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## THE USE OF LATEX AGGLUTINATION TEST AS A RAPID METHOD FOR DETECTION OF ANTIBODIES TO *MYCOPLASMA BOVIS* IN COMPARISON WITH ELISA

(With 2 Tables and 2 Charts)

By

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إستخدام إختبار تلزن اللاتكس كطريقة سريعة للكشف عن الأجسام المضادة  
للميكوبلازما بوفس بالمقارنة بالإليزا

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تسبب الميكوبلازما بوفس العديد من الأعراض المرضية في الأبقار مثل التهاب الضرع والتهاب المفاصل والالتهابات الرئوية. تم إجراء إختبار التلزن الشريحي لقياس الأجسام المضادة للميكوبلازما بوفس على عينات السيرم كإختبار حقل. تم جمع عدد 80 عينة مصل وألبان من مزرعة أبقار مصابة تم إختبارها بواسطة الزرع البكتيريولوجي والإليزا. كانت نسبة العزل 24 (30%) بينما باستخدام الإليزا على عينات اللبن كانت 33 (41,25%). بإجراء إختبار اللاتكس والإليزا على المصل كانت 54 (67,5%). تخلص هذه الدراسة الى أن إختبار اللاتكس سريع وغير مكلف لإستخدامه فى المسح الأولى وبعده يتم التاكيد بالإختبارات المعملية المؤكدة.

### SUMMARY

*Mycoplasma bovis* (*M. bovis*) induces various clinical manifestations in cattle, such as mastitis, arthritis, and pneumonia. A latex agglutination test (LAT) has been developed to detect antibodies in bovine sera as a field test. A total of 80 milk and serum samples collected from a confirmed infected bovine farm were tested by conventional cultural method and indirect enzyme linked immunosorbant assay (ELISA). Milk samples cultivation revealed positive in only 24 (30%) while ELISA

detected 33 (41.25%). LAT and ELISA on serum samples detected only 54 (67.5%) of samples. LAT allows rapid and inexpensive test that could be useful in the field for primary herd screening prior to confirmatory laboratory diagnosis of *M. bovis*.

**Key words:** *M. bovis*, latex agglutination test, ELISA, cultivation.

## INTRODUCTION

Mycoplasmas belong to the class Mollicutes which also contain ureaplasmas, acholeplasmas, spiroplasmas. They are characterized by small size (500-1100 kbp), lack of cell wall, and higher mutation rates than conventional bacteria and can rapidly develop resistance to antibiotics (Ayling *et al.*, 2000 and Nicholas and Ayling, 2003).

*Mycoplasma bovis* (*M. bovis*) is an important and emerging cause of respiratory disease and arthritis in dairy cattle and veal calves, also has a variety of other disease manifestations. *M. bovis* is certainly capable of causing acute respiratory disease. In contrast, *M. bovis* is more accepted as a cause of chronic bronchopneumonia with caseous and perhaps coagulative necrosis (Kinde *et al.*, 1993; Pftzner and Sachse, 1996, Caswell and Archambault, 2007 and Wilson *et al.*, 2007).

Mycoplasmal bovine mastitis is potentially a highly contagious disease that can cause severe economic problems in affected herds. The purchase of replacement heifers and cows are frequently the origin of mycoplasmal mastitis. Detection of mycoplasma infected cows by culture of milk is the only technique used for routine diagnosis at present. However, this process is rather laborious and time consuming where the final results are available only after several days (González and Wilson, 2003).

A number of modern tests have recently been described, including PCR (Bernadette and Robert, 2003) and ELISA, although these tests can offer advantages in term of sensitivity, specificity or reproducibility, they still require considerable investment in equipments and trained personnel and cannot be used for rapid diagnosis since they must be performed in the laboratory. As an aid to effective field control, a rapid and inexpensive test is needed for the accurate diagnosis (Martenz *et al.*, 1990).

The aim of this work was to produce a new rapid and inexpensive field test, latex agglutination test (LAT) for primary herd screening prior to confirmatory laboratory diagnosis of *M. bovis*.

## **MATERIALS and METHODS**

### **1- Samples:**

A total of 80 milk and blood samples were collected from lactating cattle farm for bacteriological isolation, ELISA and Latex agglutination test. Negative control samples were taken from 10 newly born calves 24 – 48 hours of age and 10 serum and milk samples from non- infected cow's farm.

