DEVELOPMENT AND EVALUATION OF A LATEX AGGLUTINATION TEST FOR THE DETECTION OF *MYCOPLASMA GALLISEPTICUM* ANTIBODIES IN CHICKEN SERA

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ABSTRACT
A rapid whole cell protein antigen (iron restricted *Mycoplasma gallisepticum*) based latex agglutination test (LAT) was developed to detect the presence of anti-*Mycoplasma gallisepticum* antibodies in the sera of poultry affected with mycoplasmosis. The test was found to be sensitive, specific and accurate in comparison with Haemagglutination Inhibition (HI) test. The rapidity, simplicity and economics of the LAT were found to fulfill the requirements of a screening test for *Mycoplasma gallisepticum* antibodies.

Key words: Latex agglutination test, *Mycoplasma gallisepticum* antibodies.

INTRODUCTION
Avian mycoplasmosis is one of the economically significant diseases affecting poultry. The pathogenic *Mycoplasma* species affecting poultry are *M. gallisepticum*, *M. synoviae*, *M. iowae* and *M. meleagridis*. Among this, *M. gallisepticum* is the most pathogenic and occur world-wide, causing chronic respiratory disease in chicken and infectious sinusitis in turkeys (Carpenter et al., 1981).

The most reliable method of diagnosis of mycoplasmosis is the isolation and identification of pathogens from infected birds, but this is laborious, time consuming and therefore far from a routine procedure (Zain and Bradbury, 1996). Serological monitoring system for the detection of antibodies against *M. gallisepticum* is widely used in flock screening. The most commonly used tests are the rapid serum agglutination (RSA), the enzyme-linked immunosorbent assay (ELISA) and HI tests. Another immunological detection method in use is the latex agglutination test, which is a simple macroagglutination test that combines sensitivity with low cost and ease of application in the field conditions, without the need for any trained specialist or equipment.

Iron is an essential mineral for almost all living systems and serves as a cofactor or prosthetic group for many essential enzymes involved in basic cellular functions. Bacteria have evolved several mechanisms for the uptake of iron from host cells which include the regulation of various genes in the bacterial genome, which in turn may result in the up or down regulation of various immunogenic proteins (Salyers and Whitt, 2002).

Madsen et al. (2006) conducted a transcriptional profiling of *Mycoplasma hyopneumoniae* during iron depletion (2,22 - dipyridyl at 1 mg/ml used for iron chelation) using micro arrays and identified that about 27 genes were either up or down regulated in response to low-iron growth conditions. These included genes encoding transport proteins, enzymes involved in energy metabolism, and components of translation process.

The standardization, application and usefulness of latex agglutination test to detect *M. pneumoniae* antibodies was investigated and compared with tetrazolium reduction inhibition and complement fixation and found that latex agglutination procedure was comparable with these tests for evaluating vaccine efficacy (Kende, 1969). Rurangirwa et al. (1987) sensitized latex beads with a polysaccharide isolated from a F38 culture supernatant and used it in a slide agglutination test to detect serum antibodies in goats with contagious

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caprine pleuropneumonia. The results were compared with that of complement fixation test. They found that besides being more sensitive than complement fixation, the latex agglutination test could be performed in the field using undiluted serum or whole blood and a result was obtained within two minutes.

A protocol for the sensitization of latex beads with antigen using carbonate-bicarbonate buffer was described by Ramadass et al. (1999). They indicated that the method was found to be very sensitive and the sensitized beads could be stored at 4°C for about four months and at room temperature for two months, without loss of antigen activity. Ramadass et al. (2007) developed a LAT using *M. gallisepticum* PG 31 strain and compared it with ELISA. They found that LAT and ELISA were of equal sensitivity.

Hence, the present study was undertaken to develop and evaluate whole cell protein based latex agglutination test using locally predominant strain of *M. gallisepticum* cultured in an iron restricted medium, for the rapid detection of *M. gallisepticum* antibodies.

**MATERIALS AND METHODS**

**Preparation of iron restricted whole cell proteins:** Four 500 ml flasks, each containing 250 ml cultures were incubated at 37°C to early exponential phase, as determined by a change in colour of media and optical density. To two of the four flasks, 2, 2′-dipyridyl was added to a final concentration of one mg per ml for iron chelation. The remaining two flasks were left untreated and all of the flasks were incubated for 2 h at 37°C . Local isolates of *Mycoplasma gallisepticum* from University Poultry Farm, Thrissur, were used for the study. *Mycoplasma gallisepticum* grown under iron sufficient and iron restricted conditions, were harvested at the late exponential phase of growth by centrifugation at 12,000 × g for 15 min at 4°C, washed once and re-suspended in 0.25 M NaCl.

