USING OUTER MEMBRANE PROTEINS (OMPS) OF BRUCELLA MELITENSIS BIOVAR 3 IN ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DIAGNOSIS OF BOVINE BRUCELLOSIS

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ABSTRACT

Serological profile of 40 Brucella infected cow and 50 Brucella free cow's sera was conducted using the brucellosis conventional tests in addition to ELISA with OMP of Brucella melitensis biovar 3. The percentage of positive reactors among 40 sera from Brucella infected cows using traditional serological tests were 100%, 100%, 97.5%, 100% using BAPAT, RBPT, Riv.T and TAT respectively. While the percentage of positive reactors among 50 Brucella free cow sera were (2/50) 4%, (1/50)2%, (1/50) 2%, (2/50) 4% using BAPAT, RBPT, Riv. T and TAT respectively due to false positive reaction caused by cross reaction with other microorganism which share Brucella in its antigenic structure.

The percentage of positive reactors among 40 Brucella infected cow's sera and 50 Brucella free cow's sera were 97.5 %, and 0.0% respectively using with ELISA coated with outer membrane protein (OMP) of Brucella melitensis biovar 3. in which optical density O.D were 0.795 and 0.104 respectively these indicated that ELISA coated with (OMP) of B. melitensis biovar 3. is more specific which lead to disappearance of false positive reactors (non specific reaction)among brucella free cow's which lead to cross reaction by using traditional serological tests.

Outer membrane proteins (OMPs) of B. melitensis biovar 3 profiles of SDS-PAGE revealed 8 protein bands ranging from 31.8 kDa to 91.7 kDa.

The sensitivity were 100%, 100%, 97.5%, 100%, and 97.5% for BAPAT, RBPT, RIV.T, TAT and ELISA with OMP respectively while specificity were 96%, 98%, 98%, 96%, and 100%, for BAPAT, RBPT, RIV.T, TAT and ELISA with OMP respectively. The Results concluded that the specificity of the ELISA with OMP antigen was 100%, this indicated that specified proteins antigen (OMP) increased the specificity of the test, and overcome the problem associated with cross-reactivity of antibodies due to infection with bacteria known to induce immunological cross-reactions with Brucella spp.

INTRODUCTION

Brucellosis is considered by the Food and Agricultural Organization and the World Health Organization as one of the most widespread zoonosis in the world **(McDermott, and Arimi 2002)**. Mortality rates may be

around 5% specially in calves from seropositive cows **Domenech et al., (1982)**, with high morbidity rates in adults. Brucellosis is the leading cause of contagious abortion in livestock. The most important species are B. abortus, B melitensis and B. suis causing abortions, premature births and retained placenta in livestock **Corbel, (2006)**. Risk factors for human cases often include consumption of fresh dairy products that have not been pasteurized, contact with infected animals or abortive material and handling of animals products, **Mantur et al., (2007)**. Animals are almost exclusively the source of infection for people and therefore any attempts at reducing the human disease burden is dependent on identifying the infected animal source.

Brucella is an important zoonotic disease infects both animal and man **Pappas et al ., (2006)** and considered as occupational disease infects veterinarians workers, butchers in slaughtered houses and laboratory workers, cattle breeder and farmers **Franco et al., (2007)**. Brucella species are facultative intracellular bacteria which develop mainly in the reticuloendothelia system and occasionally in other target organs, such as joints and placenta, and can cause abortion in cattle **(Cloeckaert et al., 1992 a,b.)**.

OMP, LPS and also cyto proteins are antigens whose relative importance in Brucella infections should be established to optimize diagnostic test **(Riezu -Boj et al., 1986)**. Brucella cell wall consists of peptidoglycan layer, strongly associated with the OMPs **(Dubray, 1973)**. The cell wall of Brucella abortus has been described as a complex structure populated by at least 75 proteins (OMPs) that have been reported over the past years **(Sowa et al., (1991)**. Brucella OMPs have been extensively studied because of their potential role as virulence factors, antigenic factors and molecular typing tools **(Paquet et al., 2001)**. The Brucella OMPs were investigated to seek immunogenic and protective antigens for potential diagnostic and vaccine applications **(Salhin et al., (2003)**.

