SEROLOGICAL STUDIES ON BRUCELLA IN DAKHALIA AND DAMIETA GOVERNORATES

G. Younis and Amal Abdel-Satar Ibrahim
Dept. Bacteriol. Mycol. Immunol., Fac.Vet.Med., Mansoura University

ABSTRACT

A total of 665 blood samples were first screened for Brucella antibodies by using Rose Bengal Plate test. The positive reactors were further confirmed by tube agglutination test. Results of the examined dairy cattle sera revealed that the incidence of brucellosis was 29.17%. A total of 320 milk samples were also screened and the results revealed that 102 (31.9%) were positive after tested by MRT. In corresponding, milk whey samples were tested by whey serological tests (wRBPT and wTAT). wRBPT recorded 52 positive samples (16.3%) and wTAT recorded 42 (13.1%). MRT was taken as a reference standard test for evaluating wRBPT and wTAT. The sensitivity was (50.9%) and (41.1%) for wRBPT and wTAT respectively, while the specificity was (100%) for each and the agreement was 84.4% and 81.3% respectively. On the other hand, the results showed the disagreement between serum and whey serological tests and the relative agreement between MRT and serum agglutination tests. Moreover, examination of 100 marketing milk samples was found to be negative to MRT.

Key words: *Rose Bengal, sensitivity, specificity, Brucella, milk whey, milk ring test.*

INTRODUCTION

Brucella, a genus of gram negative bacteria, is a causative agent of brucellosis, a worldwide zoonotic disease **(Corbel and Morgan, 1984)**. Brucellosis has been studied in domesticated mammals, such as cattle, sheep and pigs, and is known to cause reproductive disorders or abortions in infected animals. Brucella organisms can be transmitted from infected animals to man by ingestion of unpasterized milk and milk products, by contact with infected animals or either discharges or by inhalation of aerosols containing Brucella organisms **(Refai, 2003)**. Also, transmission via blood transfusion, bone marrow transplantation, sexual intercourse and neonatal infection had been reported **(Palanduz et al., 2000)**. Therefore, un-pasterized milk, cream, butter, unfermented cheese and other products made from untreated milk constitute a serious health hazard in area where Brucella infection is wide spread in dairy animals **(Munoz et al., 1993)**. The exeretion may stop in advanced lactation period but starts again in the subsequent lactation. The organism leads to serious economic losses for animal industry due to abortion, losses of calves, reduction in milk yield by 7-20%, some breeding troubles in infected animals and veterinary costs of diagnosis and control measures **(Soliman, 1998)**. Moreover, the organism is responsible for the disease in man ealled undulant fever

which is characterized by intermittent fever, headaehe, fatigue, joint and bone pain, psychotic disturbances and other symptoms **(Refai, 2003; and Pappas et al., 2005)**. Diagnosis of brucellosis is the eorner stone of proper eradication of the disease. Isolation of the causative agent is still the land mark for diagnosis of brueellosis **(Alton et al., 1988)** but beeause of the eost, difficulty of performance and lack of sensitivity of most culture procedures, serological diagnosis is the main tool used for detection of Brueella infeetion in animals. Although a wide range of sero-diagnostic tests are available, there is no single test is capable to identify all infected animals with brucellosis **(Davies, 1971; and Salem et al. 1987)**. Presence of antibody to Brucella infection has been reported to be deteetable later in milk than in serum therefore it is reasonable to expect that milk antibody levels refleet the serological picture of the animals **(Alton et al., 1988)**. Testing of milk has some advantages over testing of serum samples providing that a single test ean be applied to large numbers of eattle, sample eondition is not critical and sampling is less expensive **(Nielsen et al., 1996)**. It is easier for using milk and milk whey for diagnosis of brucellosis as injuring animals for collecting blood samples is difficult **(Farag, 1998)**.

MATERIAL AND METHODS

This research was direeted to detect the presence of brucellosis in dairy animals in Dakahlia and Damieta governorates using serologieal tests.

1. Samples:

A total of 665 serum samples from (500 from different cattle farms, 125 sporadic cases distributed in Dakahlia and Damieta governorates and 40 from Mansoura abattoir). Also, another 320 milk and serum samples from the same eows were collected from New-Damieta farm for eomparison between their results. Finally, 100 market milk samples were tested for presence of Brueella antibodies.

