

SOME BACTERIOLOGICAL AND SEROLOGICAL STUDIES ON BRUCELLOSIS IN RUMINANTS

By

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B.Sc. Agric. Sc. (Microbiology), Ain Shams University, 2001

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ABSTRACT

Ola Abdel-Mortada Ali "Some bacteriological and serological studies on brucellosis in ruminants" unpublished M.Sc. thesis. Department of Agric. Microbiology, Faculty of Agriculture, Ain Shams University, 2016.

Brucellosis is an incapacitating zoonosis in humans and economically disastrous disease in farm animals caused by the genus *Brucella*. It causes reproductive losses in animals in the form of late stage abortion, retained placenta, weak or dead birth and infertility in females and orchitis and epididymitis in males with hygroma and arthritis. Additionally, brucellosis is notorious for its latent infection. In Egypt, brucellosis is reported in all farm animal species and in the environment. The bacteriological method include field and lab means has a limited sensitivity and has the added difficulty of being impractical to apply at large scale in control campaigns. Therefore, the investigation was designed to evaluate the diagnostic performance characteristics of some serological tests used for brucellosis diagnosis of large and small ruminants in some governorates. In this study, a number of 11 *Brucella* isolates were isolated from five governorates (Beheira, Sharkia, Kafr El-Sheikh, Giza and Gharbia). Only four isolates were identified as *B. abortus* bv.1 and 7 isolates were identified as *B. mellensis* bv.3 according to their morphological, physiological, serological and genetic characteristics. Low diagnostic performance characteristics of Riv.T in small ruminants specially in ewes if compared to other serological tests, makes it unwise to replace the standard confirmatory CFT test recommended by the OIE, European Commissions, USDA for international trade by Riv.T even in large ruminants. Diagnostic performance characteristics of serological tests in large ruminants were better than the corresponding picture in small ruminants especially for MAT and Riv.T. Whereas the characteristics of EDTA-mMAT were better than MAT in both large and small ruminants.

Keywords:

Brucella, MAT, EDTA-mMAT, Riv.T, USDA, CFT, OIE

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INTRODUCTION

Brucellosis is a contagious disease of different animal species caused by bacteria of the genus *Brucella* (**OIE Terrestrial Manual, 2009a**). Brucellae show a wide range of host preference. Currently, twelve *Brucella* species exist (**Whatmore et al., 2014 & Scholz et al., 2016**) including three that have been reported in Egypt (**Menshawy et al., 2014 & Sayour and Sayour, 2015**), viz. *B. abortus* (affecting mainly cow), *B. melitensis* (sheep and goats), and *B. suis* (swine). *B. melitensis* infection of small ruminants is quite similar in both pathological and epidemiological standpoints to *B. abortus* infection of cow. The main clinical manifestations of brucellosis in ruminants are reproductive failure (i.e. abortion or birth of offsprings that do not survive), orchitis, epididymitis and less frequently arthritis. *B. melitensis* causes no abortion storms in pregnant cow. Moreover, brucellosis is notorious for its latent infection.

Brucellosis control programs in small and large ruminants have been suffering from failures due to lack of mapping that brings to light the distribution of the disease, inexact data about sero-prevalence and incidence of the disease, and concomitance of different livestock species in the same neighbourhood. Additionally, the vast majority of sheep and goats are distributed into small flocks owned by shepherds without identification numbers. In practice, it is much more difficult to control the disease in small ruminants kept under nomadic or semi-nomadic conditions than in beef or dairy cow kept under intensive management conditions (**Corbel, 2006**).

The diagnostic method that offers a definitive proof of brucellosis is the isolation and identification of *Brucella* microorganisms from the suspected animal. However, this method has a limited sensitivity and has the added difficulty of being unpractical to apply at a large scale in control campaigns.

The detection of specific antibody in serum or milk remains the most practical means of diagnosis of brucellosis. The most proficient and cost-

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effective method is usually screening all samples using a cheap and rapid test which is sensitive enough to detect a high proportion of infected animals. Reactors to screening assays are then tested using standard, accurate and specific confirmatory tests for the final diagnosis to be made (**Corbel, 2006**).

