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Two step immuno chromatographic screening test based detection of bovine tuberculosis

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Abstract
The present study was conducted to detect tuberculosis at an earlier stage in the apparently healthy cattle and buffaloes in and around Namakkal using a rapid antibody detection kit. The serum samples collected from the animals were tested using a point of care diagnostic kit employing a immunochromatographic or lateral flow technology. Out of the eighty-three samples tested, only three cattle serum sample were reactive suggestive of the exposure to Mycobacterium tuberculosis complex (MTC) and the remaining eighty serum samples were non-reactive in Kit-I. Hence, the results of the test imply that these rapid diagnostic kits could be uses as a rapid, cost effective, easy to perform diagnostic method for the preliminary screening of the domestic and wild animals for tuberculosis infection in the field conditions before proceeding for the labor intensive procedures like skin testing or other diagnostic methods which are expensive, require technically qualified personnel to perform the assays and time consuming.

Keywords: Tuberculosis, reactive, non-reactive, diagnosis, bovine

Introduction
Bovine tuberculosis (bTB) is a chronic bacterial disease of animals characterized by general state of illness, pneumonia, weight loss, and eventual death. The etiological agent of bovine tuberculosis is *Mycobacterium bovis*. However, other members belonging to the Mycobacterium tuberculosis complex (MTC), like *M. tuberculosis*, *M. africanum*, *M. caprae*, *M. orygis*, *and M. microti* are known to cause bovine tuberculosis in cattle (Ramanujam and Palaniyandi, 2023) [11]. Bovine tuberculosis continues to be a serious threat for animal and human health in many developing countries including India resulting in a huge economic loss to the farmers. Cattle are the main source of infection for humans apart from other domesticated animals such as sheep, goats, equines, pigs, dogs and cats, and wildlife species such as wild boars, deer, antelopes etc., The disease is usually characterized by formation of nodular granulomas known as tubercles. Although commonly defined as a chronic debilitating disease, bovine tuberculosis can occasionally assume a more progressive course. Any body tissue can be affected, but lesions are most frequently observed in the lymph nodes (particularly of the head and thorax), lungs, intestines, liver, spleen, pleura, and peritoneum (OIE 2018) [8].

In most of the occasions, infection is often subclinical; when present, clinical signs are not specifically distinctive and are difficult for the veterinarians to arrive at the diagnosis. The tuberculin skin test based on cell mediated immune response (delayed type hypersensitivity) is considered to be the standard method of tuberculosis diagnosis in live domestic animals (WOAH). The gamma interferon test is increasingly being used as a diagnostic blood test for tuberculosis in cattle and for other animals (e.g. goat, buffalo) and is available commercially. The lymphocyte proliferation test and the IgG1 enzyme-linked immunosorbent assay (ELISA) have proven to be useful as ancillary serial (to enhance specificity) and parallel (to enhance sensitivity) tests in farmed red deer (OIE 2018) [8]. Antibody assays are convenient to perform as samples can be stored for prolonged time before processing and ensure large scale screening of samples obtained ante-mortem and post-mortem and are able to diagnose the progressive disease (McNair *et al*., 2007) [5]

The diagnosis of MTC by direct polymerase chain reaction (PCR) targeting 16S-23S rRNA, the insertion sequences IS6110 and IS1081 etc., is fast and highly sensitive, showing great value in epidemiological studies (Cedeño *et al*., 2005) [1] and, it is highly useful to reach immediate treatment decisions in some species (Mikota *et al*., 2015) [6]. Specific identification of an isolate as *M. bovis* can be made using PCR targeting a mutation at nucleotide positions 285 in the *oxyR* gene, 169 in the *pncA* gene, 675/756/1311/1410 and 1450 of the *gyrB* gene

and presence/absence of RDs (Regions of Difference) (Espinosa de los Monteros *et al*., 1998; Huard *et al*., 2003; Niemann *et al*., 2000; Parsons *et al*., 2002) [2, 4, 7, 10]. The presence of *M. bovis* in clinical and post-mortem specimens may be demonstrated by examination of stained smears (acid fast) or tissue sections and confirmed by cultivation of the organism on primary isolation medium.

However, the availability of a portable, point of care diagnosis helps the veterinarians in the rapid identification of the diseased animal and thus the adoption of appropriate prevention and control measures to contain the spread of the disease. The present study reports the detection of tuberculosis in cattle and buffalo based on a rapid antibody detection kit in apparently healthy animals.

