive by the γ-IFN assay *(Table 1)*.

Table 1. *Results of γ-IFN assay and intradermal tuberculin test*
Tablo 1. *γ-IFN ve intradermal tüberkülin testinin sonuçları*

Number of Farm	Number of Animals Tested	Intradermal Tuberculin Test		γ-IFN Assay	
		Positive	Negative	Positive	Negative
1	23	15	8	11	12
2	9	9	-	9	-
3	36	36	-	33	3
4	8	3	5	4	4
5	5	-	5	-	5
6	45	4	41	5	40
Total	126	67	59	62	64

Two animals that were detected to be positive in γ-IFN assay were found negative by the intradermal tuberculin test. On the other hand, seven animals that were found to be positive by the intradermal tuberculin test were determined negative by γ-IFN assay. The sensitivity and specificity of γ-IFN assay were determined as 90% and 97%, respectively *(Table 2)*.

Table 2. *The sensitivity and the specificity of γ-IFN assay*
Tablo 2. *γ-IFN testinin spesifite ve sensitivitesi*

Results of γ-IFN Assay	Intradermal Tuberculin Test		Total
	Positive	Negative	
Positive	60	2	62
Negative	7	57	64
Total	67	59	126

DISCUSSION

In worldwide, intradermal tuberculin test is used as standard method for detection of bovine tuberculosis [1,4,5]. But, the sensitivity and the specificity of the test are impressed due to anergy, use of topical or systemic glucocorticosteroids, desensitization and operator errors [2,9]. Therefore, in last years, γ-IFN assay is developed and used with the intradermal tuberculin test in eradication programmes of bovine tuberculosis in many countries [6,7,9]. The intradermal tuberculin test is used as standard method for detection of bovine tuberculosis in Turkey, too. Despite eradication programmes, bovine tuberculosis is still an important infection among cattle in Turkey and high prevalence of the infection was detected in several studies [14-17]. Ozmen et al.[15] have reported the prevalence of bovine tuberculosis in Burdur as 0.38% in a histopathological study. When the size of the cattle population in Burdur is taken into account, the number of the infected animals is quite high. Thus, the detection of infected animals by more sensitive and specific diagnostic tests is very important. By this study, we aimed to determine the sensitivity and the specificity of the γ-IFN assay as a new test and to compare the γ-IFN assay and intradermal tuberculin test for detection of bovine tuberculosis in Burdur.

In many studies, it was showed that γ-IFN assay was more sensitive than the intradermal tuberculin test for detection of bovine tuberculosis [1,3,9,13]. In the present study, we have found that the sensitivity and specificity of the γ-IFN assay were 90% and 97% in comparison to skin test in suspect-tuberculosis cattle herds. These results are found similar to the results of the studies in Australia,Italy and New Zeland [1,9,13].

Several researchers have reported that γ-IFN assay negative and intradermal tuberculin test positive results may be caused by co-infection of the animal with an environmental mycobacterium or anergic situation of infected animals [7,8]. In this study, 62 out of 126 cattle (49.2%) were positive by γ-IFN assay and 67 (%53.2) by the intradermal tuberculin test. Seven of cattle were determined as negative by γ-IFN assay and positive by intradermal tuberculin test. In postmortem examination, the lesions were not seen in lymph nodules and tissue of all animals. Therefore, we thought that these false positive results in intradermal tuberculin test may be due to the operator's errors and nonpathogenic mycobacterial agents.

Gormley et al.[8] have reported that animals positive for γ-IFN assay and negative for intradermal tuberculin test will subsequently convert to tuberculin positivity and, pose an increased risk to the other cattle. Some researchers have also reported that γ-IFN assay could detect infection at earlier stage than the intradermal tuberculin test [2,7,8]. In the present study, we determined two animals positive for γ-IFN assay and negative for intradermal tuberculin. This result may be caused by early stages of infection or anergy, desensitization, and operator errors, such as insufficient injection to the skin.

In conclusion, we can state that γ-IFN assay is a test with high sensitivity and specificity and some advantages to intradermal tuberculin test for bovine tuberculosis diagnosis. It is a test easy to perform, does not require a wait period when retest is necessary for diagnosis shortens the time for diagnosis and eliminates the revisits to the farms. Thus, γ-IFN assay can be implemented to bovine tuberculosis eradication programs as an alternative to intradermal tuberculin test.

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