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Prevalence of Trichomoniasis in Dairy Cows with Some Reproductive Disorders in Aydin Province of Turkey

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Abstract: Trichomoniasis is sexually transmitted disease in cattle. Causative agent of trichomoniasis is *Tritrichomonas foetus* that colonizes only in the reproductive tract mucosa of cattle and causes from mild vaginitis or cervicitis to endometritis, transient or permanent infertility and abortion leading to economic losses. The parasite is transmitted from infected bulls to heifers/cows during mating. The aim of the study was to investigate the prevalence of *T. foetus* infection in dairy cows with some reproductive disorders in Aydin province of Turkey. In this study 164 dairy cows between 2-9 years old suffered from various reproductive disorders (vaginal discharge, metritis, abortus, repeat breeder and anoestrus) were used. For this purpose, smears prepared from the vagina were fixed for 15 min by fixative solution. The slides were then air-dried, stained with Giemsa for 40 min and washed with distilled water. All slides were then examined microscopically. The primary purpose of staining is to optimize the visualization of key anatomic structures to facilitate accurate identification of an organism. The percentage of infected cows was found 8.53% (n = 14). Two out of 7 cows with vaginal discharge, 3 out of 41 cows with metritis, 4 out of 4 cows with abortus and 5 out of 36 cows with repeat breeder were found as *T. foetus* positive. Anoestrus cows were not positive.

Key words: Trichomoniasis, *Tritrichomonas foetus*, dairy cow, giemsa staining, reproductive disorders, Turkey

INTRODUCTION

Bovine tritrichomonosis is one of the most prevalent sexually transmitted diseases in cattle. Causative agent of cattle trichomonosis is *Tritrichomonas foetus*. This is an extracellular, a non-invasive flagellate protozoan that colonizes only in low oxygen tension environment of reproductive tract mucosa in cattle. The infection varies in cows from a mild vaginitis or cervicitis to endometritis, transient or permanent infertility and abortion causing significant economic losses (BonDurant, 2005; Benchimol *et al*., 2007). Infertility is the primary manifestation of *T. foetus* infection which was clinically evidenced as a high percentage of non-pregnant cows (Mardones *et al*., 2008).

Tritrichomonas foetus colonizes the stratified squamous epithelial surfaces of the vagina, oviduct and mucosal surface of the uterus. Resulting inflammation of the vagina, cervix and endometrium produces a hostile intrauterine environment. *T. foetus* displays great specificity for the non-ciliated cells of oviduct epithelium. Thus this protozoan may cause early embryonic death or abortion from breeding up to 7 months of gestation with the majority of reproductive loss at 50-70 days of gestation. Abortions may be most common in older cows with partial immunity. The organism may be found in pyometral discharge, placental fluids or fetal stomach contents. Cows may remain infected with the organism for >150 days (BonDurant, 2005; Benchimol *et al*., 2006; Givens, 2006).

The parasite is transmitted from infected bulls to heifers or cows during mating. Under natural breeding conditions bulls often develop persistent asymptomatic infections and become parasite vectors thus act as carriers of the infection for life (Martin-Gomez *et al*., 1998; Mutto *et al*., 2006; Perez *et al*., 2006; Corbeil *et al*., 2008). Benchimol *et al*. (2008) demonstrated that *T. foetus* interact with sperm cells provoking damage and death of these reproductive cells in *in vitro* conditions.

Various methods have been developed to accurately diagnose *T. foetus* in cattle. The most common techniques included serological assay for antibodies, light microscopic evaluation, culture of organism and molecular-based analyses of preputial washings from bulls and cervico-vaginal secretions from female cattle (Grahn *et al*., 2005). Cobo *et al*. (2004) detected *T. foetus*

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antigens immunohistochemically and reported that changes in lectin binding pattern may have been the consequence of either an inflammatory reaction or the effects of *T. foetus* enzymes such as neuraminidase and cysteine proteinase. Corbeil *et al.* (2008) concluded that serologically tested breeding bulls could carry non-*T. foetus* trichomonads in the prepuce causing false positive diagnoses for trichomoniasis in breeding bulls as well as in non-breeding bulls.

The finding of non-*T. foetus* trichomonads indicates that these parasites may be transmitted at coitus but that infection of the female tract is transient and non-pathogenic. Monteavaro *et al.* (2008) determined that there are modifications in the carbohydrate content of the genital epithelium of infected mice. These changes could be interpreted as reflecting the reaction of the host against the protozoa or alternatively may be due to the effect of *T. foetus* enzymes which promote the adhesion and penetration of the parasite.

PCR is potentially the test for the detection of *T. foetus* (Grahn *et al.*, 2005; Mutto *et al.*, 2006; Corbeil *et al.*, 2008; Kennedy *et al.*, 2008) but is limited by the cost of individual laboratory tests ranging from $20.00-35.00 plus veterinarian charges for sample collection. To determine the presence of *T. foetus* infection within a cattle population by individual *T. foetus* PCR may be cost prohibitive (Kennedy *et al.*, 2008).

In spite of low cost, culture of organism may take up to 7-10 days and even after that the unambiguous identification of *T. foetus* from other Trichomonads may be difficult (Martin-Gomez *et al.*, 1998; Perez *et al.*, 2006; Corbeil *et al.*, 2008).

The microscopic examination for the presence of *T. foetus* which is characterized by the three anterior and single recurrent flagellum detection of an undulating and dynamic membrane, a spindle or pear shaped trophozoite and characteristic rolling and motility. (Granger *et al.*, 2000; Grahn *et al.*, 2005) In most trichomonad staining methods, smears are prepared from cultures or from the vaginal mucus.