### **2-Organisms:**

*M. bovis* reference strain was obtained from Prof. Dr. Ruhnke, Vet. Microbiol. Dept., Ontario Vet. College, Guelph Ontario, Canada.

### **3- Bacterial isolation and identification of *M. bovis*:**

Isolation and identification of mycoplasma from milk samples was done according to Ruhnke and Rosendal, (1989).

### **3- Preparation of Mycoplasma antigen:**

One ml of an active growing approximately  $10^7$  CFU/ ml estimated according to Rodwell and Whitcomb (1983) culture of *M. bovis* was first transferred into 9 ml of broth (supplemented with horse serum ,yeast extract DNA, pencillin G sodium and thallium acetate). After 72 hours incubation, the contents were aseptically poured into 90 ml heart infusion broth and then after incubation period of 72 hours the content flasks (approx. 100ml) were transferred to the final propagation flask containing 900 ml of broth. After another 72 hours of incubation, the mycoplasma cells were harvested by centrifugation at 18000 rpm in cooling centrifuge for 1 hour. Sterility checks were routinely carried out with each passage of the growth to eliminate the possibility of bacterial contaminant. After two thorough washing in sterile PBS, cells were resuspended in 15 ml of PBS/ 1000 ml of the original volume (Ruhnke and Rosendal, 1989). This stock suspension was sonicated (Bandelin electronic UW2070 Sonicator) at 20Kc/ min for 10 successive times on ice and one minute interval (Krogsgaard, 1971).

### **4- Enzyme Linked ImmunoSorbent Assay (ELISA):**

This test was carried out to estimate the *M. bovis* antibodies in serum and milk whey. In this test 80 milk samples and serum collected from lactating cows and the test done according to method of Hajkova and Jurmanova, (1986).

### **5- Sensitization of Latex microspheres:**

The following procedure is slightly modified from Kende, (1969). A latex polystyrene microspheres particles of  $0.81\mu\text{m}$  diameter (LB8; Sigma) were used in the covalent binding reaction. Prior to use,

the beads were suspended in deionized water and vigorously vortexed to ensure even distribution to break up any large particles and obtain a 10% suspension. An equal volume of latex particle and *M. bovis* sonicated antigens (0.8mg protein/ml) were incubated over night at 37 °C. The mixture was end-to-end shaken and then centrifuged at 6,000 rpm for 10 min. The supernatant was removed and the pellet was washed and brought to a volume 10% in glycine sodium chloride buffer (pH 8.2 with 1N NaOH) containing 1:500 bovine serum albumin to keep the latex well suspended (7.50g of glycine, 5.85g of sodium chloride and 0.1% sodium azide in 1 liter deionized water, and Evans blue 0.005g %), kept at 4°C.

#### **6-Latex agglutination tests:**

Agglutination test was performed on negative, field and positive ref. serum samples. Agglutination reactions were performed by adding a drop of latex solution into a drop of serum on a microscope glass slide and mixed by side to side movement of slide. The agglutination appears within 2-3 minutes in positive cases (Satynaran, *et al.*, 1988).

## **RESULTS**

#### **Bacteriological isolation:**

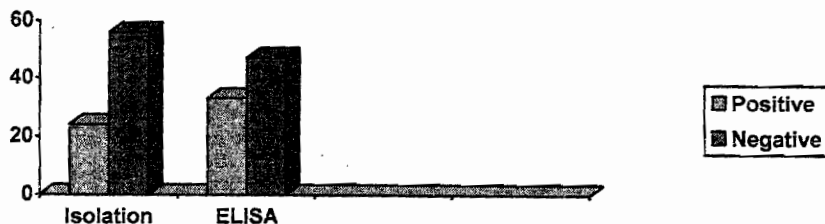
Results of *M.bovis* isolation from milk are illustrated in Table (1). Twenty four milk samples revealed positive culture (30%) while 33 (41.25%) of the milk samples revealed positive by ELISA titer to *M. bovis*.

Also the ten control milk samples were negative by isolation and ELISA

Fifty four samples were positive (67.5%) by LAT and ELISA in Table (2) the duration of latex was valid for 6 months. LAT and ELISA were applied on twenty control negative serum samples to exclude the false positive.

