

## Evaluation of Buffalo Bull Semen for Some Venereal Diseases Using PCR

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**A**RTIFICIAL INSEMINATION using unaccredited frozen semen is a major approach for diseases transmission. This work intended to scan the buffalo bull semen for *Br. melitensis*, *Toxoplasma gondii* and *Neospora caninum* using Polymerase Chain Reaction (PCR). Fifty bulls were used in this study. Semen was assessed for volume, motility, viability, and chromatin damage. Total genomic DNA was extracted from buffalo semen and amplified by PCR. The results showed that, semen samples were positive to *Toxoplasma gondii* in 6% of the semen samples, while all the semen samples were totally negative to *Br. Melitensis* and *Neospora caninum*. No significant differences were found in semen characteristics between bulls positive or negative to *Toxoplasma gondii*.

In conclusion, the present trial suggests the opportunity of venereal transmission of *T. gondii* among buffaloes. However, further investigations are required using high number of semen samples from different localities of Egypt.

**Keywords:** Buffalo bull, semen characteristics, PCR, *Brucella melitensis*, *Toxoplasma gondii*, *Neospora caninum*.

### Introduction

Control of pathogens in semen from bulls at artificial insemination centers (AICs) is mainly relied on regular testing for both the pathogen antigen as well as antibody reaction to the disease [1]. To diminish the opportunity of disease spread, criteria for the production of semen from donor bulls have been customized and bulls must be verified to qualify for entry into an AIC and be constantly monitored during semen production. Testing programs to screen donor bulls in AICs are evaluated to reduce the possibility of diseases dissemination through frozen semen [1,2].

Brucellosis in animals is a bacterial infectious and zoonotic disease. In cattle, brucellosis is frequently caused by *Brucella abortus*, less commonly by *B. melitensis* and infrequently

by *B. suis* [3]. The disease is characterized by abortion, retention of placenta, orchitis and epididymitis [3]. The *Brucella* pathogens are excreted in semen of infected bulls and the disease may be transferred to healthy cows through contaminated semen used in artificial insemination [4]. Therefore, control of spread of *brucella* pathogen, through frozen semen could be vital in artificial insemination centers [5].

*Toxoplasma gondii* is an intracellular protozoon and can infect all warm blooded animal species. Toxoplasmosis has health and veterinary importance, responsible for abortions and congenital disease in many intermediate host species [6]. *T. gondii* was isolated from semen samples of experimentally infected goats [7,8] and sheep [9] proposing

transmission of *T. gondii* by the venereal path. Although *T. gondii* had positively isolated from the cattle semen by PCR technique [10], there are no studies in the literature concerning sexual transmission of this protozoon in buffalo.

Preventing of *N. caninum* in semen is a chief objective of control platforms as it is a contributing agent of abortion all over the world [11]. *Neospora caninum* is a universal distributed obligate intracellular protozoan. It is considered as the most significant causative agent of recurrent abortions in dairy cattle in several countries and has developed adverse economic impact for their breeding, including reduced milk production, those attributed to extend calving interval, dropping stock value and raised culling rate among others [12]. Furthermore, *N. caninum* may cause disease in calves infected during pregnancy [13]. At birth these calves may have neurological signs, being below weight, missing capability to rise, or have no clinical signs [14]. *N. caninum* infection may also reduce milk production and condensed production life due to early discarding [15].

Hence, bulls' semen and artificial insemination are important ways for transmission of many diseases causing abortion in female animals, so this work aimed to examine the buffalo bull semen using polymerase chain reaction for *Br.melitensis*, *T. gondii* and *N. caninum*.

## **Materials and Methods**

### *Bulls and semen samples*

Total of 50 bulls were used in this study, semen samples were collected by artificial vagina from apparently healthy breeding buffalo bulls maintained at breeding station at Mahalet Mussa near to Sakha, Kafr el-Sheikh Governorate. Semen samples were evaluated and transported on ice to the laboratory and stored at -20 °C until further use.

