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**Rafeef M Jaber Eanzi**

College of Veterinary Medicine,  
Al-Qasim Green University,  
51013 Babylon, Iraq

**Nawras A Madlol AL-Kaabi**

College of Veterinary Medicine,  
Al-Qasim Green University,  
51013 Babylon, Iraq

## Molecular detection of *Trichomonas foetus* in women and cow in Babylon province, Iraq

**Rafeef M Jaber Eanzi and Nawras A Madlol AL-Kaabi**

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### Abstract

The present study was conducted to detect the presence of *Trichomonas foetus* from reproductive tract of women and cows in Babylon Province, Iraq. The study included the collection of 200 vaginal swabs (100 women and 100 cows). These samples were tested using microscopy, measuring trophozoites, and PCR that targeted the 18S rRNA gene of the protozoan. The results revealed the presence of the parasite in 26 (26%) of woman swabs and 95 (95%) of cow samples as detected by the PCR. For the geographical correlation, the findings demonstrated significant ( $p<0.05$ ) connection between the prevalence rate and the sample collection regions, in which high rates 25/25 (100%) was detected in cows from Hilla City, The city center of the province capital. For the age of cows, 2-4 and 5-6 years old showed significant ( $p<0.05$ ) high prevalence 40/40 (100%) and 30/30 (100%), respectively, when compared with this from older ages. For women, the findings recorded significant ( $p<0.05$ ) higher prevalence rates according to the collection regions, in which higher rates 21 out of 70 (30%) was detected in women from rural. In urban rate detected (16.66%) 5 out of 30. For the age of women, 26-35 years old showed significant ( $p<0.05$ ) high prevalence 18/40 (45%) in a comparison with this from different ages. The present study shows important data about the presence of high rates in cows, which might be an important source for a zoonosis disease.

**Keywords:** Trichomoniasis, venereal disease, reproductive

### Introduction

The protozoan *Trichomonas foetus* causes sterility only temporarily, with inflammation of the vagina, cervix and uterus. It might result in fetal mortality in early pregnancy but abortion is rarely its cause. There are usually no gross lesions. Microscopically, there is fetal placental oedema together with a mild lymphocytic and histiocytic chorionitis and focal areas of trophoblast necrosis. Fetal pneumonia can be seen in about 50% of cases, with neutrophils, macrophages and sometimes multinucleate giant cells in the bronchioles <sup>[1]</sup>. Trichomonads are flagellated protozoa which have a worldwide distribution. Some species are nonpathogenic, commensal in the digestive systems of animals and humans, while other species are pathogenic parasites. *T. foetus* are found in both animals and humans. *T. foetus* of animals (sheep, goats, bulls, camels, cats, and dogs) is well-documented to affect their reproductive efficiency. *T. foetus* is a human pathogen that affects millions of individuals worldwide by a variety of reproductive and nonreproductive tract diseases. *Foetus* causes human trichomoniasis, which is considered a sexually transmitted disease (STD) <sup>[2, 3]</sup>.

The clinical pathogenicity of *T. foetus* in humans includes vaginitis, urethritis, and cervicitis of the upper reproductive tract. Symptoms may include vulvovaginal discharge with a purulent or bloody or yellowish sample, male infertility, early preterm birth, ectopic pregnancy, and serious consequences or symptoms during pregnancy. The pathogenicity of *T. foetus* in women is considered to be "trichomoniasis", a disease that often goes unreported <sup>[4]</sup>. There is no research to identify *T. foetus* by molecular and microscopic techniques in the women's reproductive tract with genital importance <sup>[5, 6]</sup>.

*T. foetus*. are simple and particle balanoposthitis. They are living parasitic flagellated protozoa, belonging to the genus *T.* from the class of flagellates and the subclass of Trichomonadida. They are found in the colon, reproductive, and upper respiratory tracts of the host vertebrate.