An important component of any disease control effort is the ability to identify infected or infectious animals and treat or remove them from the population. In the case of brucellosis, identifying infected animals and removing from the herd is key to the control of the disease in both the livestock and the human populations. Some diagnostic or screening tests are referred to as the "gold standard", there are in fact few perfect diagnostic tests and there is always a compromise between performance and cost. For example, many screening programmes use tests with less than perfect specificity. Thus we must choice and apply test, with no false positive results, also overcome cross reactivity when using some serological tests caused by (E. coli, Salmonella dublin, Yersinia enterocolitica O:9, Pasteurella multocida, Francisella tularensis, and Pseudomonas solanacearum) which share Brucella in its antigenic structure **Mahdi and Ibrahim (2009)**.

Accurate laboratory diagnosis depends on bacterial isolation of microorganism but several problems face bacterial isolation lead us to depend on serological tests. Also bacterial isolation give false negative results in chronic cases **McGiven et al., (2006), Aliskan (2008)**.

Some serological tests give false negative results in early stage of disease also after abortion **Mandell et al., (2005)**.

The Brucella outer membrane was investigated to seek immunogenic and protective antigens for potential diagnostic and vaccine applications. The major outer membrane proteins (Omps) of Brucella spp.'s classified according to their apparent molecular mass as 36- to 38-kDa Omps or group 2 porin proteins, and 25- to 27-kDa and 31- to 34-kDa Omps which belong to group 3 proteins. Genes encoding group 2 porin proteins consist of two genes, i.e., omp2a and omp2b, which are closely linked in the Brucella genome and share a great degree of identity (>85%). In the 1990s, two genes coding for group 3 proteins were identified and named omp25 and omp31. The predicted amino acid sequences of Omp 25 and Omp 31 share 34% identity **(Cloeckaert et al., 2002)**.

Brucella melitensis strains, the expression of a fatty tissue called O-polysaccharides (OPS) on the outer membrane of the bacterium controls whether the bacterium will look smooth or round. **(Fernandez et al., 2006)**.

The absence of these O-polysaccharide chains turns the organism into a rough variant. This layer is important in identifying whether a pattern of species-specific flagellar gene." **Chain et al.,(2005)**.

Mahdi and Ibrahim (2009) reported that Salt-extractable antigen (SEA) of B. abortus S99 was used in indirect ELISA (i-ELISA) for detection of anti-Brucella antibodies in cattle sera. Results concluded that the specificity of the i-ELISA with (SEA) antigen was 100% and the sensitivity was about 95-96%.

Senthilkumar et al., (2009) stated that the sensitivity and specificity of ELISA coated with OMP was 86.84% and 95.42% respectively.

More recently, several researchers have investigated the use of the enzyme- linked immunosorbent assay (ELISA) to improve both the sensitivity and specificity of serological diagnosis of Brucella infection.

So the aim of current study was to improve both sensitivity and specificity of serological diagnosis of brucella infection and also to overcome cross reactivity of some serological tests caused by (E. coli, Salmonella dublin, Yersinia enterocolitica O:9, Pasteurella multocida, Francisella tularensis, and Pseudomonas solanacearum), which share Brucella in its antigenic structure by using ELISA coated with OMP of Brucella melitensis biovar 3. and study their OMP profiles by SDS-PAGE and compares ELISA coated with OMP with commonly used conventional serological tests.

MATERIAL AND METHODS

1- Serum Samples:

A- Brucella infected cow's sera were collected from infected farms with brucellosis during January 2009 up to March 2009. Sera of 40 naturally infected cow's from brucella infected herd. These animals were culture positive for B. melitensis biovar 3.

B- Brucella free cow's sera were collected from 50 Brucella free unvaccinated cattle

from a farm proved to be free from brucellosis on serological basis.