2. Preparation of whey:

It was done according to **Morgan et al. (1978)**. 10 ml milk samples were centrifuged at 3000 rpm for 10 minutes to separate the fat layer, the fat layer was removed and two drops of eommereial rennet were added to approximately 10 ml of skimmed milk. The tube was ineubated at 37°C for 2 hours, and then centrifuged at 2000 rpm for 10 minutes for preeipitation of easein. The obtained clear whey was either stored under deep freezing till testing by various serological tests or immediately used.

3. Antigens:

Rose Bengal plate test antigen, tube agglutination test antigen and milk ring test antigen (Haematoxyline blue antigen) were purehased from the Veterinary Sera and Vaccines Institute, Abbissia, Cairo, Egypt.

4. Serological examination of milk, whey and serum samples:

1) Milk Ring test (MRT) **(Alton et al., 1988)**:

The bottles containing individual milk samples were thoroughly mixed. From each sample, 1 ml of milk was placed in a separate Wassermann tube. One drop of haematoxyline stained milk ring test antigen (30 micron) was added to each tube. The contents were mixed gently by inverting the tube several times (avoiding foam formation). Then the tubes were incubated at 37°C for one hour.

Interpretation of the results:

Four, three and two are considered as positive while one score is inconclusive.

2) Rose Bengal Plate Test **(Morgan et al., 1978):**

Samples and the Rose Bengal Plate Test antigen were brought to room temperature before testing. One drop (30 micron) of serum under test was delivered on dry white enamel plate using a micropipette, and then one drop (30 mieron) of Rose Bengal antigen was dispensed adjacent to the sample. By using a toothpick, the antigen and the serum were thoroughly mixed in a circular movement. The plate was rocked by hand for 4 minutes. The test was read immediately after this roeking period of the enamel (4 minutes) at room temperature. Positive and negative controls were ineorporated.

Interpretation of the results:

++++ : Agglutination within one minute's coarse granules.

+++ : Agglutination within two minutes with medium granules

++ : Agglutination within three minutes with fine granules.

\+ : Agglutination within four minutes with very fine granules.

\- : No agglutination occurs within four minutes.

3) Tube agglutination test (TAT, European method) **(Alton et al., 1975)**:

It was done in clear glass Wassermann tubes and in view of the occasional occurrence of prozone phenomena at least five tubes were used per sample. The tubes were arranged in rows in a suitable rack. Using 1ml pipette, 0.8ml of phenol saline 0.5% (containing normal saline 0.85%) were added to the first tube and 0.5 ml in each succeeding tubes. To the first tube, 0.2 ml of the sample under test were added and mixed thoroughly within phenol saline, then 0.5 ml of the mixture were carried over to the seeond tube. From whieh after thorough mixing, 0.5 ml of the mixture was transferred to the third tube. The process was continued until the last tube, from which after mixing, 0.5 ml was discarded. This process of doubling dilutions resulted in 0.5 ml dilutions 1:5, 1:10, 1:20 and 1:40 and so on in each tube. To each tube, 0.5ml of antigen dilution 1:5 in phenol saline 0.5% was added. The content was mixed thoroughly resulting in final sample dilutions of 1:10, 1:20 and 1:40 and so on. The tubes were incubated at $37^{o}C$ for approximately 10 hours ± one hour before reading the results. Parallel with the test, positive and negative eontrols were ineluded. Samples with highest titres were tested with more dilutions to determine their end titres.

Reading:

The tubes were examined without shaking against a black background with the light coming from above and behind the tubes as follows:

4+: Complete agglutination and sedimentation with clear supernatant.

3+: Nearly complete agglutination with 50% clearance.

2+: Marked agglutination with 50% clearance.

1+: Slight agglutination with 25% clearanee.

-ve: No agglutination.

The highest dilution showing 50% or more clearanee was taken as the end titre for the sample.

Interpretation of the results:

A titre corresponding to antibodies levels of 20 IU/ml (++ 1:10) or higher was used as an indicative of infection.

