



Detection of Trichomoniasis in cattle in Nineveh province

W.A. Al-Obaidii¹, Q.T. Al-Obaidi² and S.D. Hasan²

¹Department of Microbiology, ²Department of Internal and preventive medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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Correspondence:

W.A. Al-Obaidii

wasanamjad@yahoo.com

Abstract

The current study was conducted on the detection of *Tritrichomonas foetus* in vaginal mucus of infected cows using conventional polymerase chain reaction technique (cPCR) in Nineveh province, Iraq. A total of 87 vaginal mucus samples were collected randomly from the vagina of Heifer cows of different ages (2-4, >4-6, >6 years old) and stages of pregnancy with different clinical status (early embryonic death, pyometra, abortion and healthy animals once) by washing cow's vagina using artificial insemination pipette, DNA extraction of *T. foetus* was done from vaginal mucus samples, cPCR was attempt using TFR3 and TFR4 primers, Results indicated that 11 cows (12.6 %) were positive for *T. foetus*. The clinical status of cows demonstrated statistically significant ($P < 0.05$) a higher percentage of Early Embryonic Death at (6.9%) compared to pyometra, abortion, and healthy cows. Furthermore, the percentage of *T. foetus* infection was significantly ($P < 0.05$) elevated among cows (>2-4 years old) at (8%) compared to (>4-6 years old) and (>6 years old) cows. This study concluded that *T. foetus* infection was an elevated percentage of infection in cows with early embryonic death and in cows (>2-4) years old. This study is the first detected *T. foetus* in cattle in Nineveh province.

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Introduction

Bovine *trichomoniasis* is defining as “a venereal disease caused by *Tritrichomonas foetus* (*T. foetus*), that belongs to the class Parabasalia, family *Trichomonadidae* and phylum Zoomastigina (1). Pereira-Neves *et al.* (2) define *Tritrichomonas foetus* as “a single trophozoitic form with a simple lifestyle and the trophozoites, which are spherically shaped, are called ‘pseudocyst.’” The parasite can transmit venereal disease from an infected male to cows through coitus or via artificial insemination using contaminated tools (3). The clinical signs in cattle infected with *Trichomoniasis* can be just mild “vaginitis” or “endometritis,” or be as serious as to severe inflammation of the whole reproductive system. Furthermore, other implications include Early Embryonic Death, abortion, and pyometra in pregnant cattle, unable to be pregnant, and decrease calving ratio (3,4). When the clinical signs are not obvious in chronically infected

bulls, it is considered as asymptomatic carriers for years, but in acutely infected bulls suffer from lesions and discharge in the genitalia for a short time after infection (5). Numerous diagnostic techniques are used for detecting *T. foetus* with different specificity, and sensitivity such as direct microscopic detection of *T. foetus* in vaginal smears stain by Giemsa stain, but this method is limited when the number of parasites is low. Another method is by inoculating the parasite in different types of culture media., this method takes from two to seven days and does not differentiate between the different types of *Tritrichomonas spp.* (6). Further, the serological test can be used for diagnosis of the parasite such as mucus agglutination test and ELISA test, but these tests have limited use because of low sensitivity, the specificity of tests, and in bulls do not develop tangible immune responses (7). The polymerase chain reaction is widely used to detect *T. foetus* DNA using different types of primers TF1, TF2, TF3, and TF4. The sensitivity of this PCR

technique was up to 90% by using specific primers TFR3 and TFR4 for *T. foetus* (8,9). Information of *T. foetus* in cattle in Nineveh governorate, Iraq is scarce and therefore, the aims of this study were to detect for the first time, *T. foetus* in vaginal mucus of infected cows using conventional PCR technique in Nineveh province, Iraq and to ascertain the percentage of *T. foetus* associated with clinical status and different ages of cows.

Materials and methods

Collection of samples

This investigation was conducted on 87 cows of different ages (2-4, >4-6, >6 years old), and stages of pregnancy were obtained from different regions in Nineveh province, Iraq. From July 2019 to December 2019, a total of 87 vaginal mucus samples were collected randomly from the vagina of Heifer cows 15 days post-coitus and at different stages of pregnancy by washing the vagina using artificial insemination pipette fitted to a syringe. The pipette was inserted into the vagina, and the syringe repeatedly pressed to flush the vagina with 10 ml Phosphate buffer saline, then aspirated, and the fluid was collected and centrifuged at 3,000 rpm, then the pellets were processed as described by Richard *et al.* (8).