2- Serological tests:

A-Conventional Serological tests: these included buffered acidiefed plate antigen test (BAPAT), tube agglutination test (TAT), rivanol test (Riv. T), rose bengal test (RBT) were applied according to **(Alton et al., 1988)**. The antigens of these tests were obtained from the Veterinary Sera and Vaccine Research Institute (VSVRI), Abbasia, Cairo 11517, Egypt.

B- Indirect Immunosorbent Assay (iELISA) :

Cattle sera samples were testes by ELISA for antibody reactively using prepared OMP antigen according to **(Hunter et al., 1986)** as following :

Microtiter plates were coated by an overnight incubation at 4°C with OMP (12.5 ug/ 100ul PBS) after 6 washing cycles, the plates were blocked for 1 h at 37°C with 200ul per well of PBS-BSA) and then washed four times. Sera were serially diluted in PBS Tween 20. Then 100ul of each diluted serum were added per well and incubated for an hour at 37°C followed by four cycles of washing .The horseradish peroxidases labeled IgG of rabbit antibovine conjugate diluted 1:1500 in PBS Tween was then added (100ul/well) and incubated for an hour at 37°C and washed as mentioned previously. The optical density was read at 540 nm using an automated plate reader. The cut off value was established at 0.2 units, which was about three times the average OD reading of negative sera.

3- Bacterial strains: Pure cultures of Brucella melitensis biovar 3 was previously isolated in the Brucella department. Animal Health Research Institute, AHRI ,Dokki. Egypt.

Each Brucella culture (smooth strain) was first grown on tryptose agar slopes at 37°C for 72 h as seed. Sterile heat inactivated horse serum was added as 5% for the growth of Brucella melitensis biovar 3 strain. The seed culture was then suspended in PBS (pH7.2) and incubated into Roux flasks of tryptose agar medium. The production culture was incubated at 37°C for 5 days. For harvest, 15 ml of sterile PBS (pH 7.2) was added to each bottle to wash the cells from the agar surface according to the method described by **Alton et al., (1988)**. The pooled cell suspension was killed by 50% acetone at 4°C for 1 to 24 h. and incubated for 2 hours at 20°C. **(Hunter et al., 1986)**.

4- Antigen preparation: OMPs was extracted from a heat killed brucella melitensis biovar 3, isolated in Brucella Department in (AHRI) according to Hunter et al .,(1986) as following:

Preparation of outer membrane proteins (OMPs):

OMPs were obtained by suspending the cell envelop (Reizu-Boj et al.,1990) in 20 mM Tris HC1 and 8 mM Mg2 S04 (100 mg/ml) and added drop wise to an equal volume of boiling 4% sodium dodecyl sulphate (final concentration 2%). The solution was kept at 100°C for 5 minutes. The mixture was then cooled and kept gently stirred at room temperature for 2 h. The insoluble fraction was recovered by centrifugation at 10.000 X g for one hour,

washed exhaustively with distilled water, and then digested by lysozyme (2% wt/ wt) for 24 h, at 37°C. The protein pellet was collected by centrifugation at 10.000 X g for 2 h. and stored at -20°C (Hunter et al., 1986). Protein concentration was measured according to **Lowry et al., (1951)**.

5- Sodium Dodecyl Sulphate Electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the procedure described by **Laemmli (1970)** on 12.5% acrylamide gel and stained by silver stain **(Tsai and Frasch, 1982)**.

6- Calculation of sensitivity and specificity: An equation was designed to calculate sensitivity and specificity by using the criteria of true negative and true positive responders from the determined brucellosis status of the animals depending upon the bacteriological examination results following the steps according to **Alton et al., (1988)** as following :

$$\text{Sensitivity \%} = \frac{\text{True positive}}{\text{True positive + False Negative}} \times 100$$

$$\text{Specificity \%} = \frac{\text{True Negative}}{\text{True Negative + False positive}} \times 100$$

The Gold Slandered test used in this study for True positive animals of brucella infection was ELISA using brucella melitensis OMP coated plates.

