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Development of BruAb2_0168 based isothermal polymerase spiral reaction assay for specific detection of *Brucella abortus* in clinical samples

Marcia Ashmi [a], Bablu Kumar [a,*], Ravi Kant Agrawal [b], Chandan Prakash [c], Abhishek [d], Karam Pal Singh [c]

[a] Division of Biological Products, ICAR-Indian Veterinary Research Institute, Bareilly, UP, India
[b] Division of Livestock Products Technology, ICAR- Indian Veterinary Research Institute, Bareilly, UP, India
[c] Centre for Animal Disease Research and Diagnosis, ICAR- Indian Veterinary Research Institute, Bareilly, UP, India
[d] Division of Bacteriology and Mycology, ICAR- Indian Veterinary Research Institute, Bareilly, UP, India

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ABSTRACT

Bovine brucellosis, predominantly caused by *Brucella abortus* is one of the most neglected zoonotic diseases causing severe economic losses in the dairy industry. The early and precise diagnosis of the disease is required to reduce the transmission of infection in humans as well as animals. In the current study, a rapid and novel isothermal amplification-based polymerase spiral reaction (PSR) was developed for the specific detection of *Brucella abortus* by targeting the BruAb2_0168 gene. The assay could be conducted at 65 °C in a water bath and results can be obtained after 60 min. The detection limit of the PSR assay was found to be 1.33 fg. The sensitivity of the assay was found to be 10^4 fold higher than conventional PCR and equivalent to real-time PCR (RT-PCR). The assay didn't exhibit cross-reaction with selected pathogenic non-*Brucella* bacteria and *Brucella* spp. other than *B. abortus*. Forty clinical samples were also tested using this novel assay and it was able to detect 25 samples as positive, however, conventional PCR could detect the targeted organism in 22 samples only.

To the extent of our knowledge, this is the first report towards the development of a PSR assay for specific detection of *B. abortus*. The assay can be used as a quick, sensitive and accurate test for the diagnosis of bovine brucellosis in the field setting. Relatively one of the paradigm-shifting aspects of this assay would be it does not require any expensive equipment and the results can be easily visualized by the unaided eye, therefore making PSR a valuable diagnostic tool in field conditions.

1. Introduction

Brucella abortus is an infectious zoonotic pathogen mainly causing bovine brucellosis. Brucellosis in bovine is one of the most neglected diseases which causes a huge economic loss to the animal health sector and is endemic in India [1]. The disease is mainly characterized by abortion, weak offspring and infertility in females and orchitis, vesiculitis and epididymitis in the males [2]. During disease progression, animals naturally recover from the infection but it continues to shed *Brucella* organisms in products of conception, vaginal discharge, feces, milk, and semen. All these products serve as a possible source of transmission of the disease to other animals and humans [3–5]. Humans are likely to get infected with *B. abortus* through consumption of unpasteurized milk/dairy products and handling of infected animal tissues. In the case of laboratory workers, the infection occurs by inhalation or through a breach in the skin [6]. Since there is no vaccine available for humans against brucellosis, diagnosis, segregation of infected animals and vaccination of the animals are the only rationale approach for the prevention and control of human brucellosis.

Thus, the early and accurate identification of the specific causative agent is important to formulate and implement appropriate control measures against brucellosis [5]. Serological tests are one of the widespread methods used for the screening of animals for brucellosis. However, they fail to detect early infections, asymptomatic heifers with latent infection [7,8], and animals with chronic infection [9]. Such animals act as a carrier of infection to other susceptible animals. Thus, for confirmatory diagnosis and to limit further transmission of infection, serology must be complemented with antigen-based detection assays viz. bacteriological or molecular diagnosis [10].

The isolation of an organism is the gold standard assay. However, it is

* Corresponding author. ICAR- Indian Veterinary Research Institute, Bareilly, UP, India.
E-mail address: babbacteriol@gmail.com (B. Kumar).

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