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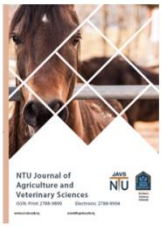

Rapid diagnosis of the most common causes of abortion in Awassi ewes in Nineveh Governorate, Iraq

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A B S T R A C T

The study aimed to determine the rates of infection with the most common causes of abortion by Rapid methods of diagnosis, and abortion rates in the governorate's areas. Rapid methods of diagnosis are important to help the veterinarian in diagnosing abortions in order to reduce the economic loss of sheep by taking the appropriate decision and treatment within a short time. This study was conducted on 92 samples (46 vaginal swabs, 46 blood) representing 60 flocks, the study was conducted from August 2022 to January 2023. The following tests were used, FASTest CHLAM Ag rapid screening test for Chlamydia, Rose Bengal Agglutination Test for Brucella, and Latex agglutination test for Toxoplasma. Results indicate that 13/46 positive samples were 28.26% for chlamydia, 29/46 positive samples 63.04% for Brucella, and 17/46 positive samples 36.95% for toxoplasma. It has been concluded that with a high infection rate of *Chlamydia abortus* in ewes in Nineveh, the rapid test was ideal for the diagnosis of Chlamydia. A high infection rate of Chlamydia in the center and northern areas of Nineveh.

Introduction

Sheep, as productive agricultural animals, occupy an important aspect of animal production in Iraq. They are one of the main sources of the production of red meat. Sheep meat is characterized by a distinct demand for it compared to other types of meat. Sheep and goats contribute in about 7% of global meat production and 3.5% of milk production. Abortion is one of the most intricate problems that sheep and goat breeders suffer from, as it causes severe economic losses. Therefore, finding quick diagnostic methods that may help in providing treatment and reducing economic losses due to abortion.

Abortion is defined as the termination of pregnancy at any stage due to several causes [1]. The most important Causes were infectious diseases like Brucella spp, Toxoplasma gondii, and *Chlamydia abortus*, which were the most common causes in Nineveh Governorate [1]. Al-Dabbah et al., 2014 [2] refer to the rates of infection with previous causes using the ELISA test. Which were 32.8% for Toxoplasma, 56% for Brucella, and 11.2% for Chlamydia. [Alameen and Dahl, 2022 [1]] conducted a comprehensive study to determine the infection rate of abortion causes by following up on all relevant studies previously conducted that used different tests, The infection rate for Toxoplasma gondii was 51.43%, and Brucella was 31.92%, The infection rate for other pathogens was 6.83%, including *Chlamydia abortus*.

Chlamydia abortus is an obligate intracellular, Gram-negative organism that has a unique biphasic life cycle; elementary body (EB), which is the infective form, and the reticulate body (RB), Invading the trophoblast cells of the fetal cotyledon, the organisms then spread to the inter-cotyledonary regions of the chorion, causing a necrotic suppurative placentitis and impairing the passage of nutrients and oxygen between the mother and fetus, which results in fetal death and abortion. [3]. There were two main methods for diagnosing chlamydial infection. The first is based on direct detection of the causative agent in tissues or swab samples. The second method is Indirect and involves serological examination of blood samples to check for the presence of chlamydial antibodies [4].

There are many diagnostic methods including a microscopic examination by special stains like modified Ziehl-Neelsen/MZN, Gimenez [5] or by isolated tissue cultures and inoculated in chicken embryos [6]. lateral flow assay LFAs (rapid screening test) is one of the most successful analytical methods, it is a test that is characterized by low cost, simple and rapid steps and is distinguished by the detection of antibodies, as well as the possibility of its use in the field, which is based on an immunochromatographic "sandwich principle" for the qualitative detection of genus-specific lipopolysaccharide (LPS) antigens of Chlamydia in different tissue, fluids of organs and feces of animals [7], with Sensitivity 93 % & Specific city 99.5 % according to the manufacturers. Molecular detection is the most important method for diagnosis [8]. In addition to indirect detection, Chlamydia Latex Agglutination reveals an aggregation of Chlamydia antibodies present in the serum of infected animals. The complement fixation test is the most widely used procedure in veterinary laboratories. Enzyme-linked immunosorbent assay is an enzymatic immunoassay technique that enables the detection of the interaction between an antigen and an antibody, using a colorimetric reaction, for the detection of Chlamydia antigens in the outer membrane protein, and for the serological diagnosis of Chlamydia in animals [4].