**Table 1:** Results of culture method and ELISA on milk samples:

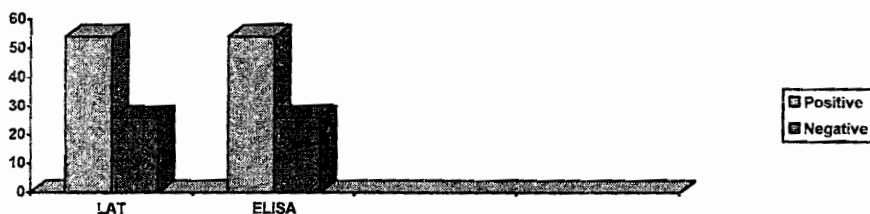
No. of samples	Isolation		ELISA	
	positive	negative	Positive	negative
80 milk samples	24(30%)	56 (70%)	33(41.25%)	47(58.75%)



**Chart 1:** results of culture method and ELISA on milk samples

**Table 2:** Results of LAT and ELISA on serum samples:

No. of serum samples	ELISA		Latex Agglutination Test (LAT)	
	positive	Negative	Positive	Negative
80	54 (67.5%)	26 (32.5%)	54 (67.5%)	26 (32.5%)



**Chart 2:** Results of LAT and ELISA on serum samples:

## DISCUSSION

The need for epidemiological data on outbreaks of *M. bovis* is crucial to prevent the spread of the pathogen and to eradicate the sources. This need is accentuated for *M. bovis* since there is no vaccine available and the organism has been shown to be highly resistant to antibiotic therapy (Adegboye *et al.*, 1995 and Byrne *et al.*, 2000).

Diagnosis of mycoplasma infection in milk has developed primarily upon microbiological culture of udder secretions. Although the reliability of this method is reasonably satisfactory, it is time consuming. Inoculated plates must be incubated at least two to three days before colonies may be observed and should not be considered as negative before seven days of incubation. Determination of mycoplasma species has required further study which may biochemical or immunological. (Martenz *et al.*, 1990).

In this work, *M. bovis* isolation bear more over prevalence (30%) from cases of clinical mastitis, these results determined the endemic status of the herd under experiment. These results agreed with Ghadersohi *et al.* (1999) that reported the isolation percentage 43% and 62% in herds positive for *M. bovis* in Victoria and North Queensland, respectively. *M. Bovis* was detected in 77% of cows of which 19% alone had *M. bovis* without any other bacteria, 17% had *M. bovis* in combination with major mastitis pathogens and 40% had *M. bovis* in combination with non-major mastitis pathogens. On the other hand, *M. bovis* was detected in 18/219 (8.2%) quarter milk samples collected from cases of bovine clinical mastitis in Northern Greece between November 1997 and March 1999. The cases occurred in 2/37 (5.4%) of the herds examined. The microorganism was isolated from bulk milk tank samples (BTS) from the two positive herds but was not isolated from 111 composite milk samples collected from clinically healthy cows from all 37 herds (Filioussis *et al.*, 2007).

The bacteriological isolation of *M. bovis* is the gold test for diagnosis but discontinues shedding of mycoplasma; progressive acidification of milk may cause reduced viability of mycoplasma. Also, a high percentage of bacterial flora may hinder the growth of mycoplasma in selective agar plates and making the identification of mycoplasma colonies difficult (Tola *et al.*, 1997). This explains why the results of IELISA and LAT were higher than the isolation and also this increase the need for the serological test. *M. bovis* antibodies were detected in 33(41.25%) milk samples by indirect ELISA while by culture only 24 (30%) milk samples were positive. These results indicated the potential value of the indirect ELISA for the detection of cows which have recently developed *M bovis* mastitis during the early stages of an outbreak.

The results indicated that the sensitivity of positive cases by LAT 54 (67.5%) was also equal to the results by ELISA. A test that allows rapid and inexpensive primary herd screening prior to confirmatory laboratory diagnosis (PCR or ELISA) should be useful in the field. In contrast to other diagnostic tests, no expensive equipments or reagent are required (e.g. ELISA plate reader, PCR machines, gel electrophoresis equipments, enzymes, etc.), and the results can be obtained in situ within few minutes. The requirements for LAT are minimal: glass slides (which are inexpensive) and disposable plastic droppers. The stability of the beads after storage for 6 months in refrigerator was not affected.

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