Serological results must be interpreted against the background of disease incidence, use of vaccination and the degree of false positive reactions due to infection with other organisms (**Gall and Nielsen, 2004; Corbel, 2006**).

In this view, the current study was designed to detect predominate species and biovars of *Brucella sp.* recovered from animal samples (large and small ruminants) that were collected from different Egyptian governorates, as well as, evaluating the diagnostic performance characteristics of some serological testes used for the diagnosis of ruminant brucellosis namely; buffered acidified plate agglutination (BAPAT), Rose-Bengal plate agglutination (RBPT), modified Rose-Bengal plate agglutination (mRBT), indirect enzyme-linked immunosorbent assay (iELISA), micro-agglutination (MAT), EDTA-modified micro-agglutination (EDTA-mMAT) tests, Rivanol precipitation plate agglutination test (Riv.T) and complement fixation (CFT). Moreover, studying the antibiotic susceptibility of the isolated field strains was performed.

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2.1. Brucellosis:

Brucellosis is a highly contagious disease affecting almost all domestic species, leading to severe economic losses due to abortion, infertility and reduced milk production. It is considered one of the most economically important zoonoses globally (**Perry and Grace, 2009 and WHO 2009**).

Brucellae are Gram-negative, facultative, intracellular bacteria showing a wide range of species-specificity and causing important diseases in both humans and animals. At present, twelve species are classified : *B. abortus* (affecting mainly cow), *B. melitensis* (sheep and goats), *B. suis* (swine), *B. neotomae* (desert rats), *B. ovis* (sheep), *B. canis* (dogs), *B. ceti* (cetaceans) and *B. pinnipedialis* (pinnipeds), *B. microti* (isolated from common voles), *B. inopinata* (isolated from a human patient) (**Blasco, 2010**), *B. papionis* (baboons) (**whatmor et al., 2014**) and *B. vulpis* (red foxes) (**Scholz et al., 2016**). The World Health Organization (WHO) laboratory biosafety manual classifies *Brucella* in Risk group III. Brucellosis is readily transmissible to humans, causing acute febrile illness – undulant fever – which may progress to a more chronic form and can also produce serious complications affecting the musculo–skeletal, cardiovascular and central nervous systems. Precautions should be taken to prevent human infection. Infection is often due to occupational exposure and is essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of dairy products constitutes the main risk to the general public where the disease is endemic. (**OIE, 2009a**).

2.2. Brucellosis in Egypt:

Brucellosis was first reported in Egypt in 1939. Egypt has mixed populations of sheep, goats, cow, and buffaloes. The number of buffaloes in Egypt is higher than in any other country in the Near East region. In addition to high prevalence rates of *B. melitensis* infections in sheep and goats, *B.*

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melitensis infections of cow and buffaloes have increased in Egypt. (**Refai, 2002**). **Salem and Hosein (1990)** isolated 44 *Brucella* strains from different animals 20 from cows, 2 from buffaloes and 22 from sheep at different localities in Egypt. Typing of these isolates revealed that 5 (11.4%) of strains were *B. abortus* biotype 3 (from cow and buffaloes), 37 *B. melitensis* biotype 3 (from sheep and cow) and 2 (4.6%) *B. abortus* biotype 7 (from cow). Authors concluded that *B. melitensis* biotypes 3 was the prevalent type among sheep and cows in Egypt. Whereas **Mahmoud (1991)** reported that the incidence of brucellosis in Kafr El-Sheekh Governorate was 2.27% and 3.09% when 176 cow and 97 buffalo's serum samples were examined in El Menia villages, the incidence was 3.03% and 5.5% in cow and buffaloes, respectively. The author added that the infection rate of brucellosis among farm animals in Egypt is not static, however, the evolutionary changes in the animal husbandry as well as extent of population movement was considered the main factors which could increase the exposure potential of individual animals or herds to brucellosis.

