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Research Article

STUDY THE PREVALENCEOF BOVINE TRICHOMONIASIS OF CATTLES IN BAGHDAD (ABU-GRAIB) BY USING IN-POUCH CULTURE DIAGNOSTIC SYSTEM

Khitam J. Yahya*[1] and May H. Kawan[2]

[1]Artificial Insemination center/Ministry of Agriculture
[2]Department of Parasitology/ Veterinary Medicine Collage/University of Baghdad

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ABSTRACT

The protozoan *Trichomonas foetus* has been recognized as an important cause of chronic reproductive failure in cattle in many countries, including Iraq. The objective of the present study was to determine the prevalence of *T. foetus* in Baghdad (Abu-Graib) by using new diagnostic culture system In-Pouch TF, Giemsa stain and study the stages of growth (morphological changes). The study based on collection and diagnosis 70 samples of preputial and vaginal washes from cattle. The study starts at October 2015 and extend to May 2016. The result showed that the total percentage of infection in cattle of Baghdad was (54.28%, 21.42 %) respectively. Through the stages of growth, the parasite manifested different morphological changes, which were ranged from a non-motile spherical shape (pseudocyst), nucleated and flagellated pear shape, bi-nucleated and flagellated spherical shape and finally slow-moving large spherical shape.

INTRODUCTION

Trichomonas foetus is a flagellated eukaryote protozoa responsible for trichomoniasis which is consider as one of the important venereal disease due to its economic significance in the cattle-raising industries [1,2] the transmission occur principally through coitus [3,1], *T. foetus* inhabits the vagina and urethra of cows and penis and prepuce of bulls [4]. The first reported of the parasite in France by Kunstler in 1888 as a cause of bovine infertility [5].

In bulls, shortly after infection, a preputial discharge combined with small nodules on the preputial and penile membranes may occur. There are no gross lesions on the infected bulls (asymptomatic), although a small number of the organisms are carrying in the preputium with some concentration in the fornix and around the glans penis. They remain asymptomatic carriers of infection for life [6]. Bulls are consider as permanent sources of infection and the infection does not affected on the fertility or the viability of their spermatozoa [7].

In the cows, it is characterized by vaginitis or cervicitis to endometritis, aberrant returns to estrus or irregular oestrous cycles, infertility, pyometra and abortion (early embryonic death), occasional late abortions led to economic loss [8].

Methods of samples collectioncan generally be classified into (a) brushing, (b) scraping, (c) washing and (d) swabbing. These sampling methods were compared with one other by multiple laboratories, the best three methods was brushing, scraping and washing, were comparable with each other, with none being significantly superior to the others. Washing is most frequently used by veterinarians due to the ready availability of pipettes and syringes, and ease of performance [9].

Several methods can be used in the diagnosis of trichomonasis: wet mount, staining method, Diamond's trichomonad medium, commercial culture kits (In-Pouch) [10,11] the specificity of (In-Pouch™ TF kit) was 100% [12] and Molecular-based techniques (PCR) [9,13,14], the Immunological tests are limited in use and are not recommended for the detection of *T. foetus* in individual animals [12].

Nowadays, isolation and identification of *T. foetus* are still a problem in many venereal clinics and laboratories in Iraq. This is usually because the available facilities and the experience of workers are limited. Also unstable electricity may lead to the culture media to be contaminated or give false results and the environment of samples transport condition is not suitable. So, bovine with trichomoniasis being left with no well treatment will lead for this parasite to be transmitted, especially in asymptomatic bull when having sexual intercourse with partners. Due to these reasons this study was done and it is consider being the first study in Iraq.

The aim of this study was Study the prevalence of *Trichomonas foetus* in Baghdad (Abo-Graib) by using the newly culture

*Corresponding author: *Khitam J. Yahya*
Artificial Insemination center/Ministry of Agriculture

system techniques (In-pouch TF system), Giemsa stain and study the morphological changes.

MATERIALS AND METHODS

70 cattle samples from both sex (male and female) at age ranging from (1.5 - 6) years old, were collected from Baghdad (Abu -Graib). The vaginal washing and perpetual washing were collected during the period from October 2015 to May 2016.

The isolation and identification of *T. foetus* was done at the Parasitology Lab/ College of veterinary medicine /Baghdad University.

***Sample collection**:* In bull, the extraneous hair and material around the preputial orifice were removed to avoid fecal contamination then a plastic pipette was attached to a 50 mL syringe and introduced into the full length of the preputial sac. The saline (Phosphate Buffer Saline (PBS) pH7.2) was placed and material was collected from the ventral fornix and dorsal surfaces of the penis and surrounding preputial mucosa [15]. The flat bevelled edge of the pipette tip was moved back and forth over the surface of the glans penis and the preputial mucosa.