RESULTS AND DISCUSSION

Accurate laboratory diagnosis depends on bacterial isolation of microorganism but problem facing bacterial isolation lead us to depend on serological tests. Also bacterial isolation give false negative results in chronic cases **(McGiven et al., (2003), McGiven et al., (2006), and Aliskan (2008)**.

Most brucellosis serological tests depend on the detection of antibodies to smooth Brucella LPS (sLPS). Even so, different Brucella species with the same LPS form will cross react as it is very similar **Abdoel and Smits (2007)**. B. melitensis and B. suis contain sLPS while B. ovis and B. canis have rough LPS **Nielsen (2002)**. There has been some suggestion **Diazaparicio et al., (1993) and Wcynants et al., (1996)**.

Reviewing the results demonstrated Table (1) the percentage of positive reactors among Brucella infected cow sera were 100%, 100%, 97.5%, 100% using BAPAT, RBPT, Riv.T and TAT respectively while the percentage of positive reactors due to cross reaction with other microorganism among Brucella free cow sera were (2/50) 4%, (1/50) 2%, (1/50) 2%, (2/50)4% using BAPAT, RBPT, Riv.T and TAT respectively which indicated that BAPAT and RBPT are more sensitive than Riv.T and Riv.T, more specific than BAPAT, RBPT and TAT as Riv. T depends on precipitation of IgM class of antibodies which include non specific agglutinating materials from serum.

The obtained results agree with that reported by **Mandell et al., (2005) and FAO/ OMS (1986)** who stated that some serological tests give false negative results in early stage of disease also after abortion, also RBPT is highly a sensitive and rapid and presumptive test and positive results should be confirmed

with highly specific tests such as CFT and ELISA.

Our results agree with that of **MacMillan and Cockrem (1985)** who mentioned that TAT produce false positive results after 7-10 days from infection, before this period its gives false negative results due to non specific reaction as a result of cross reactions.

It appeared that the BAPAT and RBPT among all tests used in this study, gave the highest rate of positive animals in cattle similar results were reported by **(Angus and Barton 1984), (Dohoo et al., 1986), (Hamdy 1992 and Hosein 1996).** The high sensitivity of these tests is mainly returned to that it detects both IgG and IgM molecules **(Nelson, et al., 1989)**. Even IgG1, which is not a good agglutinating at neutral pH, is active at low pH of BAPAT **(Macmillan, 1990)**. The BAPAT is a plate test carried out in one dilution (0.08 ml serum to 0.03 ml antigen) a method which renders the test highly sensitive due to high amount of serum. This agrees with **Nicoletti and Muraschi (1966)** who reported that BAPAT more sensitive than CFT and Riv.T.

The results also agree with **El-Gibaly et al., (1990)** who concluded BAPAT is the most sensitive test in Br. melitensis infected cows. **Refai (1989)** reported that it was decided to use the buffered acidified plate antigen test (BAPAT) as a presumptive test due to its high sensitivity. Using Rose Bengal Plate Antigen test (RBPT), The test does not show a great difference with the results of BAPAT which means that the results are nearly similar to those obtained by **Angus and Barton (1984), Hamdy (1992) and Anwar (1999)**.

It may be worthy to note that, on infection IgM appears earlier than other immunoglobulins **(Morgan et al., 1978 and Alton et al., 1988)** this would also explain the higher percentage of positive cases detected by RBPT and points out the fact that, this test could be of help in detecting cases of recent infection not diagnosed by the SAT. Moreover an advantage reported for this test is that, the acid pH of the Rose Bengal test (3.65) may inhabit the non-specific antibodies leaving the specific agglutinins **(Corbel, 1973; Patterson et al., 1976)**. As a result of this oversensitivity, the test is best applied as an initial screening test during an eradication programmes. Sera reacting to it should be subjected to confirmatory tests **(Nicoletti, 1967)**.

The RBPT has the best correlation with the results of the bacteriological studies **(Alton et al., 1975 and Mylera and Fraser, 1978)** who reported that following experimental infection, it took an average of 62 days for the tube agglutination test to detect infected animal whilst it took only 43 days for the (RBPT) to detect the same animal. A similar observation was made by **(Morgan et al., 1969) and (Morgan and Richards 1974)** where the RBPT became positive sooner than the SAT in infected animals.

