



# On-ranch finding of infectious bovine pleuropneumonia in roaming cows utilizing latex agglutination test (LAT)

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Abstract

Three outbreaks of contagious bovine pleuropneumonia (CBPP) in Kafur Local Government Area of Katsina State, involving a total of 250 animals were reported. All the animals were examined thoroughly and based on the clinical signs exhibited, 120 (group A, n= 51, B, n=29 and C, n=40) animals were selected for latex agglutination test (LAT), using serum from each of the animal. The results indicate infection rates of 11.8% (6/51) in adult animals and 19.6% (10/51) in calves in farm A, while in farm B the infection rate were 27.6% (6/29) for the adult animals and 13.8% (4/29) for calves. While in farm C, 15.0% (6/40) of the adult animals and 17.5% (7/40) of the calves are positive, as demonstrated by strong (+++) agglutination in cell 1 (Plate 2). Overall infection rate of 16.7 and 17.5% was observed for adult and calves respectively. Although this diagnostic test LAT is not new, this is the first time it's being conducted in the area of study for the diagnosis of CBPP.

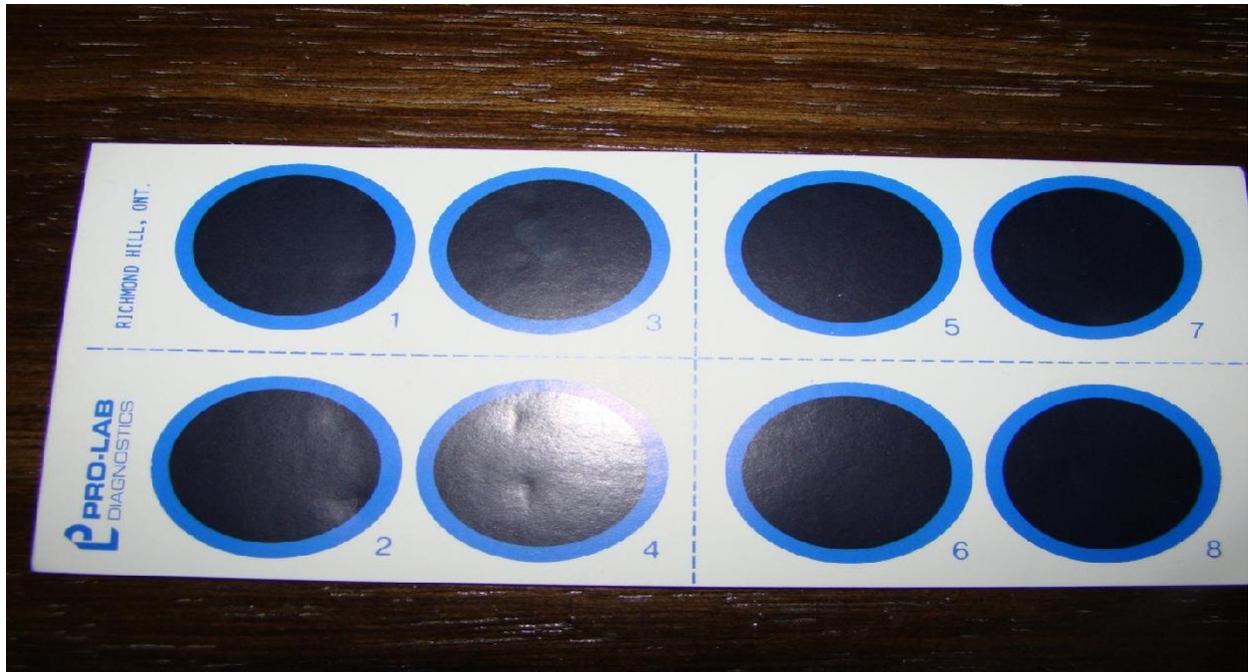
**Keywords:** *Mycoplasma mycoides* sub sp. *Mycoides*, nomadic cattle, latex agglutination test

## INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a highly contagious disease of cattle caused by *Mycoplasma mycoides* subspecies *mycoides* small colony (MmmSc) (Osiyemi, 1981; Provost et al., 1987; Taylor et al., 1992; Terlaak et al., 1992). The disease is characterized clinically by severe coughing, weakness, emaciation and sometimes by elevated body temperature (Provost et al., 1987; Egwu et al., 1996). Transmission occurs from direct and repeated contacts between sick and healthy animals. The first incidence of the disease in Nigeria was recorded in 1924 when reliable records were first available (Foluso, 2004). As at today the disease is endemic in Nigeria, West, Central, East and parts of Southern Africa (Tambi et al., 2006).