Biostatical analysis **(Crawford and Hidalogo, 1977)**.

A diagnostic test should have a high validity, which means that the percentages of false positive and false negative should be limited. Validity expressed by the sensitivity and specificity. The sensitivity of a test can be defined as the conditional probability that is test will identify all individuals with brucellosis in given population. while the specificity can be defined as the conditional probability that the test will identify all non diseased individuals.

The sensitivity and specificity of a test:

They were calculated according to **(Crawford and Hidalogo, 1977)** as follows:

$$\text{Relative sensitivity} = \frac{\text{True positive X 100}}{\text{True positive + false negative}}$$

$$\text{Relative specificity} = \frac{\text{True negative X 100}}{\text{True negative + false negative}}$$

$$\text{Agreement} = \frac{\text{both tests positive + both tests negative X 100}}{\text{Total cases examined x 100}}$$

RESULTS AND DISCUSSION

Brucellosis is still considered a serious disease affecting both man and animals **(Soliman, 1998)**. An important factor responsible for the spreading of brucellosis in Egypt is the uncontrolled importation of cattle from some countries where brucellosis is still prevalent **(Shalaby, 1986)** He also added that the lack of proper programmed of quarantine measure for imported animals to ensure their free from the disease before being introduced into our country. Diagnosis of brucellosis based on isolation of the organism from the infected animals is time consuming task due to the fact that these fastidious organisms grow slowly on primary isolation **(Meyer, 1981)**. Moreover, it is not possible to isolate Brucella every time from known infected individual (Ray, 1979), therefore, assessment of antibody response employing serological tests plays a major role in the routine diagnosis of brucellosis and supported appropriate by bacteriological examination **(Alton et al., 1988)**.

As shown in table (1), out of 665 serum samples collected from cows, 194 samples showed positive results after their screening by using Rose Bengal plate test with an incidence of (29.17%). 7 (1.1%), 45 (6.8%), 76 (11.4%) and 66 (9.9%) of samples showed titres of 1/20, 1/40, 1/80 and 1/160 respectively as demonstrated in table (2). Incidence of Brucella antibodies in cow's milk samples based on results of Milk Ring Test (MRT) as recorded in table (3) where out of 320 tested samples, 102 gave positive with MRT (31.87%). Nearly similar result was obtained by **Abdel- Ali (2001)** who recorded an incidence of (31.66%), but, lowest result (0.62%) was recorded by Kadry (1996). In reverse, highest result (76.47%) was recorded by **Ali et al. (1993)**.

Regarding whey, 52 samples (16.25%) were positive by RBPT. The obtained result is nearly similar to **Selim (1987)** who recorded (16.1%) positive result. Negative results were recorded by Onsi (2004). Meanwhile, high results were stated by **Abd El Rahman (1991)**. Concerning whey TAT, 42 positive samples were recorded with an incidence of (13.12%). 2 (0.63%), 4 (1.25%), 24 (7.50%) and 12 (3.75%) of samples showed titres of 20, 40, 80 and 160 respeetively as shown in table (4). In

contrary, a relatively highest percentage (49%) was reported by **Hamdy (1997)** and negative result was obtained by **Onsi (2004)**.

Comparing the results which obtained by wTAT and those of wRBPT, it was evident that wTAT was relatively less sensitive as it gave 13.1% positive reaetors while it was 16.25% for wRBPT. The lower sensitivity of wTAT may be attributed to the certain limitation of this test especially in early incubation and in the later chronic stage of disease **(Morgan, 1967; Nicoletti, 1969; and Davies, 1971)**. Furthermore, this agglutination test was failed to detect IgG1 antibodies, since, these antibodies can not agglutinate antigen at pH value near neutrality and when in excess. These antibodies are liable to block the agglutination activity of other isotypes resulting in false negative reaction **(Ahmed et al., 2002)**.