Log phase cultures of *M. gallisepticum* cells grown under iron sufficient and iron restricted conditions were harvested by centrifugation at 12,000 × g for 30 min at 4°C and were washed three times with PBS. The *M. gallisepticum* cells were disrupted by sonication at constant pulse for 30 seconds with 30 seconds break. The sonication was repeated for 19 times.

**Source of serum samples:** Serum samples were collected from birds showing respiratory ailments at University Poultry Farm, College of Veterinary and Animal Sciences, Mannuthy.

**Latex agglutination test:** Latex beads (Sigma, 0.88 μm) were used. Latex bead suspension (10 per cent) was washed twice by centrifugation at 6700 × g for three minutes each, in carbonate-bicarbonate buffer. Finally the latex beads were made into a two per cent suspension with carbonate-bicarbonate buffer, which was later mixed with an equal volume of *M. gallisepticum* iron restricted whole cell protein antigen (25 μg/ml) diluted in the same buffer. The mixture was incubated at 37°C for six hours with constant shaking. The sensitized beads were centrifuged at 6700 × g for three minutes and the pellet was re-suspended as a two per cent suspension in PBS containing five mg/ml of bovine serum albumin. The latex beads were left at 37°C in a water bath overnight. Finally, the beads were centrifuged as before and the pellet was re-suspended in PBS containing 0.5 mg/ml of BSA and 0.1 per cent sodium azide. Sensitized latex beads were stored at 4°C until use. The stability of latex beads was determined by storage at 4°C and at ambient temperature.

The LAT was performed on a glass slide by mixing equal volumes (20 ml) of serum sample and sensitized latex beads. The slide was rocked gently for two to five minutes. PBS and normal chicken sera were used as negative controls and rabbit anti-*Mycoplasma gallisepticum* hyper-immune serum was used as positive control. A total of 30 sera samples were subjected to LAT.

Results were read on a +1 to +4 scale, depending on the extent of agglutination and time taken for the development of agglutination. The serum samples were considered to be negative if no agglutination was observed within five minutes.

+ + + + : heavy flocculent agglutination formed immediately with clear back ground
+ + + : heavy flocculent agglutination taking 1-2 min to form with clear back ground
+ + : light flocculent agglutination against mostly clear back ground occurring in 2-3 min
+ : light flocculent agglutination against cloudy homogenous background after extended incubation
The relative sensitivity, specificity and accuracy of the test were determined in comparison with Haemagglutination inhibition test, which was performed as per OIE (2008).

Sensitivity = \( \frac{a}{a+c} \times 100 \) where ‘a’ = no. of sera positive by HI & LAT and ‘c’ = no. of sera positive by HI but negative by LAT

Specificity = \( \frac{d}{b+d} \times 100 \) where ‘d’ = no. of sera negative by HI & LAT and ‘b’ = no. of sera negative by HI but positive by LAT

Percentage agreement of LAT with HI was found out by means of kappa statistics denoted by ‘k’:

\[ k = \frac{a+d-P}{1-P} \]

where ‘P’ is the probability.

**RESULTS AND DISCUSSION**

The latex agglutination test positive samples were graded (Table 1). A total of 30 serum samples were tested using LAT and the results were compared with HI test (Table 2).

The sensitivity and specificity of the test were found to be 95.24 per cent and 93.33 per cent respectively. A kappa value of greater than 0.81 was obtained indicating that LAT was in agreement with HI.

Latex agglutination test was carried out using latex beads sensitized with sonicated *M. gallisepticum* iron restricted whole cell proteins. Hyper immune serum against *M. gallisepticum* whole cell proteins raised in rabbits was kept as positive control and phosphate buffered saline as negative control. A total of 30 serum samples from chickens were used for the test. The results were graded and compared with HI test. The LAT got a 95.24 per cent sensitivity which indicated that the LAT failed to detect two HI positive samples as positive. The specificity was 93.33 per cent which means that one sample negative in HI came positive in LAT. This was in agreement with Ramadass et al. (2007), who developed a latex agglutination test using *M. gallisepticum* PG 31 strain and compared it with ELISA. He found that LAT and ELISA were of equal sensitivity.

Because of the smaller size of *Mycoplasma* cells, rapid serum agglutination test requires large number of cells to form visible aggregates or aggregates which will settle readily by gravity. Absorbing *Mycoplasma* cells to carrier particles like latex offers a means of increasing the mass of the cells and, at the same time, maintaining the specificity of the *Mycoplasma* surface antigens (Morton, 1966).

**REFERENCES**