In Nigeria, "alive with the disease" attitude has prevailed in the last few years, farmers hardly report cases but resort to treatment with antibiotics like any other bacteria disease (Chima, 1999, 2001). Inadequate funding of cattle annual mass vaccination program, lack

of a rapid on farm screening test to aid sero-monitoring, and refusal of some farmers to allow vaccination of their animals due to post-vaccinal cellulitis, have largely contributed to the spread of the disease rendering data on infection of the disease within the country inaccurate and subjective (Chima, 2001; Molokwu, 2003). Diagnosis of CBPP in most developing countries of Africa is presently based on culture and isolation of the causal agent (which is fastidious and slow growing), serology and post-mortem (PM) examination of lungs of affected animals. Though the complement-fixation test (CFT) is commonly used as a diagnostic method in most CBPP-endemic countries of Africa, its sensitivity in detecting chronically affected animals is low (Provost et al., 1987; Nicholas et al., 1996). The inability of CFT to discriminate between natural and vaccinal exposures in animals has led to a greater reliance on PM examination of lung lesions for monitoring and surveillance of CBPP in Nigeria.



**Plate 1.** Showing the individual cells for the agglutination.

The current Office International des Epizooties-prescribed test for the diagnosis of CBPP is the modified CFT (Campbell, A.D., and Turner, 1953; OIE, 2002). Although the test is highly specific, it is relatively expensive to perform, it is slow, and it requires trained personnel and laboratory facilities. In addition, it is less effective at diagnosing animals in the early stages of the disease or of animal with chronic lesions (OIE, 2002). A number of more modern tests have recently been described, including biochemical (Rice et al., 2000), indirect and competitive enzyme-linked immunosorbent assay (ELISA) (Le Goff Thiaucourt, 1998; Nicholas et al., 1996), immunoblotting (Nicholas et al., 1996; Regalla et al., 2000), and PCR (Bashiruddin et al., 1994; Miserez et al., 1997).

Efforts currently being made in Nigeria for the eradication of CBPP involve vaccination backed up with abattoir surveillance. The test and slaughter policy recommended by the Office of Internal Epizootics (OIE) for seropositive animals is currently not practicable because of the cost involved in the payment of compensation to livestock owners. Presently, in Nigeria, the extent and pattern of CBPP prevalence is largely unknown. The aim of this paper is to report on-field-diagnosis of CBPP using latex agglutination test (LAT). LAT (Developed by Veterinary Laboratories Agency, New Haw, Addlestone. Surrey, KT15 3NB United Kingdom). This test is a rapid test for the diagnosis of CBPP. It has the advantage of not requiring electricity so quite suitable for third world countries. It is believed that this test can be beneficial in determining the extent of CBPP in Nigeria.

## MATERIALS AND METHODS

Three herds A, B and C with 99, 58 and 93 Bunaji breeds of cattle respectfully located in Kafur Local Government Area (LGA) of Katsina State, Northern Nigeria were used for these studies following a reported outbreak. One hundred twenty cattle that showed clinical signs similar to CBPP (fever mucopurulent nasal discharge, shallow, grunting respiration, painful coughing, arched back, head extended towards the direction of wind with abducted forelimbs and exercise intolerance) were clinically examined out of a total of 250 cattle in the three herd. Five milliliters of blood were collected from 120 cattle of mixed sex, and placed in a vacutainers without anticoagulant for serum extraction, the separated sera were screened on-farm for the presence of antibodies to *Mycoplasma mycoides* subspecies *mycoides* small colony (MmmSC) using LAT-PA6223 (LAT) as described by Ayling et al. (1999). Figure 1 showing the agglutination slides.

### Recording and interpretation of results

Slide test result will be recorded as follows after three minutes:

- i) Positive (+++). Strong clumping of latex beads. Agglutination should begin within one minute.
- ii) Positive (++) . Clear agglutination of the latex beads. Agglutination should begin within one and two minute.
- iii) Positive (+). Fine agglutination of the latex beads between two and three minutes.
- iv) Negative (-). No agglutination within three minutes (Plate 1).