Tube agglutination test was done on the same sera samples and results of this test are gave high percentage of positive reactions. This agrees with results of **Nicoletti and Muarschi (1966), Chappel et al., a,b (1978), Sayour (1988)** but disagrees with those of **Salem et al., (1984), Shalaby (1986), Mahajan et al., (1986) and El-Gibaly (1969)**.

In this study it was noticed the presence of some samples which reacted positively to the BAPAT, RBPT and TAT which proved negative by ELISA as a specific test for diagnosis of brucellosis may be attributed to the presence of some bacteria which share the Brucella in its antigenicity and thus cross-reacts with the antigen used. This agrees with the finding of **Morgan et al., (1978)** who suggested the presence of some bacteria as Escherichia coli, Salmonella dublin, Yersinia enterocolitica O:9 and others in the body fluids and secretions which react positively with the tests used in diagnosis of brucellosis thus causing faults or error in the interpretation of the results.

Detection of more positive reactors by RBPT than Riv.T and SAT is mostly due to its ability for earlier detection of recently infected animals as well as the longer persistence of its reaction in those chronically infected as mentioned by **Awad et al., (1977)** who reported that RBPT give positive results earlier than SAT in recent infection.

The results demonstrated in Table (2)showed that the percentage of positive reactors among Brucella Infected cow sera and Brucella free cow sera were 97.5 %, and 0.0% respectively using with ELISA coated with outer membrane protein (OMP) of brucella melitensis biovar3. and optical density O.D were 0.795 and 0.104 respectively which indicated that ELISA coated with (OMP) of brucella melitensis biovar3 is more specific which lead to disappearance of positive reactor among brucella free cow sera due to cross reaction than traditional serological tests which lead to improvement of specificity also overcome cross reactivity. In addition, **Paquet et al., 2001, Hoda (2005) and Hoda (2007)** who reported that Brucella OMPs had the potential role as virulence factors, antigenic factors and molecular typing tools ,also agree with **Salhin et al., 2003** who indicted that the Brucella OMPs were investigated to seek immunogenic and protective antigens for potential diagnostic and vaccine applications.

The serological profiles of Brucella infected and free cow were variable ac-cording to the tests. In BAPAT, RBPT, Riv.T and TAT, 40 (100%), 40 (83.3%), 39 (97.5%) and 40 (100%) were positive, respectively. Testing of these sera from Brucella infected and free cow by indirect ELISA using OMP antigen revealed that 97.5%, and 0.0% were positive, respectively, (Table 2).

The outer membrane (OM) of Gram negative bacteria contains a number of proteins. The outer membrane proteins (OMPs) of Brucella and its composition has been a subject of growing interest during the last decade. In this work, outer membrane proteins (OMP) enriched extracts of Brucella melitensis biovar 3 have been analyzed and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) profile of these strain was determined. This powerful technique allows very high resolution of protein mixture and has permitted the identification of different protein components.

Outer membrane proteins (OMPs) of Brucella melitensis biovar 3 profiles of SDS-PAGE revealed 8 protein bands ranging from 31-8 kDa to 91.7 kDa, as (Fig. 1) which shows that groups of major membrane protein of different mobilities with apparent molecular weight

between 31-8 kDa and 91.7 kDa, are visible and constitute the major OMP of Brucella melitensis biovar 3. These results are nearly similar to those recorded by **Santos et al., (1984) and Salhin et al., (2003), Hoda (2005) And Hoda (2007)** who stated that all Brucella strains regardless of biotype or geographic origin displayed major cluster of OMPs at apparent molecular weight of 88.0 kDa to 94 kDa (group 1), 35.00 kDa to 39.00 kDa (group 2) and 25 kDa to 31.0 kDa (group 3). Between these groups, additional minor bands are sometimes present. Results are also similar to that recorded by Verstreate et al., (1982) who classified the Brucella OMP in three distinct MW ranges i.e. 88 to 94 k (group 1), 35 to 40 k (group 2) and 25 k to 30 k (group 3) and other additional bands between these groups.