### *Semen evaluation*

Volume of the ejaculate, individual motility, live sperm percentage using eosin-nigrosin staining and chromatin integrity using acridine orange staining were recorded for the bulls.

### *Extraction of bacterial and parasitic DNA from buffalo semen*

Total genomic DNA was extracted from buffalo semen using a QIAamp DNA Mini Kit (QIAGEN GmbH, Germany) according to the manufacturer's protocol with minor modifications as reported by Pawar *et al.* [16] and Hasanain *et al.* [17]. Briefly, 10 µl of fresh semen and pellets of one frozen semen straw was diluted with 190 µl of phosphate-buffered saline prior to lysis with proteinase K and lysis buffer. The lysate was passed through spin column. Two washings were done with then the DNA was eluted in 20 µl nuclease-free water and stored at -80°C until further use.

### *Polymerase Chain Reaction (PCR)*

Oligonucleotide primers were selected from the gene encoding 31KDa protein of *Br. Melitensis*, B1 gene of *T. gondii* and *N. caninum*. Total of 50 µl volume containing 1µl of each primer alone (100 pmol) shown in Table 1, 25 µl of 2X Taq Master Mix (Cat. No. PLMM01, Vivantis Co., Malaysia) and 2 µl of each DNA sample. PCR was performed in a Bio-Rad T100 thermocycler which was programmed as in Table 1. PCR product was electrophoresed and examined using UV transilluminator. Positive controls (pure cultures) and negative controls (double distilled water) were used.

### *Statistical analysis*

Data were analyzed using the SPSS version 16.0. Student's t test was performed to compare between positive and negative toxoplasmosis buffalo bulls. Differences were considered to be significant at  $P < 0.05$ .

**TABLE 1. PCR primers, conditions and expected PCR products for *Br. melitensis*, *T.gondii* and *N. caninum*.**

| Target                       | Primer Sequence&PCR Product   | Program   |
|------------------------------|---|---|
| <i>Br.melitensis</i><br>[18] | <b>B1</b> TGGCTCGGTTGCCAATATCAA<br><b>B2</b> CGCGCTTGCCCTTTCAGGTCTG<br><br>The expected product (323bp) | Init.Denat. 93°C for 5min<br>Denat. 92°C for 1min<br>Anneal. 60°C for 1min<br>Extension 72°C for 1min<br>repeat for 40 cycles<br>Final Extension 72°C for 10min     |
| <i>T.gondii</i><br>[19]      | <b>B1</b> TGCATAGGTTGCAGTCACTG<br><b>B2</b> GGCGACCAATCTGCGAATACACC<br><br>The expected product (94bp)  | Init.Denat. 93°C for 3min<br>Denat. 93°C for 10sec<br>Anneal. 62.5°C for 10sec<br>Extension 72°C for 15sec<br>repeat for 40 cycles<br>Final Extension 72°C for 7min |
| <i>N.caninum</i><br>[20]     | <b>Np21</b> GTGCGTCCAATCCTGTAAC<br><b>Np6</b> CAGTCAACCTACGTCTTCT<br><br>The expected product (328bp)   | Init.Denat. 94°C for 5min<br>Denat. 94°C for 1min<br>Anneal. 50°C for 1min<br>Extension 72°C for 2min<br>repeat for 40 cycles<br>Final Extension 72°C for 8min      |

## Results

Semen samples collected from breeding station at Mahalet Mussa near to Sakha, Kafr el-Sheikh Governorate were positive to *T.gondii* in 3 (6%) out of the 50 semen samples (Fig. 1A). On the contrary all the semen samples were completely negative to *Br. Melitensis* (Fig. 1B) and *N. caninum* (Fig. 1C) using PCR.

Data presented in Table 2 showed that, ejaculate volume, individual motility, live sperm percentage and chromatin integrity were not significantly differ between *T. gondii*-ve and *T.gondii* +ve bulls.