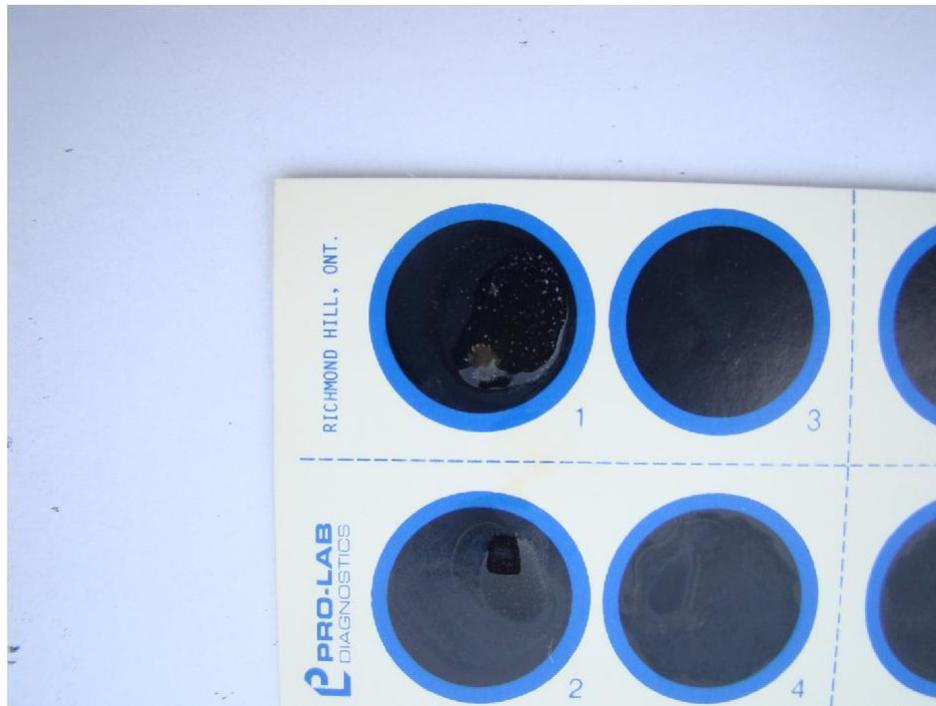
## RESULTS

Fifty one cattle were screened in herd A, made up of 34

**Table 1.** Detection of antibodies to MmmSC in Nomadic Cattle using LAT in Kafur L.G.A., Katsina State.

Herd	Herd size	No. screened	Calves	Adults	No.+ve (Calves)	No. +ve (Adults)
20 A	99	51	17	34	10(19.6%)	6(11.8%)
B	58	29	10	19	4(13.8%)	8(27.6%)
C	93	40	15	25	7(17.5%)	6(15.0%)
Total	250	120	42	78	21	

Calves = 3-12 Months, Adults =>12 Months.



**Plate 2.** Showing positive (+++) strong clumping of latex beads in cell 1.

adults and 17 calves, 11.8% (6/51) of the Adult animals were positive while 19.6% (10/51) of calves were also positive for contagious bovine pleuropneumonia (CBPP) using LAT. In herd B, a total of 29 cattle, consisting of 19 adult animals and 10 calves were screened for CBPP antigen out of which 27.6% (8/29) adults animals were positive, and 13.8% (4/29) of calves were also positive. In herd C, out of a herd of 40 cattle (25 adult and 15 calves) screened 15.0% (6/40) of the adults were positive and 17.5% (7/40) of the calves tested positive (Table 1). The agglutination observed in cell 1 (Plate 2) is due the fact that when the latex beads coated with capsular polysaccharide purified from *Mycoplasma macoides* subspecies *mycoides* cells are mixed with blood or serum from animal suspected or suffering CBPP, antibodies recognizing the capsular polysaccharide will bind and cross-link the latex particles causing agglutination (Plate 2).

## DISCUSSION

*M. mycoides* subsp. *mycoides* small colony is the etiological agent for a potentially lethal lung disease of cattle called CBPP (Thomson, 2005). The high economic impact of this disease on the cattle industry necessitates the development of rapid, sensitive and specific diagnostic assays. Ayling et al. (1999) developed the LAT for rapid diagnosis of CBPP on the field. The dynamics of infection of CBPP in herds of cattle in endemic areas appears to be more complicated than would be presumed for a directly transmitted infection with a single host species. In this study an overall infection rates of 16.7% in adults and 17.5% in calves out of 120 animals screened indicates probably a low infection rates for a disease that is transmitted through contact, ingestion and aerosol. This report is at variant with that of McDermott et al. (1987) and Dasho (2001) were they reported

serological values of 8.1 to 9.2% infection rates in southern Sudan and Ethiopia. The low values observed in this study could also be attributed to the inability of the test LAT to diagnose convalescent animals, this is partly because high level of circulating capsular polysaccharide antigen can lead to false-negative due to antibody masking effects (March et al., 2003). This study observed a slight increase though not statistically significant ( $P < 0.005$ ) in the susceptibility of calves to CBPP infection over adult, this findings is in agreement with the report of Masiga and Windsor (1978) where they reported that during an epidemic, the morbidity and mortality rates were higher in calves than adult animals, and that where the infection had been present for some time, morbidity was higher in adult animals but mortality was higher in calves. In naïve populations, calves do not appear to possess a higher level of resistance than older animals (Thiaucourt et al., 2004).

The continued presence of this important disease in Nigeria is attributed to diminished control due to the incomplete and irregular vaccination programme over the years as well as steady illegal introduction of infected cattle into these areas across the control barriers (particularly through transhumance and nomadism). Moreover, the presence in some herds of carrier animals, which might not be detected clinically or serologically could enhance the maintenance of the disease in these areas (Aliyu et al., 2000), therefore, in a third world country like Nigeria, the test LAT will allow for rapid and inexpensive primary herd screening prior to confirmatory laboratory diagnosis (using PCR or ELISA) and this will help in early recognition of the disease and allows appropriate control measures to be swiftly implemented, for example, quarantine or movement restriction.

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