Journal of Microbiological Methods 162 (2019) 83–85

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

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# Development of the Com1 synthetic peptide-based Latex Agglutination Test (LAT) and its comparative evaluation with commercial indirect-ELISA for sero-screening of coxiellosis in cattle

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ARTICLE INFO

*Keywords:*
Com1
Coxiellosis
LAT
Synthetic-peptide

ABSTRACT

A novel Com1 synthetic peptide-based latex agglutination test (LAT) was developed and evaluated against commercial ELISA kit for sero-screening of coxiellosis in cattle. The developed test is economical, has field applicability and can serve as an important rapid tool for sero-screening of coxiellosis in cattle.

## 1. Introduction

Q fever (or coxiellosis), caused by obligate intracellular, Gram-negative bacterium *Coxiella burnetii*, has been classified under category-B bioterrorism agent, and is placed among 13 priority zoonoses (Grace et al., 2012). Cases of Q fever have been reported ubiquitously, except New Zealand (Eldin et al., 2017). Ruminants serve as the major reservoirs of the pathogen. The disease in ruminants is frequently subclinical, but late abortions, stillbirths and reproductive disorders can occasionally be noticed (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; Skultety et al., 2011; Ortega-Mora, 2012; Freick et al., 2018).

The clinical diagnosis of coxiellosis is based on serological tests such as enzyme-linked immunosorbent assay (ELISA), indirect immune fluorescence assay (IFA), and complement fixation test (CFT) (OIE, 2018). However, these assays have limitations such as multifaceted protocols, inconsistent sensitivity and specificity (Bacarese-Hamilton et al., 2004), and necessitate technical expertise. Besides, these tests require purified *Coxiella* organisms which are difficult and risky to isolate and purify (Fournier et al., 1998; Xiong et al., 2012). Hence, it is noteworthy to identify novel sero-reactive proteins for the development of quick, safe and specific diagnostic assays instead of conventional serological tests (Xiong et al., 2012).

Currently synthetic peptide-based diagnostic tests have been devised as novel molecules for rapid and reliable diagnosis of infectious diseases of microbial and parasitic origin (Prado et al., 2018). The major advantages of using synthetic peptide(s) as antigen compared to the whole antigen include their ease and low cost of production, higher specificity, reproducibility with no batch-to-batch variation and further large scale production (Noya et al., 2003; Gomara and Haro, 2007; Launois et al., 2010; Kashyap et al., 2013). The use of synthetic peptide (s) as a specific antigen in developing several diagnostic tests has been employed (Kashyap et al., 2013) for the reliable detection of important bacterial agents namely, *Escherichia coli* (Modrow and Wolf, 2002), *Listeria monocytogenes* (Shoukat et al., 2013), *Mycobacterium tuberculosis* (Kashyap et al., 2013). Furthermore, Latex Agglutination Test (LAT) has more advantages as compared to i-ELISA in terms of low input cost, ease of performing the experiment and quick and easy interpretation of results (Molina-Bolívar and Galisteo-González, 2005; Farahmand et al., 2018). A number of latex based assays have been developed in the recent past for immune-detection of infectious pathogens (Thomas et al., 2017; Ábrók et al., 2018; Tsutsumi et al., 2018). However, to the best of our knowledge, synthetic peptide(s)-based diagnostics for serodiagnosis of coxiellosis in animals have never been documented. The objective of the present study was to develop a Com1 synthetic peptide-based LAT. The developed Com1 synthetic peptide-based LAT was further evaluated for its performance by comparing with commercial indirect ELISA kit (BioX Diagnostics, Belgium) using serum samples collected from

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https://doi.org/10.1016/j.mimet.2019.05.012
Received 17 May 2019; Accepted 24 May 2019
Available online 27 May 2019
0167-7012/ © 2019 Published by Elsevier B.V.

cattle ($n$ = 298). The *com1* gene is highly conserved among the strains of *C. burnetii* (Zhang et al., 1997) and encodes for a 27 kDa outer membrane-associated immunoreactive protein (Xiong et al., 2012; Flores-Ramírez et al., 2017). This Com1 protein is found in both acute and chronic disease stages of *C. burnetii* infection (Zhang et al., 1997). Also, reports on detection of IgG antibodies in human patient sera have been documented using a partially purified rCom1 antigen (Zhang et al., 1998; Chen et al., 2014).