In cow, a mare infusion pipette as described for collection of samples from bulls is fitted with a 50 mL syringe and filled with 50 mL of PBS. The pipette was introduced into the vagina and the bulb or syringe compressed several times to flush the vagina. A 5-10 mL sample was collected and the pipette and material was rinsed in universal tube containing 5 ml PBS. Fig.(1)

Fig. (1): sample collection from cow

Diagnosis: the samples collected from both sex were managed as follow after divieded to two categories

:-**first**, was stained by used Giemsa stain: 10 µL was placed onto a glass microscope slide and a second microscope slide was used to make a thin smear, air dry, the smear was fixed for fifteen minute in Coplin jar containing fixative solution (methyl alcohol), tissue paper was used to blot the excess fixative solution than stained with Giemsa stain for forty minute into a Coplin jar. The slides was rinsed briefly, washed with distilled water and dried to examine under 100x Oil-immersion objective lens. Cited by [16]. **second**, placed in the In-Pouch TF System media walls (1-1.5 cc), then the sample discarded and the top edge of In-Pouch media chamber was folded down and rolled three times, after that a wire tape was folded the end tabs behind the pouch then In-Pouch medium incubated at 37 °C for three days. The culture was examined after three days before being considered negative [17]. The positive result characterized by the observation of white sediment along the side and along the bottom of the chamber then the media was changed to dark color [18].

Reagents of In-Pouch TF system:-The In-Pouch medium contains the following: trypticase, proteose peptone, yeast extract, maltose and other sugars, amino acids, salts, antifungal and antimicrobial agents in normal saline phosphate buffer. An unopened In-Pouch should contain a clear, liquid chamber [18].Fig.(2)

Fig. (2): In-pouch TF culture system

RESULT AND DISCUSSION

The result showed that there is only (15, 38) samples showed positive test for *T.foetus* the percentage of infection in cattle in Baghdad was (21.42%, 54.28%) by using Giemsa stain and In-pouch culture system respectively, this percentage of infection is very danger in spreading of disease into the country, causing early abortion and other combined clinical sings.

The primary purpose of staining is to optimize the visualization of key anatomic structures to facilitate accurate identification of an organism. The microscopic structures of *T. foetus* were evident when smears were prepared and stained by Giemsa stain. Three anterior and one posterior flagellae stained purple. The undulating membrane stained purple and has 2–5 waves. The axostyle stained translucent purple and the nucleus appeared dark purple, the cytoplasm of *T. foetus* stained blue and contained translucent vacuoles. Examination of a number of stained organisms on a smear was usually necessary in order to visualize all of the above structures in *T. foetus*. [19]. Fig.(3)

After three days incubation, a white sedimentation appears in the bottom, indicate a positive result. It has been found to produce reliable results in less time than the standard in vitro method, have fewer problems with contaminating

microorganisms, and have significant advantages in both sensitivity and specificity.

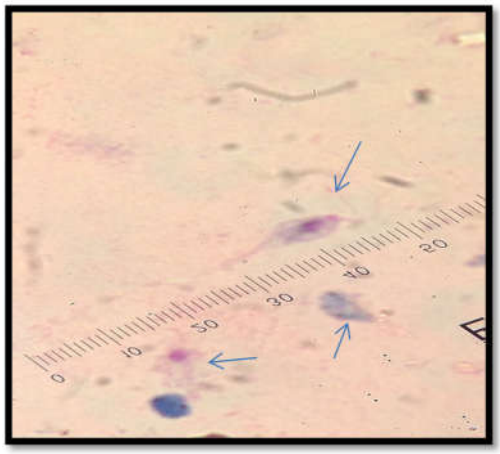

Fig. (3): *T. foetus* stained with Giemsa stain.

A white sedimentation indicated appositive result were seen by using In-pouch culture system was the same result that described by [18]. Fig.(4)

Fig. (4): positive result

In-Pouch offers some distinct advantages. Once the specimen is placed by a clinician into the In-Pouch chamber, an inoculum containing 1 to 10 trichomonads is sufficient to cause a positive test; this suggests that a positive culture would be found sooner with lower organism counts with the In-Pouch TF system. Microscopic observation can be made directly through the bag as the bag can be used as a slide on the stage of the microscope which alleviates the need to enter the broth culture. This decreases contamination problems and speeds up the examination time and this obviates the need for sampling to examine the culture for growth thereby preventing contamination. These can be conveniently transported from the site of collection to the laboratory and can be stored at room temperature, other media, once prepared, require refrigeration. Further, its cost is comparable to the ordinary culture tube. In-Pouch system can be stored at room temperature up to one year whereas other media has a shorter expiration date. The standard method of culturing and the required microscopic examination of the culture fluid are considered time-consuming and laborious [18].