Furthermore **Hegazy et al. (2009)** stated that many factors have reduced the control programme's effectiveness, such as: a lack of reliable information on brucellosis seroprevalence in sheep, a lack of adequate communication between the public health authorities, veterinarians and stakeholders, inadequate funding of surveillance and reporting systems and the free movement of small ruminants between the various governorates in Egypt.

Hegazy et al. (2011) conducted a cross-sectional study in which 791 sheep, 383 goats, 188 cow milk tanks and 173 buffalo milk tanks were randomly selected in 40 villages and tested for the presence of antibodies against *Brucella* spp. The sero-prevalence among different species was estimated and visualized using choropleth maps. A spatial scanning method was used to identify areas with significantly higher proportions of seropositive flocks and milk tanks. Authors estimated that 12.2% of sheep

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and 11.3% of goats in the study area were seropositive against *Brucella* spp. and that 12.2% and 12% of cow and buffalo milk tanks had antibodies against *Brucella* spp.

B. melitensis biovar (bv) 3, *B. abortus* bv 1, and *B. suis* bv 1 have been isolated from farm animals and Nile catfish. Brucellosis is prevalent nationwide in many farm animal species. There is an obvious discrepancy between official sero-prevalence data and data from scientific publications. The need for a nationwide survey to genotype circulating brucellae is obvious and the epidemiologic situation of brucellosis in Egypt is unresolved and needs clarification. **Wareth et al. (2014)**

Sayour and Sayour (2015) evaluated matrix-assisted laser desorption ionization time-of flight mass spectrometry (MALDI-TOF MS) for biotyping of 124 *Brucella* isolates from raw milk and tissues of cow, buffaloes, sheep and goats in 9 governorates and unknown areas in Egypt for way faster and reliable genospeciation based on protein profiles. *B. abortus* biovar 1 was found to exist more than expected, whereas 28 isolates were identified from milk and tissues of cows in Dakahlia, Sharkia, Kafr ElSheikh and Bani Suef. Two old isolates of the rare *B. suis* biovar 1 from Monofia and Bani Suef were correctly identified in addition to a recent isolate from Gamassa, Dakahlia all from cows. Authors concluded that MALDI identification at the biovar level was accurate in *B. melitensis* bv. 3. Biovar delineation of other *Brucella* species tested was hard to judge due to limitation of the library created.

2.3. Laboratory diagnosis of brucellosis:

2.3.1. Isolation and identification:

Alton (1990) stated that, in regions where *B. melitensis* is prevalent in sheep and goats, cow are liable to catch the infection from them. He added that *B. melitensis* infection in cow sometimes causes abortion but less frequently than *B. abortus*. Unfortunately, colonization of the udder is

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frequent, and the excretion of the organism in the milk, may frequently lead to epidemics of brucellosis in people working with cow or drinking milk. **Salem and Hosein (1990)** isolated 44 *Brucella* strains from different animals (20 from cows, 2 from buffaloes and 22 from sheep) at different localities in Egypt. Typing of these isolates revealed that 5 (11.4%) of strains were *B. abortus* biotype 3 (from cow and buffaloes), 37 *B. melitensis* biotype 3 (from sheep and cow) and 2 (4.6%) *B. abortus* biotype 7 (from cow). Authors concluded that *B. melitensis* biotypes 3 was the prevalent type among sheep and cows in Egypt. Also 15 *B. melitensis* biotype 3 were isolated by **Hosein et al. (1991)** from 94 cow's milk samples. Whereas, **Montasser (1995)** examined 2700 sheep and 414 goats in EGYPT. Then he stated that dominant strain isolated was *B. melitensis* biotype 3.

In this respect, **Abdel-Gwaad (1996)** examined 1816 cows in Ismailia and Port-Said governorates where eight *Brucella* strains isolated, 6 of which from supramammary lymph nodes and 2 from spleens. All isolated strains were typed as *B. melitensis* biotype 3. Moreover, **Abdel-Haleem (1998)** isolated thirty-six *Brucella* strains from milk and different tissues of reactor animals, and all isolates were typed as *B. melitensis* biotype 3. **Kadry (1999)** also carried out bacteriological examination on milk samples of 242 lactating herd cows, 134 individually raised cows, 144 herd buffaloes and 110 individually raised buffaloes from different localities at Sharkia Governorate. Bacteriological examination yielded 76 *Brucella* isolates from lymph nodes, aborted feti and milk samples from different animal groups. *B. abortus* biotype 3 was the only biotype isolated from the herd cow, while *B. melitensis* biovar 3 was isolated from other animal groups.