**Corresponding Author:**

**Rafeef M Jaber Eanzi**

College of Veterinary Medicine,  
Al-Qasim Green University,  
51013 Babylon, Iraq

The multiple host species of *T. foetus* include humans, ruminants, birds, racing pigeons, pet birds, cattle, animal exhibition, and wild animals. *T. foetus* also infect the urinary tract of humans, cattle, pigs, and horses, causing the known disease trichomoniasis. The path of transmission and the risk factors for trichomoniasis in humans include contaminated food and water, poor hygiene, hand-to-hand contact, animal bites, contact with infected animals, and sexual contact. It is reported through histories of patients diagnosed with vaginitis or balanoposthitis that they have had contact with animals with a purulent or bloody discharge [7-9].

The morphology of trichomonads is mainly divided into three generally accepted structural features that are present in all known species within this group: the presence and morphology of the characteristic axostyle, the body shape, and the presence and type of the undulating membrane. The classification of trichomonads is still subject to constant divergence, and only DNA-related studies are a more accurate tool for species identification. This tool is quickly becoming available in regions where this ability was not offered before [10-12].

The phylum Euglenozoa is closely related to this phylum and is represented by species belonging to trypanosomatids. Trypanosomatids are responsible for important human diseases such as Chagas disease, Leishmaniasis, and African sleeping sickness. Both phyla share several ultrastructural cell characteristics. Trichomonads, which are a type of protist, have five to six free flagella (from axostyle, pelta, costa, undulating membrane, and haemastrium, if present) and a prominent parabasal filament is visible in the cytoplasm. Trichomonads are cells that lack mitochondria and instead possess hydrogenosomes and paravirus, similar to parabasalids. The list of trichomonads from different ecosystems is not complete and what is known is only the tip of the iceberg [13-15].

*T. foetus* trophozoites have a life cycle marked by distinct cellular stages [16-18]. Recently, the life cycle of the organisms is thought to be relatively simple and direct. This occurs in the reproductive and urinary tracts of their hosts and has four distinct morphological stages: the trophozoite, the pseudocyst, the prepubescent cell, and the spherical cell. The trophozoite is pear-shaped, has at least three anterior flagella, and an undulating membrane. Studies aimed at elucidating the life cycle in various hosts incubated infected vaginal fluid from women-hosts of *T. foetus* with gerbils (*Meriones unguiculatus*) and demonstrated that infection was caused by the swallowed organism [19-20].

The present study was conducted to detect the presence of *Trichomonas* spp from reproductive tract of women and cows in Babylon Province, Iraq.

## Materials and Methods

### Subjects and samples

The study included the collection of 200 vaginal swabs (100 women and 100 cows). These samples were tested using microscopy, measuring trophozoites, and PCR that targeted the *18S rRNA* gene of the protozoan. This study was conducted in collaboration with the gynecology clinics in three public hospitals: AL-Imam AL-Sadiq Hospital, Al-Hilla Teaching Hospital, and Babil Teaching Hospital for Maternity and Children, and several private gynecology clinics in Babil province; Al.Qassime, Al-Hashemite, Al-Hilla, and Mahaweel Districts. Different ages and different areas of the Babylon province were investigated. The study was lasted from August, 2023 until end of January, 2024.

### Molecular study

#### Extraction of DNA

The samples were subjected to Genaid DNA purification kit and instructions, in which 0.5 ml vaginal swab was placed in 1.5 ml centrifuge tube which were followed by the procedure of the kit. The extracted DNA was NanoDrop measured.

### PCR

The complete PCR procedures are mentioned in Tables 1, 2.

**Table 1:** Contents of PCR Reaction mixture

Components	Volume (μl)
DNA	5
Forward primer	1.5
Revers primer	1.5
Master mix	12.5
Free nuclease water	14.5
Total volume	25

