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Serological detection of the latent infection of Brucellosis in calves in Mosul city, Iraq

W.S. Hassan, S.D. Hassan, K.M. Abdulrazzaq and Q.T. Al-Obaidi

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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Correspondence:
W.S. Hassan
wissamsaleem@uomosul.edu.iq

Abstract

Brucellosis is a widespread and endemic disease in the Middle East and Mediterranean regions, and it has significant public health and livestock industry concerns. It can affect a wide range of mammalian hosts, including humans. This study aimed to determine the seroprevalence of the latent infection of brucellosis in calves; Rose Bengal and indirect ELISA tests were conducted for this purpose. The serum samples were collected from 184 local calves of both sexes aged between 1 to 8 months (92 males and 92 females) from different regions of Mosul city. The current study revealed that all samples tested negative with the Rose Bengal test, whereas, in indirect ELISA 5.9%, the samples were against *Brucella* species. The seroprevalence of *Brucella* antibodies showed no statistical differences between males and females, which was 5.4 and 6.5%, respectively. This study concluded that the indirect ELISA test showed high diagnostic efficacy in detecting anti-*Brucella* antibodies in young calves.

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Introduction

Brucellosis is a critical Zoonotic disease that can infect various mammals (1). The disease causes high economic losses in the animal industry, which is difficult to control, especially in endemic areas (2,3). The disease is generally transmitted via direct and indirect routes by contaminated secretions from infected hosts. Brucellosis in cattle is caused by infection with *Brucella abortus* (4,5). However, *Brucella abortus* is more common in cattle but can also be found in other domestic and wild animals. The Brucella spp. Bacteria can retain the infectivity in vitro for a few months, especially at low temperature and high humidity conditions without direct sunlight (5). The young calves can acquire the infection with *Brucella* species via in utero and milk routes, which leads to the development of the latent infection (6,7). These animals show negative results in the serology for the presence of anti-*Brucella* antibodies; however, they become seroconverts at the time of first calving and abortion of these animals (8,9). After that, the infection is considered active, and the infected animals start shedding the bacteria. The latent infection of brucellosis interferes with disease control programs, as conventional serological tests cannot detect such infections (4,10). Therefore, it considers a potential infection source and disease transmission in affected herds and flocks. Hence, productive diagnostic tools are recommended for implementation in the control programs to effectively eliminate the infected animals and reduce the disease prevalence, especially in endemic areas (10,11). Different serological tests, such as Rose Bengal and complement fixation tests, have been used to detect anti-*Brucella* antibodies in cattle. However, these tests have some false positive and negative results; therefore, alternative tests have been developed, such as indirect ELISA (ELISA). The latter is a highly accurate test and easy to handle. It has high sensitivity and specificity compared to the other serological tests, and therefore it is considered a confirmatory and diagnostic test for brucellosis (12). ELISA can detect all

types of immunoglobulins (Igs) in serum, which is an essential feature from a clinical perspective in diagnosing brucellosis (13).

Materials and methods

Ethical approval

This work was ethically permitted by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Mosul (UM.VET.2021.43) on Oct 25, 2021.

Animals and sample collection

The study included 184 serum samples from unvaccinated local calves in Mosul city; they were 1-8 months of age, and the samples were collected from both sexes (92 males and 92 females). They were collected between December 2021 till April 2022. Sterile syringes were used to collect 6 ml of blood from the jugular vein, and the blood was stored at 4C for 24 hours. After that, the sera were obtained by centrifugation of blood at 3000 rpm for 5 minutes and then stored at -20ºC for subsequent applications.

Serological tests

The Rose Bengal antigen is produced by (GÖKHAN, Turkey) which contains the antigen of *Brucella abortus*, and it was used according to the manufacturer. Briefly, 200 µl of serum sample was placed on a card, then 200 µl of Rose Bengal antigen was added and mixed with the serum for 4 minutes. The presence of agglutination during the specific time (4 minutes) is considered a positive result; otherwise, it is a negative result. Also, the indirect ELISA containing *Brucella abortus* antigen was used according to the manufacturer's instructions (VMRD, Inc./ USA). In short, ten µl of serum sample was added to each well of the ELISA plate and incubated for 1 hour at room temperature. Then, the plate was rinsed with the washing buffer three times, followed by adding the Horseradish Peroxidase (HRP)-conjugated antibodies, and the plate was incubated for 1 hour. The plate was again rinsed with the washing buffer three times, the substrate solution was added to the plate, and the final step was reading the plate at 450 nm by the plate reader (Bio-TEK/ USA).

Statistical analysis

The difference in the seroprevalence between males and females was assessed using a two-sided Chi-square, and the P values < 0.05 were considered significant.

Results

The study was designed to reveal the presence of anti-*Brucella* antibodies in the sera of young calves by using Rose Bengal and indirect ELISA tests. These sera were all tested negative with the Rose Bengal test (Table 1). Whereas the indirect ELISA showed positive results in 6.5% of female calves, which represents the infection of 6 animals out of 92, and in male calves, the positive results were 5.4% which represents the infection of 5 animals out of 92, which indicated that there are no statistical differences between them (Table 2).

