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Investigation of *Tritrichomonas Foetus* in Cattle Using Different Methods in Basrah City – Iraq

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ABSTRACT

Tritrichomonas foetus causes a venereal disease in cattle called bovine trichomoniasis. *T. foetus* lives in the reproductive tract of the cow, the surface of the penis and prepuce of the bull and causing early fetal loss and sometimes late term abortions. It can also extend breeding / calving season. In this study, 155 cows of different ages (2-6 years old) and with a variety of clinical signs (repeat breeders, abortions, and discharge only) were examined from different areas in Basrah Province, Iraq from November 2020 to July 2021, and 75 samples collected from healthy animals (without any symptom). The infection rate was detected in wet mount techniques found fewer positive findings than in pouch techniques, with 12 (40%) recorded and in pouch systems, 30 (100%) recorded while in the culture 24(80 %), Giemsa-stained smears 19(63.3%) and Acridine orange was 20 (66.6%). The present study improved that cows more than three years are more susceptible to be infected than cows less than three years. The study concluded that if no control method is applied in Basrah, there is a serious risk of spreading *Trichomonas foetus*.

1. Introduction

Trichomonas foetus that belongs to the parabasalia phylum causes a venereal disease in cattle called bovine trichomoniasis. *T. foetus* lives in the reproductive tract of the cow, the surface of the penis and prepuce of the bull and causing early fetal loss and sometimes late term abortions. It can also extend breeding / calving season (Youngquist & Threlfall 2006; Taylor *et al.,* 1994). This parasite has a pyriform shape with rounded anterior and pointed posterior ends, and has a diameter ranging from 10 to 25 mm in length and 5-10 mm in width. Three flagella extend forward and the fourth extends backward on the organism. *T. foetus* has one single nucleus and four flagella. The undulating membrane on one side of the organism vibrates characteristically with three to five waves. (Rae *et al.,* 2004). When cows become infected with a bull, they develop trichomoniasis. Within 1-2 weeks, the pathogen enters the reproductive tract by vaginal passage, pyometra, and abortion, which are the first physiological signs of the disease, leading to repeat births, irregular heat cycles and reduced fertility (Gregory *et al.,* 1990). A uterus can become infected and the most economical clinical sign of the disease may be infertility due to early embryonic death (BonDurant *et al.,* 1990).

The diagnosis of trichomoniasis can be made using a variety of methods, including wet mounts, staining methods, Diamond's trichomonad medium, commercial culture kits (In-Pouch TF) (Bryan *et al.,* 1999; Parker *et al.,* 2003). which had specificity 100% (Jebara *et al.,* 2012) as well as

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molecular-based techniques (PCR) (Yao 2013; Campero *et al.,* 2003; Cobo *et al.,* 2007) while immunological tests are not recommended for detecting *T. foetus* in individual animals (Jebara *et al.,* 2012).

In this study, the rate of *T. foetus* infection in Basrah Province, Iraq was assessed compared two staining methods (Giemsa and Acridine orange) with Diamond's media culture and using the newly developed culture technique (In-pouch TF system).

2. Materials and Methods

In this study, 155 cows of different ages (2-6 years old) and with a variety of clinical signs (repeat breeders, abortions, and discharge only) were examined from different areas in Basrah Province, Iraq from November 2020 to July 2021, and 75 sample collected from healthy (without any symptom).

2.1. Samples Collection

Three specimens Samples from cows are obtained by washing the vagina. The first swab was then soaked in normal saline for one minute. This was then squeezed onto the tube wall and used in a wet mount smear and staining within one hour. The second swab was immersed and squeezed for cultivation in Diamond modified medium culture tubes. The third swab was carefully inoculated into a pouch TF culture system (Krieger *et al.,* 1993; Kittel *et al.,*1998).

2.2. Wet Preparation Method

Using the procedure (Nasir *et al.,*2022; Bauer *et al.,* 1974). (Fig. 2- A-).

2.3. Staining

2.3.1. Giemsa-Stained Smear

Following the procedure (Lun & Gajadhar 1999). one drop of vaginal swab solution was applied to the microscopic glass slide, and it was then fixed in methanol for 30 minutes. After the staining was applied, they were rinsed under running water, then dried vertically. Giemsa dye solution was used for staining for 2 to 3 hours (timing modified based on preliminary trials). On the slides, violet, pear-shaped trophozoites were detected using 1000X microscopy. (Fig.2-B-).

2.3.2. Acridine Orange Staining

A microscopic glass slide was stained with a vaginal swab suspension, air dried, heat fixed, then immersed in the solution for 20 seconds. Slides were stored at room temperature in the dark after exposure to pH 7.2 buffers (Lun & Gajadhar 1999) and then scanned under a fluorescent microscope at 400X using a TS 510 nm selective beam splitter. Various filter components are included, including a barrier filter at 247 nm, an additional filter at 249 nm, and an excitation filter at 255 nm for narrow-band excitation. (Fig. 2-C-) *T. foetus* trophozoites with a brick red nucleus and yellow green color. It is easy to distinguish bacteria and yeast from trichomonads because they are smaller and morphologically different from each other.