Conventional PCR technique

Genomic DNA of *T. foetus* was acquired from the cow's vaginal mucus samples using the QIAamp DNA mini kit (Qiagen, Cat No./ID: 51306) and following the product manual. The concentration and purity of the DNA obtained from infected cows were estimated by using Nanophotometer™ P-Class (IMPLEN, Germany). The concentrations of samples were verified by absorbance (A) at wavelength 260nm. They were revealed to be between 55 and 92 ng, with the purity of A_{260}/A_{280} nm ratio of 1.8-1.9.

The hypervariable of ITS gene of *T. foetus* from the cow's vaginal mucus samples (n=87) was subjected to amplification as a target in the conventional PCR approach. The oligonucleotide primers were those of Robert *et al.* (10) and acquired from First BASE Laboratories Sdn. Bhd. Malaysia (Table 1). PCRs were performed, and reaction mixtures (50 ul) contained 5 ul GeneAmp PCR 103 buffer, 2 U Taq -polymerase, 100 mM of each deoxyribonucleotide, 10 pmol of each primer, and 3 ul of the DNA sample. The mixture was first denatured at 94°C for 90 seconds and denatured again by 40 cycles at 94°C for 30 seconds, followed by annealing at 67°C for 30 seconds and extended at 72°C for 90 seconds before concluding with a final 15-minutes extension at 72°C. The DNA samples after being amplified were put through electrophoresis on 2% agarose gel stained with Ethidium Bromide to visualize the amplified DNA fragment in the Gel Doc™ imager (BIO RAD/ USA).

Table 1: Oligonucleotide primers used for amplification of the parasite *ITS gene*

Primers	Sequences 5'- 3'	Expected size (bp)
TFR3	CGGGTCTTCCTATATGAGACA GAACC	347
TFR4	CCTGCCGTTGGATCAGTTTCG TTA	

Statistical analysis

The difference in the percentages of infection between the various clinical status and ages of cows were assessed by using two-sided Chi-square test in IBM-SPSS statistics version 19 program (11).

Results

In the current study, *T. foetus* was detected in 11 cows with a percentage of 12.6% in Mosul City, Iraq, by using conventional PCR technique (Table 2). For *T. foetus*, the positive bands were at nearly 347 bp (Figure 1). Furthermore, the clinical status of cows showed that statistically significant ($P<0.05$) elevated percentage of Early Embryonic Death (EED) was 6.9% compared to pyometra, abortion, and healthy cows (Table 2). The percentage of *T. foetus* infection was significantly ($P<0.05$) higher among cows >2-4 years old at 8% compared to cows >4-6 years old and >6 years old cows (Table 3).

Table 2: The percentage of *Tritrichomonas foetus* associated with the clinical status of cattle using cPCR

Clinical status	Number of examined cows	Number of positive cows (%)
EED	23	6(6.9) ^a
Pyometra	19	1(1.1) ^b
Abortion	8	3(3.5) ^c
Healthy animals	37	1(1.1) ^b
Total	87	11(12.6)

Values significantly different ($P< 0.05$) between cows clinical status are labeled with superscript letters (^{a,b,c}).



Figure 1: Gel electrophoresis image showing: Lane (M) Exact Mark 100-1500bp DNA ladder; Lanes (2, 3, 6) negative samples to *T. foetus* DNA; Lanes (1,4,5) positive samples to *T. foetus* DNA in approximately band size 347bp.

Table 3: The percentage of *Trichostrongylus axei* associated with the ages of cows using cPCR

Age of animals	Number of examined cows	Number of positive cows (%)
2-4 years	39	7(8) ^a
>4 -6 years	22	4(4.6) ^b
>6 years	26	0(0) ^c
Total	87	11(12.6)

Values significantly different ($P < 0.05$) between cows of different ages are labeled with superscript letters (^{a,b,c}).

Discussion

This current investigation reported the percentage of *T. foetus* infection in cows in Nineveh province was 12.6%, which is higher compared to previous studies in Iraq. Baqer (12) indicated that the percentage of *Trichostrongylus axei* in slaughtered bulls was 2% in Basrah province using microscopic examination of slide smear stained with Giemsa stain. Studies undertaken in other countries reported different percentages of *T. foetus* infection in Sheridan cattle, USA reported 2.9% of infection in cattle employing PCR technique (13); in Canada it was 6% using PCR (14); in California it was 15.8% using culture media (15); in Nevada it ranged between 26.7 and 44.1% (16); In Australia 122 of

689 (17.7%) cattle were found to be positively infected with *T. foetus* (17), and in Costa Rica it was 6.7% in cows over 4 years old (18). The percentage of *T. foetus* infection differs from country to country and between regions within the same country, which might be due to different management practices, how sensitive and specific the diagnostic tests are, as well as the presence and the efficacy of the control programs (19).