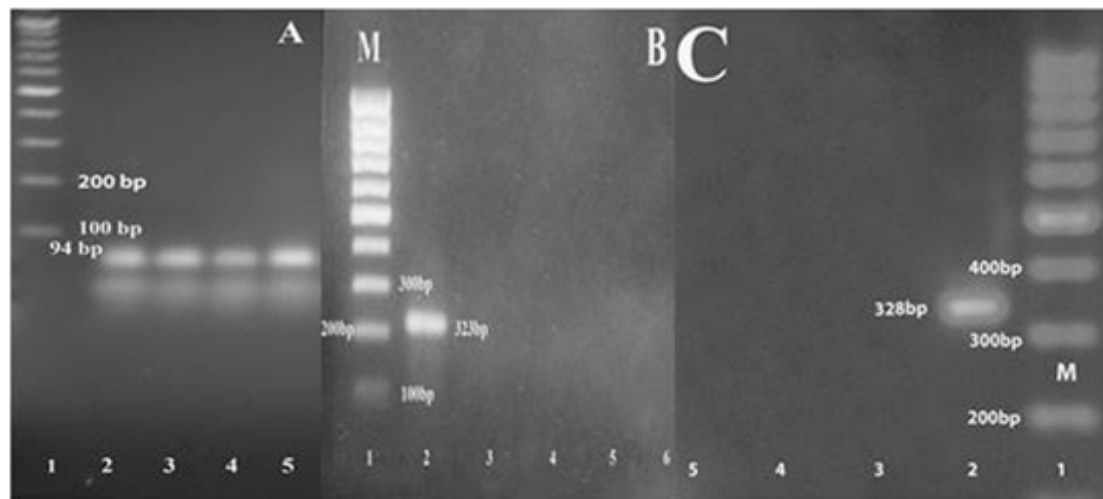
## Discussion

Diagnostic approaches such as microbiological isolation and identification of organisms and nucleic acid amplification built techniques have been applied for revealing of brucella pathogens in bull semen [3]. Although microbiological isolation is the gold standard for brucella detection in semen, this technique is time consuming and there is hazard to laboratory personnel. A sensitive nucleic acid built method, polymerase chain reaction has been used in recognition of brucella genomic DNA in bovine semen [4, 21, 22]. Slight

expert personnel (for reaction set up and result investigation) limit PCR technique being used as a classical diagnostic test for Brucellosis under field situation and less equipped laboratories at breeding bull stations.

In the present study, all the buffalo semen samples were totally negative to *Br. melitensis*. In contrast, Dehkordi *et al.* [23] recorded 24.50% and 3.92% in bovine semen samples while 15.38% and 1.09% in buffalo semen samples were positive for *Brucella abortus* and *Brucella melitensis* respectively.

In the current study, the semen samples were positive to toxoplasmosis in 3 (6%) out of the 50 semen samples. This was agreed by Scarpelli *et al.* [10] who revealed the existence of the parasite in semen samples of animals infected with oocysts (GI) and tachyzoites (GII) which suggest the opportunity of sexual transmission of *T. gondii* in the bovine species. This also was supported by the isolation of *T. gondii* in the semen of sheep [9], goats [24] and swine [25]. Moreover, the *T. gondii* DNA was identified in tissues of stillborns



**Fig.1. PCR targeting *T.gondii* from bulls semen DNA(1-A) . Lane 1: 100 bp molecular size marker. Lane2: positive control, Lanes 3-5: *T .gondii* PCR products from bulls semen DNA samples (94bp). PCR targeting *Br. melitensis* (1-B) & *N.caninum* (1-C) from Bulls semen DNA. Lane 1: 100 bp molecular size marker. Lane 2: positive controls, Lanes 3-5: PCR amplification of bulls semen DNA samples.**