Brucella spp. Is a gram-negative, non-spore-forming, non-motile, coccobacillus or short rods. It is an aerobic, facultative intracellular bacterium that frequently causes abortions in a variety of animals [9]. The most prevalent species of Brucella in farm animals are Brucella abortus, Brucella melitensis, Brucella canis, and Brucella suis. Abortion is most frequently occur in cases of infection in pregnant ewes or goats [10;11].

There are many methods to diagnose including are based on bacterial isolation and identification of Brucella [12]. Molecular methods are a significant advancement in the field of diagnosis [13;14]. In addition to immunological methods, detect an immune response to Brucella antigens [14] However, they are mostly used for simplicity of execution and interpretation and are based on antibody detection. Including milk ring test (MRT), Rose Bengal test (RBT) is a simple method of Brucellosis diagnostics and is the most widely used for the serological diagnosis of sheep and goat brucellosis which was used in this study [15]. In addition to other tests that depend on the presence of antibodies such as Card Test (CT), complement fixation test (CFT), and enzyme-linked immunosorbent assay (ELISA) [16].

Toxoplasma gondii (T. gondii) is an intracellular protozoan parasite that affects almost all warm-blooded animals, with cats, wild cats, and other felines serving as definitive hosts as well as sheep, goats, pigs, and humans serving as intermediate hosts [17]. Toxoplasmosis is an important disease and one of the common zoonotic diseases, and it has widespread all over the world. It is transmitted to the intermediate host either by picking up the oocysts found in cat feces or eating the tissue cysts found in meat. Toxoplasmosis causes fetal malformations and abortion in farm animals and humans [18].

Tachyzoites are passed from the infected mother to the fetus through the placenta, resulting in fetal infection. The severity of the illness for a fetus depends on the stage of pregnancy during which the infection occurs, [19]. T. gondii antibodies "IgM" detection is especially useful in acute or primary infection diagnosis [20].

T. gondii can be diagnosed in several ways, including Microscopic examination (printing method). While there were Serological assays like Latex agglutination test (LAT) which was used in this study, which detect the presence of Toxoplasma gondii antibodies in blood serum or meat juice at room temperature[21], also an Enzyme-linked immunosorbent assay (ELISA) can be automated to test numerous samples at once and can be modified to test both antibodies and antigens. In addition to Molecular methods based on the detection of parasite nucleic acids [22]. [Hade B.F2014 .23] did a study in Iraq using two tests, In order to conduct an ELISA serological examination to look for parasite infection, The same sample was subjected to a molecular analysis using the polymerase chain reaction method to look for the presence of the parasite gene (B1 gene) in the serum sample. To determine the preference between the two tests.

Our study aimed to determine the rates of infection with the most common causes of abortion by Rapid methods of diagnosis, determine the areas with abortion rates in the governorate, and determine the rate of infection of Chlamydia using the rapid test.

Materials and Methods:

Ethical approval This study was ethically permitted by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Mosul (UM.VET.2022.069) on 15 Sep 2022.

Animals of study: Our study was conducted on 92 samples (46 vaginal swabs, 46 blood) representing 60 flocks (10903 ewes) who suffered from abortion. Samples were attended from Awassi ewes 1-3 years of age 1-2 weeks after Suffered from abortion from various areas of Nineveh (Mosul, Al – Shoura, Al - Shikhan , alkoush, Bardarash, Bashiqa, Tilkaif ,Hammam Al – alil, Rabia , Al – Kuwair, Wana), the study was conducted from August 2022 to January 2023.