The clinical status of infected cows demonstrated that statistically significant elevated rate of Early Embryonic Death (EED) was 6.9% compared to pyometra, abortion, and healthy cows, findings which are in agreement with Joanna *et al.* (20) how observed that the clinical signs of cattle infection with *T. foetus* rang from moderate vaginitis or metritis to severe inflammation of genitalia. Furthermore, in times of pregnancy, it results in early embryonic death, abortion, and pyometra. Infertility and abortion in infected cows occur because the parasite is able to infect the mucosal membrane of the genital tract which causes adherent *T. foetus* cells and release cysteine protease (CP30) that creates cytopathic effects in oviduct cells, epithelial cells of the vagina and uterus, with resultant apoptosis, and the cysteine protease is able to cleave IgG2 and evade the immune response of the host (21).

The findings of this investigation indicate that the percentage of *T. foetus* infection is higher among cows >2-4 years old, this finding corresponding with that of BonDurant, (22) who found that the majority of cows and young bulls (younger than 3 years old) could possibly achieve spontaneous clearance of the infection. Further, cows typically clear their infection in 3 months or less and gain a brief period of immunity to *T. foetus* no less than a year and sometimes as long as three years. However, it is inconsistent with the findings of Yao, (23) who recorded a high percentage of infection in heifers in comparison with cows because it is possible for cows to retain immunity against infection for as long as three years after being infected while cows regain from infection, in general, possess immunity against infection for one to three years but there can be variations between animals. In order to control *T. foetus* infection in a flock, bulls under 3-4 years of age are used instead of animals older than 3-4 years (17). In this study, conventional PCR was used to detect *T. foetus* because of the high specificity and sensitivity of this technique for the detection of the parasite (9).

Conclusions

T. foetus infection was first detected in cows in Nineveh province, with a higher percentage of infection in cows with early embryonic death and in cows >2-4 years old. Therefore, *T. foetus* infection must be addressed through early diagnosis and preventive treatments against the parasite, as well as using specific vaccines against it.

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Conflict of interest

Author declare no conflict of interests of the manuscript.

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الكشف عن داء المشعرات في الأبقار في محافظة نينوى

وسن أمجد العبيدي^١، قيس طالب العبيدي^٢ وصادم ظاهر حسن^٣

^١ فرع الإحياء المجهرية، ^٢ فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

أجريت هذه الدراسة لغرض الكشف عن طفيلي المشعرات في مخاط المهبل للأبقار في محافظة نينوى وباستخدام تفاعل البلمرة المتسلسل التقليدي، حيث تم جمع ٨٧ عينة من الأبقار والاباكير وأعمار ومرحل حمل وبأعراض سريرية مختلفة (موت الجنيني المبكر، تقيح الرحم، إجهاض) فضلاً عن السليمة منها تم غسل المهبل للأبقار باستخدام الماصة الخاصة بالتلقيح الاصطناعي، ومن ثم استخلاص الدنا للطفيلي من العينات والكشف عنه بتفاعل البلمرة المتسلسل التقليدي باستخدام بادئين. أثبتت النتائج أن ١١ من الأبقار ١٢,٦% إيجابية للإصابة بالطفيلي، في حين سجلت الأبقار التي تعاني من موت الجنين المبكر أعلى نسبة إصابة ٦,٩% مقارنة بالأبقار التي تعاني من الإجهاض وقيحية الرحم وبفروق معنوية واضحة، ارتفعت نسبة الإصابة بالطفيلي معنوياً في الأبقار التي تبلغ أعمارها أكبر من سنتين إلى ٤ سنوات ٨% مقارنة بالفئات العمرية الأخرى. نستنتج من هذه الدراسة أن طفيلي المشعرات غالباً ما يصيب الأبقار التي تعاني من موت الجنين المبكر وبأعمار تتراوح من أكبر من سنتين إلى ٤ سنوات. وهذه الدراسة تعتبر الأولى من نوعها للكشف عن الطفيلي في محافظة نينوى.