**TABLE 2. Effect of toxoplasmosis on fresh semen characteristics of buffalo bulls (Mean  $\pm$ SE).**

| Bulls                | Ejaculate volume(ml) | Individual motility % | Live sperm %   | Chromatin damage % |
|----------------------|----------------------|-----------------------|----------------|--------------------|
| <i>T. gondii</i> -ve | 3.3 $\pm$ 0.7        | 80.5 $\pm$ 1.2        | 85.3 $\pm$ 1.2 | 0.6 $\pm$ 0.1      |
| <i>T. gondii</i> +ve | 2.9 $\pm$ 0.04       | 79.3 $\pm$ 0.5        | 83.0 $\pm$ 0.7 | 0.5 $\pm$ 0.1      |

and placenta of sheep inseminated with infected semen suggesting the potential infection by contaminated semen [9].

With regard to semen analysis, no significant differences in all studied semen characteristics between bulls positive and negative to toxoplasmosis. To our knowledge, there are no studies in buffalos registered a correlation between toxoplasmosis and semen parameters. In this respect, Barakat *et al.* [26] recorded low quality semen of rabbits infested with *T. gondii*. In Egypt, high seroprevalence of toxoplasmosis and neosporosis in cattle were recorded by Ibrahim *et al.* [27]. So, it is essential to test the animals annually or semi-annually especially bulls in AIC centers to monitor the disease status.

In the present study, *N. caninum* infection is not reported in buffalo semen. The chance of *N. caninum* transmission by semen can indicate great depth repercussions on cattle semen trade.

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Sharifzadeh *et al.* [12] reported higher percentage (17.14%) of semen samples (9.71% and 7.43% of fresh and frozen semen) in Iranian bulls infected with *N. caninum* using PCR that lead to raise possibility of the disease spreading. Moreover, 10.53% of positive samples for *N. caninum* were recorded by Doosti *et al.* [28] in bovine frozen semen used for artificial insemination. The presence of *N. caninum* DNA in semen from naturally infected bulls was also reported by Ortega-Mora *et al.* [29] and proved the probability of *N. caninum* transmission by semen.

In conclusion, the present finding demonstrated the possibility of toxoplasmosis transmission by buffalo semen. Further testing may be needed relying on the predominant diseases such as brucellosis and neosporosis.

*Conflict of interest*

None.

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### تقييم السائل المنوي لطلائق الجاموس لبعض الأمراض التناسلية باستخدام تفاعل البلمرة المتسلسل

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يمثل التلقيح الصناعي باستخدام السائل المنوي المجمد وسيلة لنقل الأمراض التناسلية. وتهدف الدراسة الحالية إلى فحص السائل المنوي لخمسين من طلائق الجاموس من الإصابة بأمراض البروسيللا (*Br. melitensis*) و التوكسوبلازما (*T.gondii*) و النيوسبورا (*Neospora caninum*) باستخدام تقنية تفاعل البلمرة المتسلسل وقد تم تقييم خصائص السائل المنوي مثل حجم القذفة و نسبة الحيوانات المنوية الحية و نسبة الحركة الفردية الطبيعية النشطة و أيضا نسبة سلامة الكروماتين. وقد تم استخلاص الحمض النووي الديوكسى ريبوزى من عينات السائل المنوي و اكتثاره باستخدام تفاعل البلمرة المتسلسل. وقد اوضحت النتائج ان الإصابة بالتوكسوبلازما كانت 6% من عينات السائل المنوي للخمسين طلوقة بينما كانت سلبية لكل من البروسيللا و النيوسبورا . وقد خلصت النتائج إلى إمكانية الإصابة التناسلية للجاموس بالتوكسوبلازما (*T.gondii*) و تبدو الحاجة إلى المزيد من إجراء فحوصات على أعداد كبيرة من السائل المنوي للجاموس للتأكد من سلامته في مختلف نواحي مصر.

**الكلمات الدالة :** طلائق الجاموس، مواصفات السائل المنوي، تفاعل البلمرة المتسلسل، بروسيللا ميلنتسس، توكسوبلازما جوندى، نيوسبورا كانينم.