Sample collection:

Samples included in the study were taking swabs from the vagina of aborted ewes for the rapid test to detect *Chlamydia abortus*, A 10 blood samples obtained from the jugular vein were collected in tube without coagulant; sera were harvested after centrifugation at 3000 xg for 15 minutes. The superannuated serum was collected to perform an antibodies-antigen reaction test to detect antibodies for *Brucella abortus* and *Toxoplasma gondii.*

Laboratory tests

FASTest CHLAM Ag rapid screening test: The FASTest CHLAM Ag rapid screening test prepared by MEGACOR DIAGNOSTIK, (Austria) was used, which is based on an immunochromatographic "sandwich principle" for the qualitative detection of genus-specific lipopolysaccharide (LPS) antigens of Chlamydia in different tissue, fluids of organs and feces of animals. The special sterile swabs were placed in the dropper bottle then 22 drops equivalent to (0.9 ml) of the diluted Buffer material were added. The swab was mixed until the sample dissolved in the diluent, then the sample was left for (10-15) mins at room temperature for extraction purposes. The swab was squeezed against the wall of the tube to remove all fluid. A special filter cap was placed to purify the impurities, and then 4 drops (about 150 microliters) were added to the cassette window a flat-based test cassette, the result was taken after 20 minutes according to the manufacturer's (MEGACOR DIAGNOSTIK, Austria).

Rose Bengal Agglutination Test (RBT): The RBT is the serological diagnosis of sheep and goat brucellosis and is the most frequently used straightforward brucellosis diagnostic technique. At a pH of 3.6–3.7, RBT is carried out using a stained B. abortus suspension. RBT uses a straightforward spot agglutination test in which drops of serum and rose Bengal-stained antigen are combined on a plate, and any agglutination that appears denotes a positive reaction. [24]. The RBT is a screening test that is quick, easy, affordable, and effective. It is used to diagnose diseases in humans, animals, and herds. Results can be seen in a short time [25]. For the detection of anti-Brucella antibodies, serum samples were screened by a Rose Bengal Agglutination Test (RBT) (SPINREACT, S.A/S.A.U Ctra, Santa Coloma, Spain), Brucella abortus suspension, strain S99, in lactate buffer 1 mol/L, phenol 5 g/L, Rose Bengal, pH 3.6, The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result, according to the manufacturer's instructions (SPINREACT, S.A/S.A.U Ctra, Santa Coloma, Spain).

Latex agglutination test (LAT): In this test, latex particles are coated with a soluble antigen, and agglutination is seen when a positive serum is added. Anti-T. gondii IgG antibody detection with LAT is quick and simple. In sheep, LAT has a low specificity of 61.9% and a low sensitivity of 78.6%. [26;2]. Thus, Due to its ease of use, LAT is frequently used as a screening tool in epidemiologic surveys, but a positive result necessitates additional investigation using other serological tests. [28], LAT has also been altered to identify anti-T. gondii IgM antibodies in humans to determine if they have recently become infected. [29],

Detection of anti-*T. gondii* antibodies, serum samples were screened by a commercial latex agglutination test (LAT) (SPINREACT, S.A/S.A.U Ctra, Santa Coloma, Spain). The positive reactors were then diluted; two-fold dilution, 1:2 up to 1 Sera showing titer of ≥1:2 were considered positive results according to the manufacturer's instructions (SPINREACT, S.A/S.A.U Ctra, Santa Coloma, Spain).

Results:

The results of the present study showed different infection rates for each test, as the results of the FASTest CLAM Ag test for Chlamydia were 13 positive samples out of 46 vaginal swab samples, at a rate of 28.26%, and the results of the Rose Bengal Agglutination Test for brucellosis were 29 positive samples out of 46 serum samples, at a rate of 63.04%, and the results of the Toxo-Latex agglutination test for Toxoplasma showed 17 positive samples out of 46 serum samples, with a rate of 36.95%, Table 1, Figure 1.

Table 1. The number of positive samples and the percentage of infection of enzootic abortion, brucellosis, and toxoplasmosis in Nineveh province.

Name of test	Name of disease	No. of samples	No. of Detection	Percentage %
FASTest CHLAM Ag	Enzootic abortion	46	13	28.26%
Rose Bengal Agglutination Test	Brucellosis	46	29	63.04%
Toxo Latex agglutination Test	Toxoplasmosis	46	17	36.95%

The results showed that there are positive results for chlamydia, brucellosis, and toxoplasma in various areas of Nineveh Governorate, which are considered endemic diseases in Iraq Table 2.

Table 3. Geographical location of infection with brucellosis, enzootic abortion, and toxoplasmosis in Nineveh Governorate.