The synthetic peptide employed in this study was designed employing the *com1* gene sequence (Accession No. CP020616) of *C. burnetii* RSA 493 from NCBI database. The retrieved outer membrane protein (*com*1 gene) sequence from NCBI database was subjected to identification of trans-membrane regions using TOPCON software (http://topcons.net). These identified peptides were further screened for their antigenicity (B-cell epitopes), surface probability, hydrophilicity plot, and turn and flexibility scores using protean module of DNAStar software. Based on the best score of above parameters and protein BLAST analysis a Com1 peptide which showed very high specificity was selected (QALQKKTEAQQEEHAQQAIKENAKK-Amidated) and finally got synthesized commercially (Shanghai Science Peptide Biological Technology, China). The purity of the synthesized peptide was checked (≥95%) using HPLC. The peptide was then resuspended in PBS (final stock concentration of 10 mg/mL) and stored at -20 °C until further use.

In brief, the latex test reagent was prepared using varying concentration (1.0%, 1.25%, 1.5%) of carboxy blue dyed latex beads (Cat#: 19120, Polysciences, USA) and varying concentration (50 µg/ml, 100 µg/ml, 200 µg/ml) of the com1 synthetic peptide. The conjugation of latex beads with com1 synthetic peptide was performed using covalent coupling method as described earlier (Charleux et al., 1992; Bale et al., 1989). The test was optimized by using varying volumes of test reagent and known positive and negative test sera supplied with commercial ELISA kit (Bio-X Diagnostics, Belgium). In brief, 10 µl of conjugated latex reagent was dispensed in each well of a concavity glass slide. Then equal volume (10 µl undiluted sera) of known-positive and the known-negative sera were added to wells and the contents were mixed properly with the help of sterile tooth picks. The glass plate was then manually rotated in clock-wise and anti-clockwise directions for about 1 min to observe for visible agglutination, if any. In case of positive serum sample, the agglutination was observed within one minute as clear-cut coloured flakes accumulating on the periphery of the wells, while, in case of negative serum, there was no agglutination. Based on several optimization trials, latex beads (1.25%) coated with com1 synthetic peptides (100 µg/ml) and serum samples showed optimal visible agglutination pattern within 1 min with known-positive serum (Fig. 1). Auto-agglutination of the prepared latex reagent was also verified using phosphate buffered saline.