These results indicate the importance of the presence of the long lipopolysaccharide O side chains in the accessibility of OMPs on smooth Brucella strains and should be considered when undertaking vaccine development. **Cloeckaert et al., (1990) and Cloeckaert et al., (1991)** who stated that according to the specificity of the competitive ELISA, OMPs useful for the detection of infected animals are the OMPs of 10, 16.5, 19, 25 to 27, and 36 to 38 kDa. It therefore seems that a combination of several protein antigens is necessary for the development of an immunoassay with a sensitivity comparable to that of the smooth lipopolysaccharide ELISA **Cloeckaert et al., (1992 a,b)**.

Moreover **Mahdi and Ibrahim (2009)** reported that Salt-extractable antigen (SEA) of B. abortus S99 was used in indirect ELISA (I-ELISA) for detection of anti-Brucella antibodies in human and cattle sera. By using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), specificity of the I-ELISA with (SEA) antigen was 100% and the sensitivity was about 95-96%, this indicated that specified proteins antigen increased the specificity of the test, also overcome the problem associated with cross-reactivity of antibodies due to in infection with bacteria known to induce immunological cross-reactions with Brucella spp .

Our results are also similar to that of **Limet et al., (1993)** who reported that the antibody response of cattle to the minor 89-kDa outer-membrane protein (OMP) of brucella was measured by indirect ELISA with the purified protein and compared with the antibody response to smooth lipopolysaccharide (S-LPS), suggesting the presence of one or more cross-reactive epitopes on this protein. Results indicate that specific epitopes of the 89-kDa OMP in combination with those of other OMPs could be useful for diagnosis of brucellosis in cattle. The author concluded also that the OMP ELISA has the potential to achieve greater specificity for Brucella.

In this study out of 40 examined cow sera 39 Brucella infected cow sera were positive reactors 39/40 (97.5%) using ELISA coated with OMP and disappearance of positive reactors among Brucella free cow sera .table (2) in comparison with the results of positive reactors by screening tests BAPAT 40/40 (100 %) and 40/40 (100%) using RBPT, 39/40(97.5%) using Riv.T and 40/40 (100%) using TAT respectively among Brucella infected cow sera and appearance of positive reactors among

Brucella free cow's sera (2/50) 4%, (1/50)2%, (1/50) 2%, (2/50)4% using BAPAT, RBPT, Riv.T and TAT respectively, table (1) which indicated that ELISA coated with OMP was more specific than BAPAT, RBPT, Riv.T and TAT. Our results agree with **MacMillan and Cockrem (1985), Omer et al., (2001).**

Due to the high probability of occurrence of false positive and false negative results by using the commonly used serological tests in brucella diagnosis, overcome all of these faults we must use ELISA coated with outer membrane protein (OMP) of brucella.

It is important to use a more specific test such as ELISA coated with outer membrane protein (OMP) of brucella to overcome these problems.

Looking to table (3) the sensitivity were 100%, 100%, 97.5%, 100%, and 97.5% for BAPAT, RBPT, RIV.T, TAT and ELISA with OMP respectively while specificity were 96%, 98%, 98%, 96%, and 100%, for BAPAT, RBPT, RIV.T, TAT and ELISA with OMP respectively. The Results concluded that the specificity of the ELISA with OMP antigen was 100% and the sensitivity was about 97.5%, this may be attributed to the specified proteins antigen that increased the specificity of the test, also overcome the problem associated with cross-reactivity of antibodies due to in infection with bacteria known to induce immunological cross-reactions with Brucella spp.

As the OMP ELISA has the potential to achieve greater specificity for Brucella ,the outer membrane proteins (OMP) useful for the specific detection of Brucella infection .We advise to use this test in control and eradication programmes of brucella in all animals species.