2.4. In Vitro Cultivation of *T. foetus*

The swab specimen was incubated in Diamond's culture medium at 37°C for seven days in anaerobic conditions (Radonjic *et al.,* 2006; Waters & Gard 2021). Wet mount smears were examined daily. A solution without agar, containing 10% inactivated horse serum, was used for incubation of *T. foetus* in Diamond's medium.

A milliliter of cultured *T. foetus*, which is two days old and in log phase growth, was placed into the incubator at 37°C. The pellet was centrifuged at 16000 g for ten seconds and resuspended in Diamond's medium containing 50 percent newborn lamb serum, as well as resuspended in Diamond's parasite suspension medium in 50 ml.

2.5. In Pouch TF Culture System

Using an In Pouch TF culture system (Biomed Diagnostics, Santa Clara, CA, USA), vaginal swabs were inoculated into the upper chamber. Following incubation at 37°C, samples were immediately pushed into the lower chamber. Using the microscope, the cultures were examined on days 2, 3, and five after inoculation. The results showed motile trichomonads (Lun & Gajadhar 1999).

2.5.1. Reagents of In-Pouch TF System

An in-pouch should contain a clear chamber containing: trypticase, proteose peptone, yeast extract, maltose and other sugars, amino acids, salts, antifungals and antimicrobials (Radonjic et al., 2006; Diamond, 1957). Fig. (1).

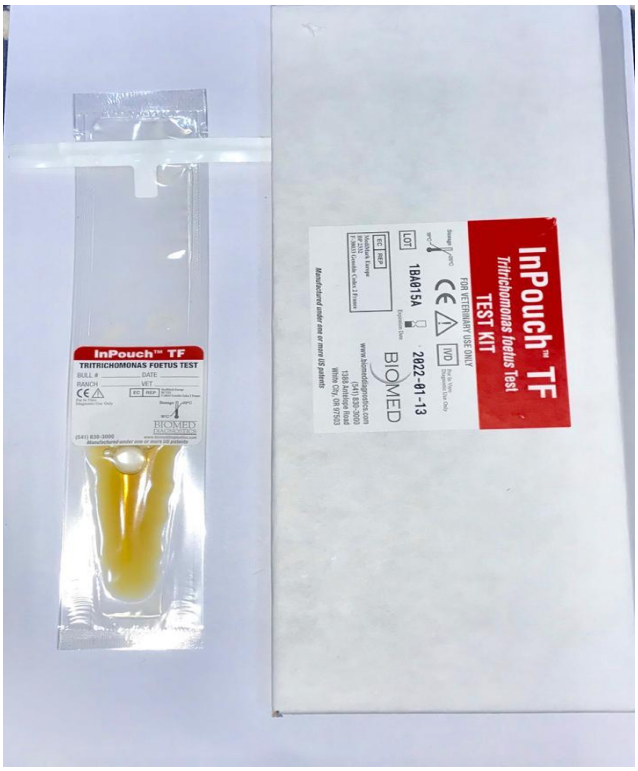

Fig. 1. In-pouch TF culture system

3. Result and Discussion

3.1. Rate of *T. foetus* Infection in Different Methods

The infection rate detected in wet mount techniques found fewer positive findings than in pouch techniques, with 12 (40%) recorded and in pouch systems, 30 (100%) with

statistically significant difference between them (p<0.001). Further, culture was recorded 24 (80%), Giemsa-stained smears 19 (63.3%), and Acridine orange 20 (66.6%) (Table 1).

Table 1
Comparison of five different methods of diagnosing trichomoniasis

Diagnosis	N. of positive %	N. of negative %
Wet smear	12 (40%)	18 (60%)
Giemsa stain	19 (63.3%)	11 (36.7%)
Acridine orange	20 (66.7%)	10 (33.3%)
Culture	24 (80%)	6 ((20%)
In pouch TF	30 (100%)	0(0.0%)

