Biomedical Signal Processing and Control 46 (2018) 59–66

Contents lists available at ScienceDirect

Biomedical Signal Processing and Control

journal homepage: www.elsevier.com/locate/bspc

An advanced intelligent ELISA test for bovine tuberculosis diagnosis

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ARTICLE INFO

Article history:
Received 6 November 2017
Received in revised form 18 April 2018
Accepted 28 May 2018

Keywords:
Bovine tuberculosis (bTB)
Enzyme linked immuno sorbent assay (ELISA)
Intra dermal reaction (IDR)
Fisher's linear discriminant (FLD)
Artificial neural network (ANN)

ABSTRACT

The control of bovine tuberculosis (bTB) relies first on an optimal diagnosis of the disease. Several tests have been implemented for bTB detection which are generally complex, slow of use and relatively expensive especially in poor countries. A simple rapid, cost effective and efficient automated method for bTB assessment is still needed. Here, we propose a combination of the simple Enzyme Linked Immuno Sorbent Assay (ELISA) test with either the artificial neural network (ANN) analyzing method to effectively diagnose TB in cattle. The proposed method has been experimented on 30 bTB+ and 43 bTB- subjects in the north part of Tunisia, as assessed by the intra dermal reaction test (IDR). The obtained results have reached a 94% of accuracy when applying the ANN. Moreover, the proposed methodology enabled us to reduce the number of the used pathogens-derived antigens to three instead of the standard five antigens-based ELISA. Compared to previous works, the proposed expert system seems to be promising and may prove helpful for the veterinary diagnosis of tuberculosis.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) in humans, *Mycobacterium bovis* (Mbv) in cattle and *Mycobacterium avium* in poultry [1]. However, in several circumstances Mbv, the agent of bovine tuberculosis (bTB), can cause TB in human [2,3]. A prerequisite first step toward the eradication of TB consists of the efficient diagnosis of the disease. Such diagnostic method should be rapid, specific and easy to implement in low income countries where the disease is mainly present.

bTB is a main concern for both the industrialized and developing countries. It is part of World organisation for animal health (OIE) and food and agriculture organization of the United Nations (FAO) [4,5] which contains animal diseases, relevant socio-economic and/or hygienic can have serious consequences for trade and production of cow. For the most part of developing countries, Zoonotic bTB is present in animals where control activities are frequently unavailable or insufficient. 70% of OIE member countries are affected by bTB. Though rare, human tuberculosis due to (Mbv) is still a public health problem of interest to both medical and veterinary professions and there is a required for maintain attentive bacteriological surveillance. This disease cause nearly 2000 human deaths per annum (6%) worldwide [6,7]. In Tunisia, bTB is a major zoonosis [8].

The diagnostic conditions of microscopy and the limited time needed for traditional culture methods have based attention on developing rapid methods for *M. bovis* detection in clinical specimens and the early identification of mycobacterial isolates. The main common current test for bTB diagnosis, intradermal reaction test (IDR), is not a routine practice for bTB control in development countries in term to political, social and economic limitations. In spite of the fact that the IDR remains the international field diagnosis method of bTB [9], benefits of the IDR and reasons for its large use are low costs, high availability, long history of employ and, for a long time, the lack of alternative methods to detect bTB. This test allows; as location and investigation; the condition of a robust precision which is the merely validated test, and highlights the existence of a cell-mediated immunity against Mtb. Nonetheless, IDR seems to lack sensitivity [10]. Their main drawbacks are the complexity, the slowness of use for a long time and the lack of alternative methods to detect bTB. The analysis and the interpretation of the results are extremely difficult; by non specific responses induced by other species of mycobacteria. For the reason that a

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https://doi.org/10.1016/j.bspc.2018.05.031
1746-8094/

Table 1
Signification of ELISA antigens.

Antigens of ELISA test	Signification
A	Crud BCG proteins
B	The recombinant 10 kDa culture filtrate antigen (CFP-10)
C	The recombinant 6 kDa Early Secretory Antigenic Target (ESAT-6)
D	The recombinant Esat-6/CFP-10 heterodimer
E	The Tuberculin purified protein derivative (PPD)

positive skin examination result is frequently focuses on measuring small differences in skin thickness, reading of the test is likely to be subject to error. Due to known limitations, this test need for a second-step visit, low degree of standardization, and imperfect test accuracy. Immunosuppression caused by stress (manipulation, etc.) or anergy because of an advanced stage of bTB possibly account for the low sensitivity of IDR in some circumstances. A low sensitivity still constitutes a serious problem for the sustainable success of control programs.

Enzyme Linked Immunosorbent Assay (ELISA) is a simple and rapid method that has been proposed to diagnose various studies; in specific Mycobacteria-derived five antigens. Compared to other immunoassay methods, ELISA tests are more accurate. They are considered highly sensitive, specific and compare favorably with other methods used to detect the TB. ELISA possesses the added benefits of financially greatly cheap. These advantages of ELISA [11] make it a useful biotechnical tool with many applications, either in scientific research or clinical diagnosis of diseases.