The developed Com1 synthetic peptide-based LAT assay was further evaluated for its performance by comparing with commercial iELISA kit (BioX Diagnostics, Belgium) using serum samples collected from cattle ($n$ = 298) housed in cattle shelters of Rajasthan, India. In brief, cross-bred (*Bos taurus* x *Bos indicus*) cattle were analyzed in the present study. The blood samples from the cattle were collected aseptically by following ethical guidelines under veterinary supervision. Briefly, aliquots of blood samples (3 ml) were collected aseptically into 5 ml capacity tubes of BD Vacutainer® spray-coated $K_2$EDTA tube for blood analysis and BD Vacutainer® SST II Advance (Vacutainer®, Becton Dickinson, Franklin Lakes, USA) for serum separation. The collected samples were transported to the laboratory in a pre-chilled container containing ice from the place of their collection and processed as soon as possible. In order to collect sera, the clot activator tubes (BD Vacutainer® SST II Advance) containing 5 ml blood samples were kept at 4 °C and then centrifuged at 2500 x*g* for 10 min for separating serum. The collected serum samples were divided in two aliquots and finally stored at −20 °C until further use. The commercial i-ELISA kit used for comparison had a sensitivity and specificity of 100% and 99.49%, respectively. Initially the sera were first screened by employing commercial i-ELISA, which exhibited a seropositivity of 14.09% (42/298) for coxiellosis. However, on screening the same sera by the developed LAT test, a higher seropositivity of 31.87% (95/298) was found. The results are presented in Supplementary Table 1. The relative diagnostic efficacy (relative diagnostic sensitivity and specificity) of the developed LAT was calculated by using 2 × 2 contingency table using commercial i-ELISA as reference test. The relative diagnostic sensitivity for the developed LAT test was found to be 76.19%, while, relative diagnostic specificity was 75.39%. The relative sensitivity of LAT assay observed in our study was in agreement with earlier reports, wherein the relative diagnostic sensitivities ranged between 35% to 97% for differential diagnosis of *Mycobacterium bovis* and *Mycobacterium avium* subsp. *paratuberculosis*; diagnosis of scrub typhus, detection of norovirus and diagnosis of enteropathogenic and enterohemorrhagic *Escherichia coli,* (Koo et al., 2004; Wongchotigul et al., 2005; Lee et al., 2010; Rocha et al., 2014), however, the specificity observed in our study (75.39%) was found to be lesser than the earlier reports (Koo et al., 2004; Wongchotigul et al., 2005; Lee et al., 2010; Rocha et al., 2014). Moreover, a fair agreement between these two tests was observed in this study (κ = 0.34). Further, we performed approximate cost analysis of both the tests. The cost involved to analyse a single serum sample by the developed LAT test was nearly ten times cheaper than the commercial i-ELISA test, although the developed test was moderate in relative diagnostic sensitivity and specificity. Moreover, it is rapid and can be performed under field conditions as an initial screening test at ease.

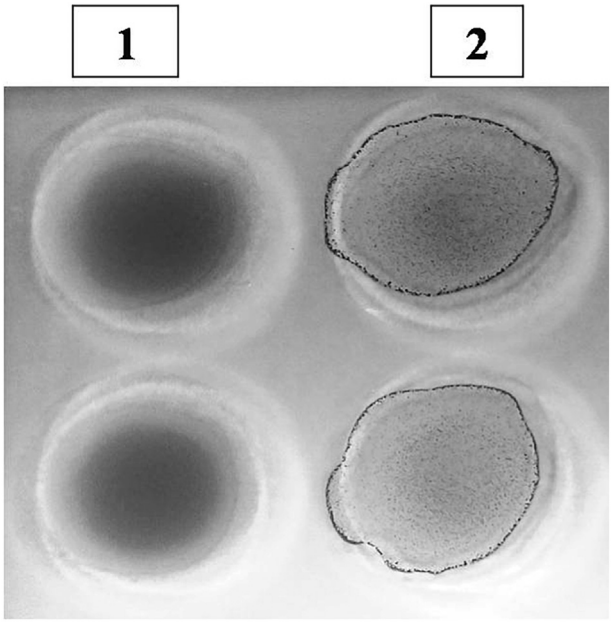


**Fig. 1.** Com 1 synthetic peptide based Latex Agglutination Test; Lane 1: Known-Negative sera, Lane 2: Known- Positive sera.

To conclude, our study explored the utility of the Com1 peptide based LAT as an alternative to the conventional serological assays for serodiagnosis of coxiellosis for the first time. Further research is warranted to improvise the sensitivity and specificity of the developed assay, either by selection of another peptide of the, com1 protein, or any other protein which are reported to be immunogenic, such as chaperonin GroEL, Chaperone DnaK and 34 kDa outer member protein (ybgF) (Skultety et al., 2011; Xiong et al., 2012). It would be rational to evaluate the efficacy of this approach using more number of serum samples and other commercially available ELISA kits.

The authors thank Director, ICAR- Indian Veterinary Research Institute, Izatnagar for providing facilities to carry out the research. The research is supported by grants from the Indian Council of Agriculture

Research-Outreach Programme on Zoonotic Diseases (Grant no.1000494) to SVSM. The technical assistance by Mr. K.K. Bhat and Dr. Deepa Ujjawal is duly acknowledged.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2019.05.012.

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