Al-Talafhah et al (2003) used a combined cross-sectional and longitudinal design to estimate sero-prevalence of *Brucella* antibodies in Awassi sheep and the incidence of abortion due to brucellosis during one lambing season, and to test risk factors. The *Brucella* organisms isolated from aborted fetuses and vaginal swabs were characterized as *B. melitensis*

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biotype 3. The animal-level incidence of abortion was 20% and the specific incidence risk of abortion due to brucellosis was 13%.

Sayour (2004) studied the differentiation of 357 *Brucella* isolates and 15 reference *Brucella* strains at the species, biovar and sub-biovar levels. The substrate specific tetrazolium reduction (SSTR) classified the isolates as *B. melitensis* with 4 main metabolic patterns. Multi-phage typing identified the isolates as *B. melitensis* with 4 lytic patterns. Antibiotic resistogram detected seven field strains within the 343 isolates of *B. melitensis* bv. 3, two strains in the 13 isolates of biovar 2, and one strain unique to the isolate of biovar 1. Five epidemiological maps were drawn. Combined SSTR (a simple alternative to classic metabolic tests) and multi-phage typing guaranteed accurate speciation. Antibiotic resistogram was very helpful for strain typing.

Abdel-Hamid (2007) stated that bacteriological trials resulted in recovery of 14 *Brucella* isolates from 492 milk samples (10 from sheep and 4 from goats) and all the isolates were typed as *Brucella melitensis* biovar 3.

Sahin et al (2008) isolated *B. abortus* from 48 (32.21%) of 149 lung samples and stomach contents of the aborted fetuses in Turkey. Based on the biochemical tests and the agglutination tests with monospecific A and M antisera, only 3 of the isolates were found to be *B. abortus* biotype 1 and the remaining 45 were biotype 3. This study also revealed that the dominant biotype of *B. abortus* was biotype 3 in this region. Whereas, **Ica et al (2008)** isolated 75 strains of *B. abortus* from aborted bovine fetuses collected from several regions of Turkey Between 2002 and 2007. The isolates were all identified as *B. abortus* biovar 3 by conventional bacteriology.

Herr et al (2010) prepared modified *Brucella* selective (MBS) medium, which contains improved antibiotic mixtures, erythritol as the only carbon source, and neutral red as a pH indicator, showed good selectivity for the *B. abortus* strains. Erythritol in the MBS medium was able to promote and/or recover the delayed growth of the *B. abortus* strains through the

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antibiotic mixtures. The *Brucella* colonies, which assumed a pinkish color at their central part, were easily differentiated from other organisms. The MBS medium also allows the isolation of the *Brucella* strains even in contaminated specimens and/or in specimens containing small numbers of viable organisms. Different seropositive samples from sheep, cow and goats from May 2009 to May 2010. Species of *Brucella* were isolated from, 9 of 32 in cow, 25 out of 69 in sheep and of 5 out of 5 in goats, from lymph nodes and spleen tissues. The species examined by biochemical characteristics and had identical reactions with the standard strain. Oxidative metabolic tests performed, by substrate specific tetrazolium reduction (SSTR) test on the species, confirmed them as *B. melitensis*. Based on the production, growth in the presence of thionin and basic fuchsin dyes, and agglutination test with monospecific A and M anti-sera) the strains were determined biochemical, oxidative metabolic, and biotyping tests (CO₂ requirement, H₂S as *B. melitensis* biotype 3. Afifi (2011)

Abdel-Hamid et al. (2012a) isolated Forty three field of *Brucella melitensis* biovar 3, as confirmed by phenotypic bacteriological identification at the genus, species and biovar levels in addition to molecular identification of *Brucella* isolates at the species level by multiplex PCR. *B. melitensis* bv. 3 were recovered from specimens of 101 cows, 70 buffaloes, 116 ewes, 123 goats, 64 bulls and 34 buffalo bulls.

