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ISOLATION OF *Brucella abortus* FROM ABORTED LAMBS IN THE GOVERNORATE OF SULAIMANIA /SAID-SADIC DISTRICT

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ABSTRACT

Brucella abortus was isolated and identified from aborted lambs in the Governorate of Sulaimani /Said-Sadic district. The blood agar, candle jar and 37°C incubator were successfully used. Microbiological and biochemical tests were used for identification of the isolates. Although previous incidence of brucellosis prevalence has been reported serologically in the area but the present study could represent the first attempt for B. abortus isolation, with local available facilities in the Governorate of Sulaimani.

Keywords: Brucellosis, Zoonosis, Abortion, Sheep.

عزل جراثيم *Brucella abortus* من حملان مجهضة فى محافظة السليمانية /ناحية سيد صادق

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الخلاصة

تم عزل و تشخيص جراثيم *Brucella abortus* من الحملان المجهضة فى منطقة سيد صادق العائدة لمحافظة السليماتية. أستعملت فى هذه الدراسة أطباق أجار الدم و ناقوس الشمعة (كاندل جار) مع حاضنة ذات ٣٧ °م. أجريت الفحوصات المايكروبايولوجية و البايوكيميائية المتاحة لتشخيص العزلات. رغم توفر شواهد سيرولوجية سابقة على حدوث داء البروسيلا فى المنطقة، ألا أن هذه الدراسة يمكن أعتبارها كأولى المحاولات لعزل جراثيم *Brucella abortus* فى منطقة السليمانية.

INTRODUCTION

Brucellosis is among the dangerous contagious diseases which effect animals and transmitted to man through contact or consumption of animal products, particularly milk and milk product, precisely the white cheese. The devastating effects of this disease include a heavy economical loss and actual threat to the community health. Different types of animals are infected, including domestic animals like goats, sheep and cattle. In these animals, the disease is characterized mainly by abortion in females and inflammation of gonads in males which may leads to sterility (1). In Middle East countries including Iraq, the number of aborted animals is progressively increased in the last 30 years (2).

Several authors have reported positive serological results in sheep, goats and cattle in Baghdad, northern Governorate of Iraq and Sulaimani Governorate respectively (3-5). Particularly in Nainava Governorate several investigations have been demonstrated the isolation of Brucella mellitensis from man and animals (6-8). The prevalence of Brucellosis among man and animals in Syria, Sadui Arabia and Egypt has been also reported (9-11). Brucella can be diagnosed bacteriologically or serologically (12). Isolation and identification of Brucella species are more reliable method for diagnosis. Brucella is well known as fastidious organism on primary isolation, slow-growing, requiring incubation for 3-21 days (13). Moreover incubation in a carbon-dioxide enriched atmosphere is required for Brucella abortus. Although preliminary identification can be done by microscopic and colonial morphology, there are several biochemical tests that can be used for identification (14). Several types of complex selective culture media have been recommended for Brucella (15), but these are not available locally due to impose of different embargos in the region. So the present study was an attempt to apply simple and available technique for isolation and identification of Brucella in the area.

MATERIALS AND METHODS

Eight aborted full grown lambs from Said-Sadic district 50 Km east of Sulaimani city were submitted to the Sulaimani central veterinary laboratory. The lambs were obtained from a non Brucella vaccinated flock of sheep, with history of frequent abortion. Direct culture from abomasal fluid was carried out aseptically, using duplicate blood agar plates. The inoculated plates were incubated at 37 °C under aerobic and microaerophilic atmosphere. The candle jar was applied for microaerophilic purpose. A damp sterile cotton wool was added to prevent the desiccation of the inoculated plates. The plates were examined every 3-5 days till 21 days. The growth colonies were subcultured on fresh blood agar plate, this process was repeated after 3 days, whenever it was necessary. Preliminary identification was done by observation of colonial morphology, nonmotility and microscopic examination, using Gram's stain technique. Agglutination with specific B. abortus antiserum (CHIMICA OMNIA, ITALY) was determined, negative sera were used as control. Biochemical tests such as catalase, oxidase, urease, H_2S production and Indol production were also applied for the obtained isolates (14).

RESULTS

After 72 hours of incubation 6 from total 8 microaerophilic inoculated blood agars have showed growth of pinpoint bluish transparent colonies, after second subculture the growth was noticeably good. Aerobically cultured plates showed no growth. The obtained microorganisms were not motile. Gram's stained smears have revealed negative staining coccobacilli. Isolates were non hemolytic, non motile but required CO_2 for growth. All isolates demonstrated positive rapid agglutination with specific B. abortus antiserum. The biochemical tests of all 6 isolates have demonstrated similar results; catalase positive, oxidase positive, urease positive, H_2S production, Indol production negative. According to the presented results all isolates seems to be Br. abortus.

DISCUSSION

Comparatively with the serological investigations a limited number of reports are available regarding isolation and identification of Brucella from animals and man in the region. This may be due to the fastidious and slow-growing nature of this organism (12). Nevertheless Br. ovis and Br. mellitensis have been isolated from aborted foetal stomach content in Serwan district of Sulaimani Governorate and Northern Governorates of Iraq, by using selective culture media (2). Isolation of Br. mellitensis from aborted foetal stomach in Nainava Governorate has been reported by several workers (6-8). Br. mellitensis from Beni-Suef Governorate of Egypt was also reported using selective media (16).

According to some reports, complex media such as serum dextrose agar, serum potato infusion agar, trypticase soy agar, tryptose thiamine agar, Albimi brucella agar or broth must be used for primary isolation (17,18). However these media were not available during performance of this study, so instead of using these complex media, the blood agar has been used successfully. It is worthy to mention that growth of Br. abortus is enhanced by serum or blood (12). Candle jar was applied in this study besides aerobic culturing, since sealed container is absolutely necessary for initial cultures of Br. abortus. Moreover Br. abortus that obtained directly from infected animals will not grow in the air (14). However a Brucella culture when becomes adapted for growth in vitro will grow very well on any of the basic media (14). This agrees with above observation in our sub culturing process. Brucella culture require prolonged incubation, hence the surface of a solid medium become too dry, so we have found that addition of a moisten cotton wool is an important requirement to keep essential rate of moisture in the candle jar. It is well known that Br. abortus is not among principal pathogenic Brucella species that isolated from sheep, since the sheep appears to be most susceptible to Br. melitensis, but may also be infected with Br. abortus, which causes occasional infections in sheep resulting in abortions (13,17). Br. ovis cause epididymitis in rams and occasionally abortion in ewes, some time infection without abortion may results in weak lambs (14). Br. ovis like Br. abortus requires CO_2 for growth but Br. melitensis is not, the above results have showed growth only in the candle jar which indicates CO_2 requirement. Br. abortus produces H_2S, Br. ovis is urase negative and not produce H_2S but Br. melitensis produces little or no H_2S (12-14). Moreover both Br. melitensis and Br. ovis are not reactive with specific Br. abortus antiserum. Hence our isolates are almost appearing to be Br. abortus rather than any other species of Brucella. However the PCR technique could be useful for detection of Brucella organisms, since it is more rapid and sensitive than the traditional cultural methods, it detect Brucella-DNA even in low numbers of Brucella organisms in field samples (19, 20).

Finally several authors have recommended specifically equipped Brucella reference laboratories for definitive identification and biovars determination of new isolates (12,13,18).

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