Table 1: Seroprevalence of anti-*Brucella* antibodies in young calves by using the Rose Bengal test

Samples	Number of samples	The Rose Bengal	Percentage
Male	92	0	0
Female	92	0	0
Total	184	0	0

Table 2: Seroprevalence of anti-*Brucella* antibodies in young calves by using the indirect ELISA

Samples	Number of samples	indirect ELISA	Percentage
Male	92	Five [a]	5.4%
Female	92	Six [a]	6.5%
Total	184	11	5.9%

Different small letters vertically indicate significant differences at level P<0.05.

Discussion

The results showed that all included samples were tested negative for brucellosis by the Rose Bengal test, which agrees with the study (9). It has been reported that the born calves from infected dams with brucellosis were tested negative by both Rose Bengal and tube agglutination tests. Also, Hassain *et al*. (14) showed negative results for the presence of anti-*Brucella* antibodies in the sera of lambs and kids by using the Rose Bengal test. Therefore, it is likely that the tests used in our study were unable to detect antibodies to *Brucella* spp. at all stages of the infection process (15-17). The studies of Al-Hankawe *et al.* and Hussain *et al.* (18,19) in Mosul city reported that the infection rate in cows was 10.7% and 18.25%, respectively, using the Rose Bengal test. Those reports differed from our results because of the variation in the age of animals included in their studies, as they included mature animals and others with the previous calving. The infection rate with brucellosis is higher in the mature animals compared to the young and immature ones, which may occur due to in utero infection, or it may occur via the infected milk that might lead to the occurrence of latent infection (20,21). Conventional serological tests cannot detect latent infection. However, the infected animals may seroconvert when they become sexually mature and

have their first calving and /or abortion. At this point, the disease is developed, and the animals become active shedders (7,22).

Our study showed that the infection rate with brucellosis using indirect ELISA was 5.4% in males and 6.5% in females. These results are similar to the study of Al-Hankawe *et al.* (18). They reported that the infection rate was higher in females compared to the males, in which 5.9% of total animals included in their study were reported as infected by using indirect ELISA. Indeed, those reports confirm that ELISA can detect latent infections with brucellosis at the early stages in calves (23,24). Also, it has been reported that indirect ELISA is considered an efficient diagnostic tool with high sensitivity, and this test is more sensitive than Rose Bengal and complement fixation tests (25,26). The Rose Bengal test uses a bacterial suspension of the whole cells of smooth strains of *Brucella* spp. as an antigen (27).

In contrast, the purified lipopolysaccharides (known as O-chain) have been used as an antigen in the indirect ELISA, which enables the test to have a high sensitivity in the diagnosis (28,29). Therefore, indirect ELISA is a confirmatory and efficacious test for diagnosing brucellosis (30,31). However, our study was different from Al-Hankawe *et al.* (18), who reported an infection rate of 23.01% in cows in Mosul city using indirect ELISA, because of the variation in the age of animals included in our study, and the difference in the stage of the disease at the time of sampling the animals as well.

Conclusion

In conclusion, this study showed that indirect ELISA has a practical diagnostic ability to detect the latent infection of brucellosis in young calves. Therefore, it is recommended to use that test in control and eradication programs of brucellosis and for screening and detecting latent and active infections in animals.

Acknowledgment

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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لكشف المصلي للإصابة الكامنة بالبروسيللوسز في العجول في مدينة الموصل، العراق

وسام سالم حسن، صدام ظاهر حسن، كرم مظهر عبدالرزاق و قيس طالب العبيدي

فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

يعد مرض البروسيللوسز من الأمراض واسعة الانتشار والمستوطنة في مناطق البحر الأبيض المتوسط والشرق الأوسط. يعتبر المرض مهم من ناحية الصحة العامة وكذلك في مجال تربية الحيوانات، يمتلك المرض القدرة على إصابة أنواع عديدة من الثدييات وبضمنها الإنسان. استهدفت هذه الدراسة تحديد نسبة انتشار الإصابة الكامنة بالبروسيللوسز في العجول باستخدام اختباري وردية البنكال والاليزا غير المباشر. تم جمع عينات المصل من ١٨٤ رأس من العجول المحلية ومن كلا الجنسين وبأعمار تراوحت بين ١ الى ٨ أشهر (٩٢ من الذكور و ٩٢ من الإناث)، حيث تم جمع العينات من مناطق مختلفة من مدينة الموصل. أظهرت نتائج الدراسة الحالية بان جميع العينات المشمولة بالدراسة كانت سالبة باختبار وردية البنكال، بينما في اختبار الاليزا غير المباشر أظهرت ٥,٩% من العينات نتيجة موجبة. أظهرت نسبة الانتشار لأضداد جراثيم البروسيلا عدم وجود فرق معنوي بين الذكور والإناث حيث سجلت نسب ٥,٤ و ٦,٥% على التوالي. استنتج من هذه الدراسة بان اختبار الاليزا غير المباشر اظهر كفاءة تشخيصية عالية في الكشف عن تواجد أضداد البروسيلا في العجول صغيرة العمر.