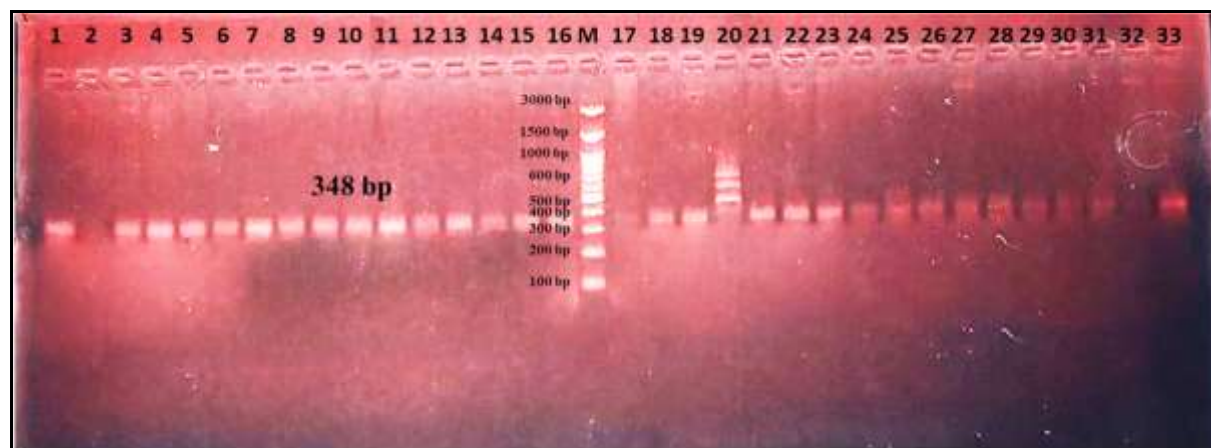
**Table 2:** PCR Program for *18S rRNA* gene amplification

Temp.	PCR	Step (Temp °C)	Time (Sec)	Repeat
	Initial Denaturation	94	5X60	
	Denaturation	94	45	35 cycle
	Annealing	50	35	
	Extension	72	60	
	Final extension	72	5X60	

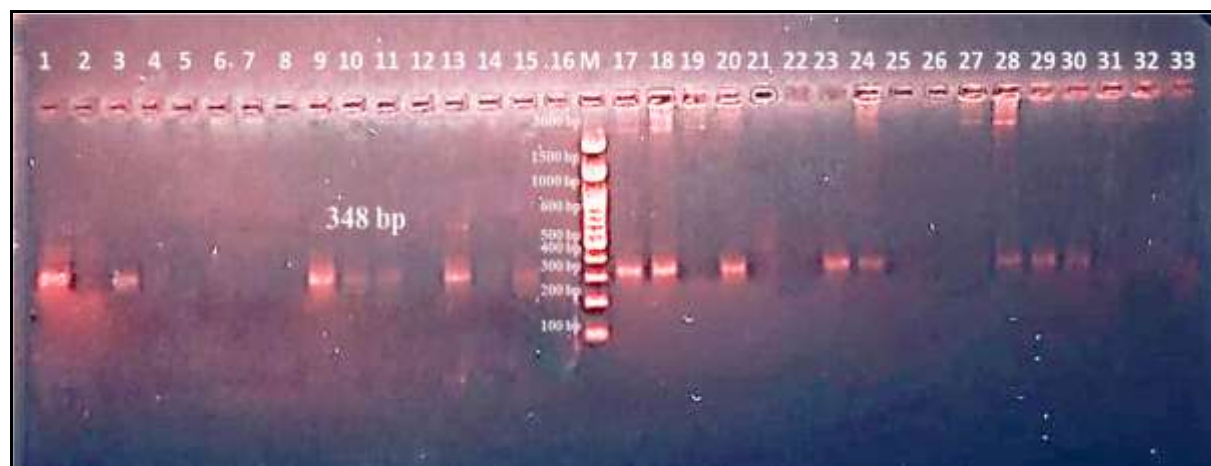
A 1.5%-agarose gel electrophoresis was utilized at 80 volts for 60 mins. The products were visualized by a UV-machine.

### Results

The results revealed the presence of the parasite in 26 (26%) of woman swabs and 95 (95%) of cow samples as detected by the PCR (Figure 1).