Geographical area	Number of infected		
	Enzootic abortion	Brucellosis	Toxoplasmosis
Mosul	2	4	1
Al – Shoura	0	1	0
Al - Shikhan	0	5	2
alkoush	0	0	2
Bardarash	0	5	2
Bashiqa	2	2	4
Tilkaif	3	1	1
Hammam Al - alil	1	1	0
Rabia	1	1	0
Al - Kuwair	1	6	5
Wana	3	3	0

From the results, it was found that the rates of Infectious abortion in the three diseases generally increase in the months of October and November, which is the birth period in Nineveh Governorate, and in particular for Chlamydia and Brucellosis, as these two diseases are characterized by the occurrence of abortions in the last stage of pregnancy or immediately before birth Table 3.

Table 3. The number of positive samples according to the months of the year.

the months of the year	Chlamydia abortus	Brucella abortus	Toxoplasma gondii
August	0	2	1
September	1	4	5
October	9	8	4
November	2	10	6
December	1	4	1
January	0	1	0

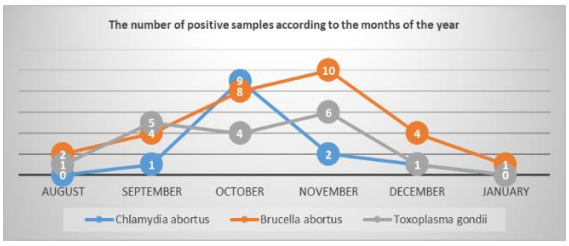

Figure 2. The number of positive samples according to the months of the year.

Discussion:

Our study aimed to determine the rates of infection with the most common causes of abortion by Rapid methods of diagnosis, determine the areas of highest abortion rates in the governorate, and to determine the rate of infection of Chlamydia using the rapid test. The results showed an infection rate of 28.26% for chlamydia, 63.04% for brucellosis, and 36.95% for toxoplasma. The results of the present study did not agree with [2], because of the differences in Chlamydia detection, and results obtained by the research showed a lower infection rate. while it agreed when talking about Chlamydia [1] because the researcher took the infection rate from more than one study of abortion in sheep in Nineveh Governorate. The results of the present study agree with [30] regarding the infection rates in other provinces of Iraq, this was due to the movement of animals affected by grazing and the movement of sheep trade. The results of the present study also agreed with [7] because it used the same type of test, which detects chlamydial antigen (2 of 5 samples). Among the results of infection with brucellosis, infection rates in the present study did not agree with [31], which gave a lower infection rate when compared with our results, and the reason is explained by the fact that the researcher detected antibodies against

brucellosis in milk samples. In addition, the results did not agree with [32], the reason may be that blood samples were collected randomly from ewes and rams, in addition to the fact that this test detects antibodies in general, which are present in the case of previous infections and vaccinations. From the results, the rates of Toxoplasma infection were in agreement with [33] and regarding serological tests using the Toxo Latex agglutination test. The reason is attributed to the use of the same test and the same type of sample. Data from the present study agreed with [24] in infection rates by adopting samples and serological tests to detect antibodies, the infection rates agreed for the two studies were 50%, and 59% respectively [23; 33]. The results of the present study showed that there are positive results for chlamydia, brucellosis, and toxoplasma in different areas of Nineveh Governorate, especially the areas surrounding the center of the governorate, which are characterized by high livestock population, closeness of areas, and ease of movement of animals. Among the results, it was found that the rates of abortion for Chlamydia and brucellosis increased in general in the months of October and November, which is the lambing season in Nineveh Governorate. The reason may be that Chlamydia and brucellosis cause abortion in the last stages of pregnancy and for Chlamydia in particular, abortion occurs a few days before birth. The infection rates were also high in the center and Northern areas of Nineveh Governorate this agrees with Another report [2] due to the intensive breeding of sheep in these areas, bad management practices, and poor Prevention procedures during abortion.

Conclusions:

The present study concluded that a high infection rate of *Chlamydia abortus* in ewes in Mosul compared to previous studies of Chlamydia [2;1]. For diagnosis, the rapid test was ideal because it detects the antigen, unlike other tests that detect antibodies(Rose Bengal Agglutination Test and Toxo Latex agglutination Test), which do not provid an accurate diagnosis if the animals are vaccinated or have been exposed to a previous infection. The infection rates were also high in months October and November.

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Conflict of interest: There is no conflict of interest.

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