Bakhiet et al. (2013) isolated *Brucella melitensis* bv.3 from retropharyngeal lymph node and other lymphoid organs of sheep and goats from Almonofiyah Governorate & Albehera Governorate, Egypt.

Elbauomy et al. (2014a) studied the epidemiological situation in 5 related unvaccinated ovine farms of baladi breed located in Nobaria district belonging to single owner and managed with one field veterinarian. Sudden onset of abortions followed the entry of purchased replacement infected rams used for mating estrous synchronized ewes. On evaluating the performance of serological tests statistically, buffered acidified plate antigen (BAPA) and

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modified Rose-Bengal tests (mRBT) had higher relative sensitivity of 99.4%, 96.8 respectively on the expense of specificity and good kappa (κ) agreement with complement fixation test (CFT). Three field isolates of *Brucella melitensis* biovar 3 were recovered from slaughtered aborted *Brucella* infected ewes.

Wareth *et al.* (2014) reviewed that *B. melitensis* biovar (bv) 3, *B. abortus* bv 1, and *B. suis* bv 1 have been isolated from farm animals and Nile catfish, Brucellosis is prevalent nationwide in many farm animal species. There is an obvious discrepancy between official sero-prevalence data and data from scientific publications. The need for a nationwide survey to genotype circulating *Brucella* is obvious and the epidemiologic situation of brucellosis in Egypt is unresolved and needs clarification.

El-Gohary *et al* (2015) evaluated in-vitro the germicidal efficacy of commonly used disinfectants including, Virkon® S (Potassium peroxy-monosulfate and sodium chloride, 1%), Suma sol (chlorinated powder, 0.6%) and QACs (Quaternary ammonium compounds) and two antiseptics; including bovadine iodine (iodine, 1%) and Dettol on *Brucella abortus* strain isolated from dairy cows and their surrounding environment under different interfering conditions. The results revealed that virkon S and dettol exhibited high efficacy against *Brucella abortus* at different concentrations and contact periods either in presence or in absence of organic matter. Authors concluded that, periodical assessment of the disinfectants formulations in-vitro and also in-vivo conditions, with the target of enhancing and improving of brucellosis prevention and control in animals and humans is highly required.

Sayour and Sayour (2015) evaluated matrix-assisted laser desorption/ ionization time-offlight mass spectrometry (MALDI-TOF MS) for biotyping of 124 *Brucella* isolates from raw milk and tissues of cow, buffaloes, sheep and goats in 9 governorates and unknown areas in Egypt for way faster and reliable genospeciation based on protein profiles. *B. abortus* biovar 1 was found to exist more than expected, whereas 28 isolates were

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Warethet al (2016) highlighted the role of dogs and cats as symptomatic carriers and reservoirs for *Brucella* and represented the first report of feline infection with *B. abortus* bv 1 globally. These pet animals may contaminate environment and infect both livestock and humans. Surveillance and control programmes of brucellosis have to include eradication of the disease in dogs, cats and companion animals.

2. 3.2. Molecular characterization of *Brucella* Spp. by PCR:

The detection of *Brucella* using PCR was highly sensitive and specific, but the reaction is fairly expensive to perform. However, it is faster than bacteriological test which can take a week or more and it is more accurate than immunological methods because it detects the presence of organism directly. The application of two synthetic oligonucleotides as probes and as primers in PCR were presented for a specific, sensitive and quick identification of *Brucella* species. **Fekete et al (1992) & Herman and Herman (1992)**

The main problem which remains in the bacteriological diagnosis of brucellosis is the lack of definitive test for the determination of long standing active infection. It was also stated that culture is rarely successful in the absence of identifiable localized lesions and the current serological tests often give inconclusive results. However the PCR which is not dependent on the