**Fig 1:** PCR amplification image of the ITS1 of *T. foetus* recovered from human vagina. Positive product bands: 348 bp



**Fig 2:** PCR amplification image of the ITS1 of *T. foetus* recovered from cow vagina. Positive product bands: 348 bp.

For the geographical correlation, the findings demonstrated significant ( $p < 0.05$ ) connection between the prevalence rate and the sample collection regions, in which high rates 25/25 (100%) was detected in cows from Hilla City, The city center of the province capital. For the age of cows, 2-4 and 5-6 years old showed significant ( $p < 0.05$ ) high prevalence 40/40 (100%) and 30/30 (100%), respectively, when compared with this from older ages. For women, the findings recorded significant ( $p < 0.05$ ) higher prevalence rates according to the collection regions, in which higher rates (30%) 21 out of 70 was detected in women from rural. In urban rate detected (16.66%) 5 out of 30. For the age of women, 26-35 years old showed significant ( $p < 0.05$ ) high prevalence 18/40 (45%) in a comparison with this from different ages (Tables 6).

**Table 3:** Rate of infection of *T. foetus* of cows by PCR according to the geographical areas.

Geographical area	Total number	Positive cases	Percentage (%)
Al-Hilla	25	25	100
Western Hamza	30	30	100
Al-Qassim	35	35	100
Al-mssayb	10	5	50
total	100	95	95
$\chi^2$		47.36	
P value		<0.0001 (Highly significant)	

**Table 4:** Rate of infection of *T. foetus* in cows by PCR according to the age group.

Age interval	Total number	Positive	Percentage (%)
2-4	40	40	100
5-6	30	30	100
>6 years	30	25	83.33
$\chi^2$		12.28	
P value		0.002 (Highly significant)	

**Table 5:** Rate of infection of *T. foetus* in women by PCR according to the geographical areas.

Geographical area	Total number	Positive cases	%
Urban	30	5	16.66
Rural	70	21	30
$\chi^2$		1.94	
P value		0.164	

**Table 6:** Rate of infection of *T. foetus* in women by PCR according to the age group.

Age interval	Total number	Positive	Percentage (%)
20-25	30	5	16.66
26-35	40	18	45
36-40	30	3	10
total	100	26	25
$\chi^2$		12.85	
P value		0.002 (Highly significant)	

## Discussion

*Trichomonas foetus* can be infected to women due to sexual transmission, which may cause vaginitis and cervicitis and subsequently infertility and abortion. The molecular detection of *T. foetus* in Iranian women with Bacterial vaginosis (BV) showed a high prevalence of 27.5% compared to wet mounts and culture, in which only 6.9% were negative for *T. foetus*. In addition, their special attention to the wet preparations with respect to identifying the active mobility of the organisms and their quantitative abundance in this study. Their study represents that probably the majority of women examined for BV in their laboratory were over the phase of parasite growth and multiplication in vaginal secretions, and most of the samples were part of the late phase, beginning phase of the detection with the quickly art method amongst wet mount method. Nenoff *et al.* without reporting the prevalence, and another study in their results showed 1.69%, although the molecular technique used in this study was a real-time PCR method [21-24]. Little is known about the prevalence in the general population of symptomatic and asymptomatic individuals, about the prevalence in women and men looking for general medical consultation, and about the treatment response. The discrepancy in the prevalence rates of *T. foetus* is noticed, which may be due in part to the different methods used for detection. It is confirmed that the wet preparation and cultivation are not very sensitive. The highest rates of *T. foetus* are reported on cultures performed in microaerophilic and macronutrient-rich medium [25, 26].

In Iraq, until today and to the best of our knowledge, there is no or little previous studies using molecular techniques to detect *T. foetus* from women or cows. In general, these methods are relatively few and have been used for the detection of *T. foetus*. Some other studies have detected *T. vaginalis* with a different rate of infection [27, 29]. Alkan *et al.* detected *Trichomonas* in 32% of women with BV using the culture method on Diamond's media. On the other hand,



Smayevsky *et al.* detected 8% *T. vaginalis* with BV-negative cultures by wet mount microscopy and the culture method on the modified Diamond's media. Based on the molecular method, our results showed that the prevalence of *T. foetus* among women was high. This variation in the rate of infection is due to being affected by several factors. Some are personal hygiene, social and demographic factors, behavioral lifestyle, and immunity <sup>[21, 30]</sup>.

## Conclusion

The present study shows important data about the presence of high rates in cows, which might be an important source for a zoonosis disease.

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