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الملخص العربى

استخدام البروتين الخارجى للبروسيلا فى اختبار الاليزا لتشخيص مرض البروسيلا فى الأبقار

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تم الفحص السيرولوچى لعدد ٤٠ عينة سيرم لأبقار مصابة بالبروسيلا وعدد ٥٠ عينة سيرم لأبقار خالية من البروسيلا باستخدام الاختبارات السيرولوچية الشائعة بالإضافة لاختبار الاليزا مع بروتين الغشاء الخارجى للبروسيلا ملتنيسز النوع الثالث، وكانت نتائج نسبة الإيجابى لسيرم الأبقار المصابة بالبروسيلا كالتالى :

١٠٠٪ و ١٠٠٪ و٩٧,٥٪ و ١٠٠٪ باستخدام الاختبارات الطرق السيرولوچية الشائعة مثل اختبار المحمض المخمد والروزبنجال والريفانول والتلزن الأنبوبى على التوالى.

بينما كانت نتائج نسبة الإيجابى لسيرم الأبقار الخالية من البروسيلا كالتالى ٤٪ و ٢٪ و ٢٪ و ٤٪ باستخدام الاختبارات الطرق السيرولوچية مثل اختبار المحمض المخمد الروزبنجال الريفانول والتلزن الأنبوبى على التوالى نتيجة لظهور الإيجابى الكاذب بسبب التفاعل التداخلى لبعض الميكروبات التى تتشابه فى التركيب الچينى للبروسيلا.

وكانت نتائج نسبة الإيجابى لسيرم نفس الأبقار المصابة بالبروسيلا والأبقار الخالية من البروسيلا السابقة باستخدام اختبار الاليزا مع بروتين الغشاء الخارجى للبروسيلا ملتنيسز النوع الثالث ٩٧,٥٪ و ١٠٠٪ على التوالى وكانت الكثافة الضوئية ٠,٩٥ و ١,٠٤ على التوالى مما يبين أن اختبار الاليزا باستخدام البروتين الغشاء الخارجى للبروسيلا أقل حساسية وأكثر نوعية من الاختبارات السيرولوچية الشائعة مما أدى إلى اختفاء الإيجابى الكاذب (تفاعل غير نوعى) فى الأبقار الخالية من البروسيلا نتيجة للتفاعل التداخلى كما فى الاختبارات السيرولوچية الشائعة.

أظهرت نتائج الترحيل الكهربى لفصل المكونات البروتينية لبروتين الغشاء الخارجى للبروسيلا ملتنيسز النوع الثالث على أعمدة هلال متعدد الاكريل أميد بوجود المادة الماسخة (SDS-PAGE) باحتوائه على ٨ حزم بروتينية تراوحت أوزانها الجزيئية بين ٣١,٨ - ٩١,٧ كيلو دالتون.

كما وجد أن الحساسية للاختبارات السيرولوچية كالتالى ١٠٠٪ و ١٠٠٪ و ٩٧,٥٪ و ١٠٠٪ و ٩٧,٥٪ كما فى اختبار المحمض المخمد والروزبنجال والريفانول التلزن الأنبوبى واختبار الاليزا باستخدام البروتين الغشاء الخارجى للبروسيلا على التوالى، بينما وجد أن نوعية

هذه الاختبارات السيرولوجية كالتالى ٩٦٪ و ٩٨٪ و ٩٨٪ و٩٦٪ و ١٠٠٪ كما فى اختبار المحمض المخمد والروزبنجال والريفانول والتلزن الأنبوبى واختبار الاليزا باستخدم البروتين الغشاء الخارجى للبروسيلا على التوالى.

خلصت النتائج أن النوعية لاختبار الاليزا باستخدام أنتجين البروتين الغشاء الخارجى للبروسيلا كانت ١٠٠٪ بينما كانت حساسية ٩٧,٥٪ وذلك يبين على أن استخدام هذا البروتين النوعى زاد من نوعية اختبار الأليزا وجعله يتغلب على ظاهرة الأجسام المضادة فى التفاعل التداخلى نتيجة للإصابة ببعض الميكروبات والتى تؤدى إلى التداخل التفاعلى المناعى مع ميكروب البروسيلا.

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