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Article

Using The Rose Bengal Plate Test to Diagnosis Contagious Abortion (Brucellosis) in Aborted Ewes in Al-Muthanna Province

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Abstract: Along with being a zoonotic disease that is spread to humans, the purpose of the presented work was determining the prevalence regarding brucellosis, which results in recurrent abortions in ewes in the province of Al-Muthanna based on the Rose Bengal plate test (RBPT). This has been done for controlling the disease and lessen the resulting financial losses. Serum samples from 40 ewes have been gathered in various parts of the province of Al-Muthanna between February and April 2024, throughout the lambing season. With the use of RBPT, the data indicate that 45% of ewes have brucellosis (18/40). According to the research, the percentage of ewes infected with Brucella throughout the lambing season was high, and there was a highly significant correlation between the two variables. The animal's symptoms, case history, and the abortion time—which occurs following the third month of pregnancy—were all factors in the diagnosis. Since the germs grow and multiply in the case when they reach the reproductive system, particularly in uterine placentomes, which causes them to suppurate and separate the fetus from its dam, resulting in abortion, along with the clinical signs regarding brucellosis, retained placenta, arthritis, mastitis, corneal inflammation, laterals, or bilateral orchitic in rams, we can confirm that the cause of abortion is mechanical rather than toxic. According to our findings, RBPT is a screening test utilized for identifying the prevalence of Brucella species in serum samples. Ewes in this area have been found to be infected with Brucella, necessitating additional investigation and research into additional risk factors linked to isolation and infection of Brucella in this region.

Keywords: Rose bengal plate test, Brucellosis, Ewes

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1. Introduction

No less than 500,000 human cases of brucellosis are reported each year, which makes it one of the most prevalent zoonotic diseases worldwide. It results from the species Brucella and affects both animals and humans. Just North Europe, North America, Oceania, and South-East Asia are immune to brucellosis, which is endemic in goats and sheep throughout the majority of Mediterranean [2] basin, Central Asia, and the Middle East [3] [4]. Sheep brucellosis is classified as either non-zoonotic ram epididymitis, which results from agent B. ovis, or typical zoonotic brucellosis, which results from Brucella melitensis. Brucella melitensis, a common human Brucella species, primarily infects goats and sheep [5]. The main way that diseases between humans and animals occur is through direct contact with bodily fluids like urine and birthing products from diseased animals. Stillbirths, abortions, decreased production, and infertility are among the signs of B. melitensis in animals [6], whereas undulant fever, muscle soreness, headache, arthritis, and lumbar pain are the main symptoms of brucellosis in humans [7]. In spite of ongoing efforts to reduce zoonotic brucellosis, which poses a serious threat to public health, the disease is nevertheless endemic in the great

majority of Middle Eastern nations and is thought to be responsible for tens of thousands of new cases each year [8]. The most prevalent zoonotic disease, brucellosis, affects around half a million people annually globally [9]. Ingestion of contaminated product, direct contact with diseased animals and their materials, and inhalation of the contaminated particles are the three ways that brucellosis is spread to humans [10]. Just 10 Brucella melitensis organisms cause illness, and the infectious dosage of the causal agent is extremely low [11]. In distant locations, the Rose Bengal plate test (RBPT) could be utilized as a herd screening test because it is straightforward, effective, quick, and simple to administer [12]. Additionally, brucellosis is diagnosed using the Complement Fixation Test (CFT) as confirmatory test [13].

Strict vaccination and hygiene protocols serve as the foundation for control efforts. The goal of vaccination is to lower the disease's prevalence to a point where it could be eradicated through testing and slaughter. Although conjunctival vaccination is less harmful than subcutaneous vaccination, it is not safe enough to administer to animals in spite of their pregnant state [14], and there is debate on how long the immunity provided by this vaccine technique lasts. The most widely utilized methods for diagnosing brucellosis are polymerase chain reaction (PCR) testing, serological testing, and agent identification by culture [15].

2. Materials and Methods

Study Area:

The research has been based on cases that were received at the Veterinary Teaching Hospital in Al-Muthanna, where the case history and time of abortion were adopted, in addition to clinical signs and other laboratory examinations that confirm the case and diagnosis. Forty aborted ewes were included in the study.

Case History and Clinical Signs:

All cases that arrived at the hospital suffering abortion in the 3-4 month of pregnancy (approximately >100 days), which was a basic sign that was relied upon in addition to other signs, which included: high temperature, loss of appetite, red eyes, and placental retention in some cases (12 out of 40) (30%). According to the case history and what the owner said that the rams suffering from epididymitis and orchitic impair fertility which considered the key economic effect.

Samples Collection:

With the use of a sterile syringe, five milliliters of blood have been extracted from the jugular vein of ewes who had been aborted. The samples were then placed in an anticoagulant tube and sent straight to the lab to be incubated at room temperature. After being separated, clear serum can be kept for up to a week at a temperature between 2 and 8°C or for longer lengths of time at -20°C, for a period of ten minutes.

Rose Bengal Plate Test:

A quick slide agglutination technique called the Rose Bengal plate-agglutination test, also known as the buffered Brucella antigen test (BBA), was created to directly detect Brucella antibodies in both humans and animals (figure 1).