Materials and Methods

Tuberculosis Antibody Rapid Detection Kit (Bovine TB Alert Kit)

The Bovine TB Alert Kit for *in vitro* diagnosis of Tuberculosis was provided by Translational Research Platform for Veterinary Biologicals (TRPVB), Tamil Nadu Veterinary and Animal Sciences University, Chennai.

Collection of Blood and Serum Samples

The apparently healthy crossbred and Kangayam cattle, Murrah buffaloes maintained at the Livestock Farm Complex, Veterinary College and Research Institute, Namakkal and crossbred cattle brought to Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal during the year 2016 has been screened in the study. The blood samples were collected from eighty-three bovines including seventy-one crossbred cattle, two Kangayam breed of cattle and ten Murrah buffaloes. About 5 ml of blood samples were collected from the jugular vein of the animal in a sterile vacutainer tubes and kept undisturbed in a slanting position for about two hours and in refrigerator for overnight. The tubes were centrifuged at 3000 rpm for 10 min at room temperature for the separation of the serum and stored at 4 °C until use.

Screening of serum samples

The serum samples were screened for the presence of antibodies against *Mycobacterium tuberculosis* complex using an *in-vitro* diagnostic kit "Bovine TB Alert kit" of TRPVB, TANUVAS for rapid detection of tuberculosis in bovines. The serum samples and required number of devices (Kit-I) were brought to room temperature. The device was placed on a flat surface and labelled according to the sample ID. One drop (10 µl) of serum sample without any air bubbles was drawn using a disposable dropper and added to the sample well by holding vertically. Then two drops of diluent supplied in the kit was added dropwise into the sample well and the left undisturbed for 20 minutes. The results were read at 20 minutes and interpreted.

Results and Discussion

Lateral flow test kits have great practical applicability in wildlife because of its easiness to perform and immediate test results, although their Se is limited (Thomas *et al*., 2021) [12]. The rapid antibody detection kit is a point of care diagnostic approach at field conditions where the adoption of intricate diagnostic procedures are challenging. The kit used in this study is based on immunochromatographic or lateral flow technology in which a cocktail of tuberculosis specific recombinant proteins and crude protein purified derivatives are bound to the membrane solid phase. The gold conjugated with carrier molecules are used as detection system.

The results were interpreted as non-reactive (negative) if there is a development of single colored line in the control (C) area and no visible colored line in the test area of Kit -I which indicates the absence of antibodies to either *M. tuberculosis or M. bovis*. The results were interpreted as reactive (positive) if there is a development of three colored lines, one in the control (C) area and two visible colored lines in the test area (T-I & T_2) of Kit -I indicate the presence of antibodies to either *M. tuberculosis and or M. bovis*. The development of a very faint line in the test area of the kit within 20 minutes indicates the tuberculosis (MTC) infection. The development of three visible colored lines, one in the control (C) area and two visible colored lines in the test area of Kit -II (T-I & T_2) indicate the presence of tuberculosis antibodies.

Interpretation Chart

Kit-I results		Kit-I results		Result
T_1	T_2	T_1	T_2	
Line	Line	No need to use kit 2		TB sero-positive
No line	Line			TB sero-positive
No line	No line			TB sero-negative
Line*	No line*	No line	No line	TB sero-positive/ Inconclusive

* This needs to be tested with kit 2 for reconfirmation

Out of the 83 serum samples tested using the tuberculosis antibody rapid detection kit, three samples collected from cattle were reactive in the Kit -I showing the development of colored line in T_2 along with the control line in one sample and development of colored line in T_1 as well as T_2 along with the control line in two samples respectively. These reactive serum samples when tested using the Kit-II to confirm the presence of MTC antibodies revealed to be non- reactive. The results of the study provide a preliminary hint on the exposure of animals to the Mycobacterium tuberculosis complex.

Based on the results of the rapid diagnostic kit, a confirmatory test like tuberculin test using purified protein derivative (PPD) or interferon gamma release assay (IGRA) to assess the cell mediated immune response which are critical in the mycobacterial infection could be employed. Though the skin test, an ante-mortem TB diagnostic test is efficacious in definitive diagnosis of TB, it is not usually applicable to wildlife due to the need to handle animals twice over a 2-3-day interval (Palmer *et al*., 2001) [9].The recent addition to the ante mortem diagnosis of TB in wild animal similar to that of IGRA is the quantification of IFN-γ-inducible protein 10 (IP-10) which is a chemokine induced by IFN-γ that plays a role in type IV hypersensitivity reactions (Goosen *et al*., 2016) [3].

Conclusion

The Bovine TB Alert Kit could be deployed as a user friendly, rapid diagnostic kit for the preliminary detection of Mycobacterium tuberculosis complex in animals in the field level.

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