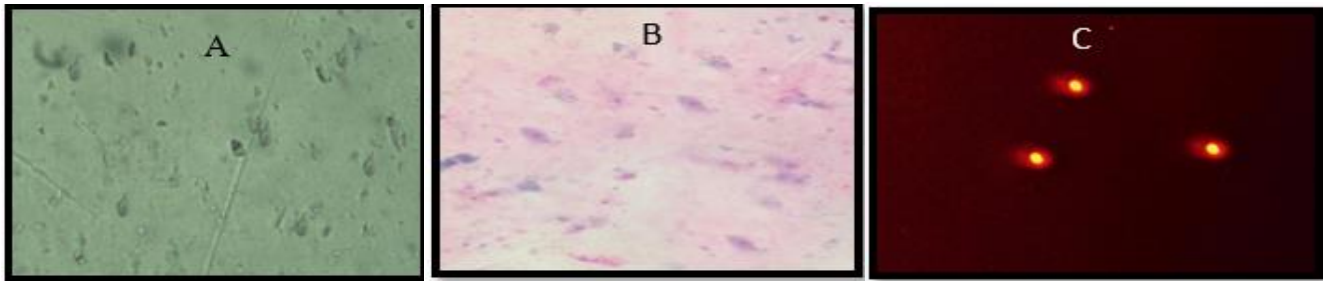

Fig. 2. (A) The picture of *T. foetus* taken under a 40X microscope without staining. (B) microscopic examination of *T. foetus* parasites using Giemsa stain under 40X magnification. (C) Acridine orange (trophozoite stained brick red with a yellowish nucleus, X400)

It is found that the percentage of cows that were infected with *T. foetus* in Basrah province was 12 (40%), 19 (63.3%), 20 (66.7%), 24 (80%), 30 (100%) by wet mount, Giemsa stain, acridine orange, culture and in pouch TF system, which is higher compared previous studies in Iraq.

Hassan, 2013 recorded that the detection of bovine trichomoniasis bulls in Basrah slaughterhouse and reported the percentage of infection (2%) by using Giemsa stain. The percentage of infected cows in Turkey was (8.53%) using Giemsa stain and Alobaidii *et al.,* 2021 indicated that 11 cows (12.6 %) were positive for *T. foetus* by using PCR method in Nineveh province Also, the prevalence of bovine trichomoniasis was 34% positive in the cows at the Kumasi abattoir by using wet preparation (Sallu *et al.,*2020). In Pakistan, Murtaza *et al.,* 2015 recorded a total of eighty samples (preputial flush N.= 40 and vaginal secretions N. = 40) only four (10%) were positive using In-pouch TF kits. Saba (2012) in Iraq recorded that the percentage of infected women was (21%) using the In-pouch TV culture system. These results were disagreement with the current study because poor reporting system, some technical limitations such as participant selection and sample size in addition to sampling methods, intervals, shipping medium, temperature, the procedures that have been used to identify the parasite and because of the long distance between sampling location and the laboratory (Dahab *et al.,* 2012).

An In-Pouch offers some distinct advantages. With the In-Pouch TF system, a positive culture is found sooner for each lower organism counts after a specimen is placed in the chamber; a positive culture can be found sooner with one to ten *T. foetus* inoculums. The bag can be used as a slide on the microscope stage, so that observations can be made directly through it. Thus, the need to enter a broth culture is eliminated. This reduces contamination problems and speeds up the examination process. Furthermore, sampling is not required to inspect the culture for growth, thereby preventing contamination. As a result, these can be conveniently transported from the collection site to the laboratory, where they can be stored at room temperature while other media require refrigeration once prepared. In addition, the cost of this system is comparable to the cost of a conventional culture tube. In Pouch TF can be stored at room temperature for one year, while other media have a shorter expiration date. Normally, culture fluids are culturing and microscopically examined, which is a time-consuming and labor-intensive process.

3.2. Infection Rates of *T. foetus* In Cows According to Their Age

The infection rates for cows equal or more than 3 years 10 (7.14%),15(10.7%), 16(11.i4%)16 (11.4%) and 22(15.7%) in addition cows less than three years recorded 2 (2.2%), 4 (4.4%), 4 (4.4%),8(8.8%) and 6 (6.6%) by using wet amount, Giemsa stain, acridine orange, Culture in diamond medium and in pouch TF respectively (Table 2).

Table 2
The rate of infection with *T. foetus* in bovine by using different diagnostic methods.

Age groups	No. of examined	N. of positive by wet mount	N. of positive by Giemsa stain	N. of positive by acridine orange	N. of positive by Culture	N. of positive by in pouch TF
Cows ≥ 3 years	140	10(7.14%)	15(10.7%)	16(11.4%)	16 (11.4%)	22(15.7%)
Cows < 3 years	90	2 (2.2%)	4 (4.4%)	4 (4.4%)	8(8.8%)	6 (6.6%)
Total	230	12(5.2%)	19(8.2%)	20 (8.6%)	24(10.4%)	30(13%)

As a result of the present study, older cows more than three years are more susceptible to infection than cows younger than three years. This contradicts the claim of many researchers that: Females of all ages were not affected by age group; this could be due to: - stable hormone levels in older cows make the vaginal environment more conducive to the growth of *T. foetus*, Partial immunity develops in infected females, which does not prevent reinfection but enables the animal to overcome the infection in a shorter period of time (Corbeil *et al.*,1998). Old cows are more able to become pregnant and aborted, which led to shedding large numbers of organisms and many farmers depend on artificial insemination with virgin cows.

4. Conclusion

The results of this study suggest that In-pouch culture diagnostic system is an excellent diagnostic technique for determining the presence of *T. foetus* with high sensitivity and specificity**.**

Competing Interests

The authors have declared that no competing interests exist.

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