In this study, an advanced development of a rapid ELISA test is reported to diagnose the bTB. A simple ELISA method of Mbv specific secreted antigens (see Table1) was firstly extracted antigens to select the most significant. New screening tests are possible to be soon available and to propose more opportunities for bTB supervision. The use of automatic analysis system with either the artificial intelligence methods deals for a reliable diagnosis tool. ELISAs; as large-scale screening tests in zoonotic countries; may be well recommended for assessing the precision of TB prevalence in cattle.

The first idea of ELISA feature optimization was presented in a previous work [12]. In this paper, an extended version of [12] is presented in which many details are considered to get accurate performances' evaluation of the proposed procedure:

- (i) Evaluation criteria of learning performance: An enhanced training network structure based on a combination between fisher linear discriminant (FLD) and artificial neural network (ANN), cross validation application and statistical analysis method.
- (ii) The used classification parameters are displayed to highlight their impact on the classification results.

This paper is organized as follows: Section 2 describes the proposed serological ELISA analysis, antigens extracted process. In Sections 3 and 4, experimental results and discussions of the entire proposed approach are reported on two a large dataset of ELISA. The conclusions are provided in Section 4.

2. Materials and methods

2.1. Database description

In this work, the coverage procedure of infected bovine species is examined in Tunisia during 2012–2014 to evaluate the tuberculosis assessment approach in this period. The data related to the control program of bTB and domestic animal population in Tunisia were taken from the Agriculture Ministry.

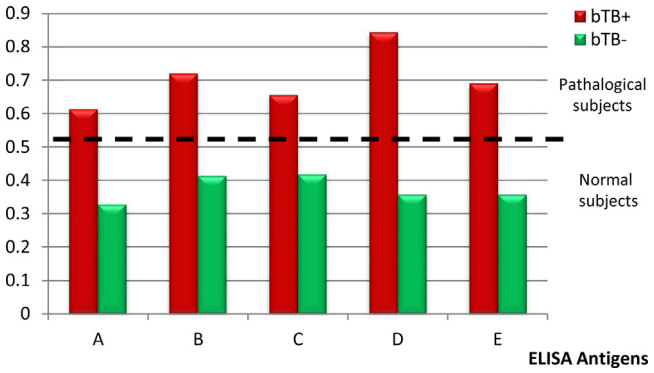

Fig. 1. Histogram of both biological tests: IDR and ELISA tests.

2.1.1. Study subjects and sampling

The study was conducted on 152 dairy cattle belonging to 8 randomly selected dairy farms in north of Tunisia. All animals whose age was more than 6 months were included. Blood samples were collected before IDR. Blood samples were taken into serum tubes (serum clot activator tubes; Vacuette; Greiner-Bio-One), transported at room temperature, and then stored at 2–8 °C until processed. Following centrifugation (3000 ˍ *g* for 30 min at 2–8 °C) the serum was removed, aliquoted, and stored at ˍ20 °C and were tested by an indirect IgG-ELISA.

All examined subjects are obtained from the Tunisian Pasteur institute, which are generated using the IDR test. Due to the removing of outcomes, only 73 topics have been used in this study. 30 bovines of them are assessed with a TB symptom (bTB+) and 43 seen normal (bTB-). In Table 2, we show some samples from the collected data base which highlight the difference between the outcomes and selected subjects used for the classification process.

From these measures, we can see clearly that there is a great accordance between the ELISA test parameters and IDR sign. The age feature (per month) was not considered in the classification system because our database contains only statistical parameters that do not contribute significantly in the categorization procedure. In fact, in the case of bTB+ (pathological subjects), the five feature values are substantially higher than to 0.5 and for bTB- (normal subjects), the ELISA responses are less than 0.5 (see Fig.1).

2.1.2. Tuberculin skin test (TST) or intra dermal reaction (IDR)

TST for diagnosis of tuberculosis were performed. Briefly, 0.1 mL of bovine PPD (2500 IU/mL bovine PPD, Bovituber; Rhône Mérieux, France) was injected in the cervical area. After 72 h, the injection site was measured with callipers. A cow was considered reactive (positive, bTB+) if a swelling >4.0 mm was detected.

2.1.3. Antigens preparations

The construct pET23b-Rv3875 (esxA) was from Karen M Dobos (Colorado State University, CO USA) under the TB research materials contract NIH, NIAID NO1-AI-40091. We have such construct to purify from *E.coli* BL21 a His-tagged form of ESAT-6 on a Nickel column. pET28-CFP-10/ESAT-6 expression constructs was a gift from Philip S. Renshaw (Department of Biochemistry, University of Leicester, Adrian Building, University Road, Leicester United Kingdom).This construct was used to express either CFP-10 alone or the hetero-dimmer ESAT-6/CFP-10. PPD from *M. bovis* was purchased from 0.1 mL of 20,000 IU/mL bovine PPD (Bovituber; Rhône Mérieux, France).