Figure 1. Rose Bengal Plate Test

Procedure

a. Allow the samples and test reagents to come to room temperature.
b. Gently suspend the antigen vial. To get a complete mixing, aspirate the dropper multiple times.
c. Fill one of the card's circles with one drop (25 µl) of the serum being tested. Fill two more circles with one drop of the positive control serum and one drop of the negative control serum.
d. To each circle adjacent to the material to be analyzed, add 1 drop (25 µl) of Rose Bengal Antigen [16].
e. Using a disposable stirrer, combine the contents of each circle and distribute them throughout the whole ring-enclosed area. For every mixture, use a different stirrer.
f. Spend four minutes slowly rotating the slide by hand.
g. If there was any agglutination, the sample was categorized as positive; if not, it was classed as negative.

3. Results

According to the findings, 18 of the 40 (45%) tested samples were positive (reactive: any level of agglutination apparent macroscopically), and all reactive specimens should undergo additional testing to elucidate the problem (table 1) and (figure 2).

The results of this test were correlated with the case history (abortion in ewes and orchitic in rams) as well as the clinical signs mentioned above, which confirmed that these animals were infected with brucellosis.

The results also show that the most infections with brucellosis in sheep were occur during the lambing season 45% (18/40) (table 2).

Table 1. Results of Rose Bengal Plate Test

Test	**Rose Bengal Plate Test**		
Animals	Total Samples	Positive	Percent
Ewes	40	18	48%

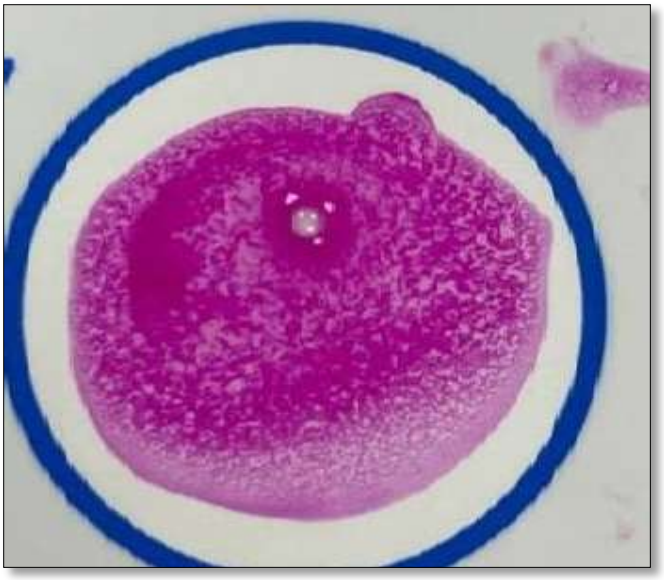

Figure 2. Agglutination of positive Rose Bengal Plate Test

4. Discussion

This research, which assessed the risk variables thought to be connected to the incidence of brucellosis and measured the prevalence rate of the disease in aborted ewes, confirmed the existence of brucellosis in Al-Muthanna Province. The incidence rate of brucellosis across sheep flocks has been found to be in close accord with a prior report that has been carried out in the region [17], suggesting that brucellosis has been an endemic health issue for sheep in the area for the past ten years.

Since it was carried out by numerous other studies in the same location and its serological rate was reported to be between 45 and 56 percent in flocks of sheep and goats with the use of ELISA and RBPT tests [18]. Every year, SC routes sheep to receive a complete dose of the Rev1 live attenuated strain vaccine, which has no age restrictions. Even while the vaccination is not required to be taken during pregnancy, some farmers might choose to do so due to the fact that they are often the ones administering it and many of them lack education. Despite lowering dosages administered conjunctively or subcutaneously, it was observed that this vaccination is unsafe to use during pregnant [19]. Another possible risk factor for brucellosis in sheep ranches was grazing on common pasture. Along with the fact that culture methods aren't available in labs in endemic nations, the diagnosis is mostly based on serologic testing because it is quick and easy [20]. In several nations, RBPT has been successfully employed to eradicate brucellosis for many years [21].

As a result, RBPT was utilized in this study to determine brucellosis frequency in humans and sheep across numerous nations. Overall, 45% of ewes had brucellosis by RBPT throughout the lambing season, according to the research's findings (18/40). Our findings closely matched those of [22], who verified that the prevalence among sheep was 12.2%, [23] who came to the conclusion that the total prevalence of brucellosis in ovine has been 10.0%. Along with people's reporting practices, sampling timing and location might also have an impact on the variations in brucellosis prevalence.

The best test for detecting human brucellosis is RBPT. This is because the procedure is quick, easy, and extremely sensitive [24]. The largest infection rate occurred in sheep during the lambing season, according to an analysis of the seasonal variation of brucellosis. Our findings ran counter to those of [25], who concluded that the majority of cases occurred in the spring, summer, and early fall, and [26], who verified that the seasonal occurrence of human brucellosis cases peaked in the summer.

5. Conclusion

Our findings and other reports support the notion that brucellosis is an endemic illness in the province of Al-Muthanna that affects sheep. In such species, it is regarded as a major cause of abortion. Their financial support is much appreciated by the authors. They want to express their gratitude to all of the owners, lab workers, and vets for their assistance and hospitality.

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