The rate of infection with *T.foetus* recorded in the present study were much higher than the other previous studies reported by many researchers in Iraq:- Hassan (2013) study the detection of bovine trichomoniasis of bulls in Basrah slaughterhouse and reported the percentage of infection (2%) by using Giemsa stain [20], Serin *et al*. (2010) in Turkey recorded that the percentage of infected cows was (8.53%) by using Giemsa stain [16] and Murtaza *et al*. (2015) in Pakistan recorded a total of eighty samples (preputial flush n= 40 and vaginal secretions n =40) only four (10%) were positive by using In-pouch TF kits [21]. 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 [22,23,24,9].

After less than 24 hours incubation in culture media, the parasite grew in In-pouch culture media with pear shape, normal sizeand jerky motion. Through the stages of growth, the parasite manifested different morphological changes, which were ranged from a non-motile spherical shape (pseudocyst) Fig (5a), nucleated and flagellated pear shape Fig (5b), binucleated and flagellated spherical shape (Fig 5c) and finally slow-moving large spherical shape Fig. (5d). this agree with [25,26,27,28].

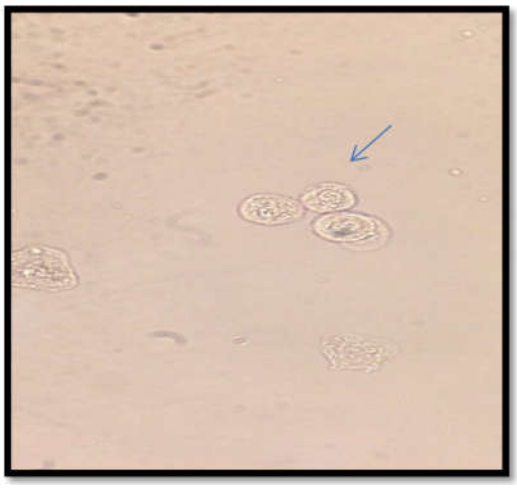

Fig. (5a): non-motile spherical shape (pseudocyst)

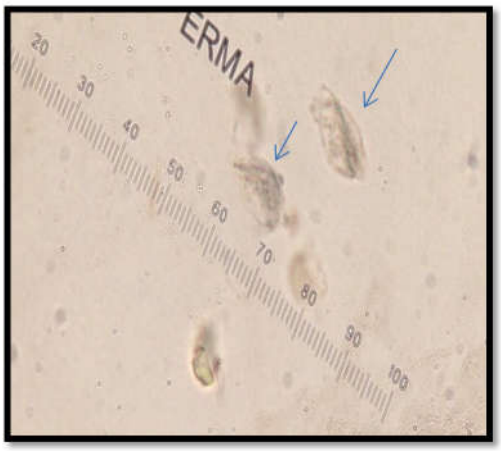

Fig. (5b): nucleated and flagellated pear shape

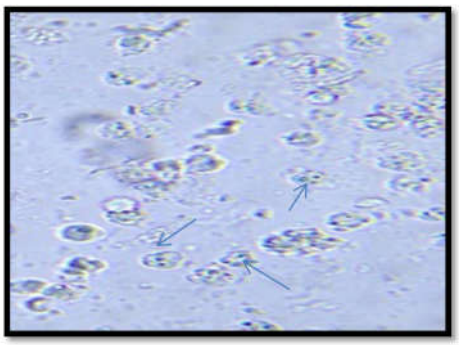

Fig. (5c): binucleated and flagellated spherical shape

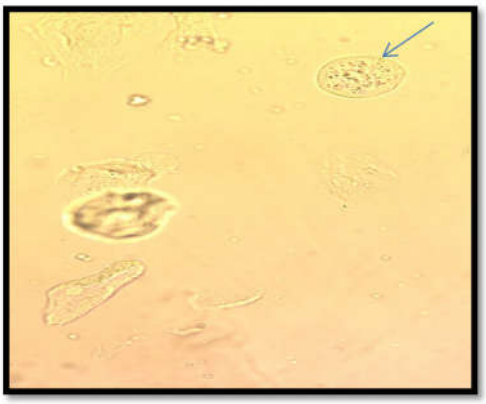

Fig. (5d): slow-moving large spherical shape

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