From data presented in table (5), it was stated that low sensitivity of wRBPT and wTAT (50.9% and 41.1%) in comparison to MRT could be attributed to cretin factors such as removal of solid part in milk with rennin, the change in pH, change in moleeular weight of some immunoglobulins and the presence of majority of immunoglobulins in comparison to raw milk. Therefore the whey contains fewer amounts of immunoglobulins in comparison to raw milk with cream **(Sutra et al. 1986; Hamdy, 1997; and Abd-Alla et al., 2000)**. In addition, the whey tests are less sensitive, but less influenced by non- specific factors than MRT and produce more confirmatory results **(Cruickshank and Duguid 1968, Morgan et al. 1978; El-Gibaly and Rashad, 1990; and Hamdy 1997)**.

MRT may give false positive reaction when the samples were taken near the end of lactation cycle from mastitic milk quarters with hormonal disorders shortly after parturition and when the colostrums included in the samples **(Corbel and Morgan, 1984; and Macmillan, 1990)**.

Also, non specific reaction to MRT was reported in case of increase in protein levels in milk to about 3-15 times than the normal level **(Hajdu, 1964)**. Moreover, non specific reaction of MRT was reported due to presence of cross- reacting bacteria **(Stuart and Corbel, 1982; and Corbel and Morgan, 1984)**.

By testing the serum samples with RBPT and TAT, 91 serum samples produced positive results for each with an incidence of (28.43%). These results show disagreement between blood serum and whey serological tests that were also reported by **El-Gibaly and Rashad (1990) and Hamdy (1997)**. It could be attributed to the defatining process before the performance of the whey tests, since; most of the immunoglobulins are present on the surface of the fat globules. Moreover, removal of solid parts by rennin, change in pH and change in the molecular weight of immunoglobulin could be other additional factors that lead to low sensitivity of whey agglutination test **(Hamdy, 1997)**.

Agreement between MRT and blood serum serological tests revealed that the MRT has highest ratio of agreement with RBPT and TAT. On the other hand, MRT failed to identify many samples which gave positive reaction with blood serum serological tests. These may be attributed to the stage of infection, where udder antibodies appear later than serum antibodies (40% in 4 week, 60% in 8 week, 70% in 13 week, 100% in 20 week) after the appearance of serum antibodies or to the irregularity in the filtration of agglutinins from

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الملخص العربى

دراسات سيرولوچية على البروسيلا فى محافظتى الدقهلية ودمياط

جمـــــال يـــونس أمـــل عبدالستار إبراهيم
قسم البكتيريا والفطريات والمناعة - كلية الطب البيطرى - جامعة المنصورة

استهدفت هذه الدراسة الكشف عن الأجسام المضادة لميكروب البروسيلا وذلك بفحص ٦٦٥ عينة سيرم من الماشية وذلك باستخدام إختبار الروزبنجال وتم التأكد من النتائج بإجراء إختبار التلزن الأنبوبى وكانت نسبة حدوث المرض ١٧ر٢٩٪، وأيضاً تم تجميع عينات لبن وسيرم من ٣٢٠ حيوان وتم إختبار مدى تواجد الأجسام المضادة للبروسيلا فى عينات اللبن باستخدام إختبار اللبن الأنبوبى الذى أسفر عن تواجد ١٠٢ عينة إيجابية بنسبة (٧٨ر١٣٪) وباجراء اختبار الروزبنجال والتلزن الأنبوبى على شرش اللبن وكانت النتائج الإجابية لكلا من الاختبارين ٥٢ ، ٤٢ على التوالى تم أيضاً مقارنة نتائج إختبار اللبن الأنبوبى ونتائج الاختبارات السيررلوچية للشرش واتضح أن حساسية الروزبنجال) ٢٩ر٥٠٪ أما بالنسبة حساسية التلزن الأنبوبى فكانت (١ر٤١٪) وكان التخصص لكلا الاختبارين ١٠٠٪ من ناحية أخرى كانت نسبة التوافق ٣ر٨١٪ و ٤ر٨٤٪. كما أوضحت النتائج عدم توافق بين الاختبارات السيرولوچية للسيرم والاختبارات السيرولوچية للشرش ولكن كان هناك توافق بين اختبار اللبن الأنبوبى والاختبارات السيرولوچية للسيرم، وبعد إجراء اللبن الحلقى على ١٠٠ عينة من ألبان التسويق كانت نسبة تواجد الأجسام المضادة للبروسيلا معدومة.