The smears are then fixed and treated with Giemsa, silver, iron Bhaematoxylin or other stains. Although, many diagnostic features of trichomonads could be observed with silver or iron Bhaematoxylin stained smears, these methods are cumbersome, laborious and expensive. Giemsa staining is more rapid than silver and iron Bhaematoxylin (Lun and Gajadhar, 1999).

The purpose of this study was to investigate prevalence of *T. foetus* infections in dairy cows and relationship with some reproductive disorders in Aydin province of Turkey. This is the first study on *T. foetus* prevalence in Turkey.

MATERIALS AND METHODS

The investigation was carried out in Aydin region between November 2007 and March 2008. About 164 dairy cows between 2-9 years old suffered from various reproductive disorders (vaginal discharge, metritis, abortus, repeat breeder and anoestrus) were used in this study. Before taking samples, reproductive history of each cow was recorded.

For this purpose, smears prepared from the vagina were fixed for 15 min by dipping the slides in fixative solution (methyl alcohol). The slides were then air-dried, stained with Giemsa for 40 min and washed with distilled water. All slides were then examined microscopically with oil immersion at light microscope 100 x magnification. The primary purpose of staining is to optimize the visualization of key anatomic structures to facilitate accurate identification of an organism.

RESULTS AND DISCUSSION

A total of 164 dairy cows with some reproductive problems were examined in this study. These problems determined as vaginal discharge (n = 7), metritis (n = 41), abortus (n = 4), repeat breeder (n = 36) and anoestrus (n = 76). The percentage of infected cows was found 8.53% (n = 14). Two out of 7 cows with vaginal discharge, 3 out of 41 cows with metritis, 4 out of 4 cows with abortus and 5 out of 36 cows with repeat breeder were found as *T. foetus* positive (Table 1). Anoestrus cows were not positive.

All known microscopic structures of *T. foetus* were evident when smears were prepared and stained by the method used in this study. The parasite has three anterior, one posterior flagella that were clearly observed at stained smears but axostyle, blepharoplast/pelta and costa were not clearly observed at some stained smears.

Tritrichomonas foetus infects the mucosal surfaces of the reproductive tract and adheres to Bovine Vaginal Epithelial Cells (BVECs) in species-specific manner and this interaction induces apoptotic cell death in normal BVECs. *In vitro* co-incubation of *T. foetus* with BVECs resulted in host cell cytotoxicity (Singh *et al.*, 2004, 1999). Agnew *et al.* (2008) reported that vaginal IgA and mucosal inflammatory response were present in the *T. foetus* infected heifers. Infection by *T. foetus* seems responsible for uterine inflammatory lesions usually associated with pregnancy loss. *T. foetus* infected heifers in which inflammation was characterized by moderate submucosal infiltration by lymphocytes and plasma cells in both the vagina and uterus. Some lymphocytes were transmigrating through the mucosal epithelium of *T. foetus* infected heifers.

Table 1: The percentage of reproductive disorders in all and *T. foetus* positive cows

No, of reproductive problems	Vaginal discharge	Metritis	Abortus	Repeat breeder	Anoestrus
All cows (n = 164)	6.6% (n = 7)	25.0% (n = 41)	2.43% (n = 4)	21.95% (n = 36)	4.34% (n=76)
T. foetus positive cows (n = 14)	28.5% (n = 2)	7.3% (n = 3)	100% (n = 4)	13.8% (n = 5)	0.0% (n = 0)

In the study, vaginal discharge, metritis and abortus were seen in 2 cows (28.5%), 3 cows (7.3%) and 4 cows (100%) (Table 1) in *T. foetus* positive cows, respectively. Adherence of parasites to BVECs, apoptotic cell death in these cells elevated levels of IgA and other inflammatory changes may be associated with these reproductive disorders in *T. foetus* positive cows in the investigation.

Tritrichomonas foetus displays great specificity for the non-ciliated cells localized in the deeper oviduct folds. *T. foetus* is able to adhere to damage and cause detachment of bovine oviduct cells leading to cell death. To attack the oviduct, which is the natural passage for the early stage of embryo could contribute to infertility in cows (Benchimol *et al*., 2006; Midlej *et al*., 2009). Benmichol *et al*. (2007) demonstrated that when a large number of *T. foetus* interacts with oocytes *in vitro* damage and apoptosis are provoked in the cows reproductive cells and the behavior of this parasite as one of the causes of cattle infertility. On contrary Bielanski *et al*. (2004) reported that *T. foetus* has no detrimental effect on the fertilization and development of IVF embryos and the potential risk of transmission of trichomonosis is unlikely due to the limited survival of the parasite in IVF culture conditions.

Barbeito *et al*. (2008) reported that embryonic loss was significantly higher and occurred in the early and middle phases in pregnant mice accordance with the time of embryo death in infected bovines. In infected animals at the early phase of pregnancy there was evidence of embryonic death without inflammatory changes in the uterus suggesting a pathogenic mechanism that does not involve direct tissue damage. In the later days, pregnancy loss was associated with endometritis and changes in the decidua. Stewart *et al*. (1998) reported that the overall pregnancy rates were 84 and 93.5% in the breeding groups with *T. foetus* infected and non-infected bulls. These rates were found significantly different. A cow was 2.97 times less likely to be pregnant if she had been exposed to *T. foetus* positive bulls.

Benchimol *et al*. (2008) reported that a decrease in the spermatozoa motility was observed as well intense semen agglutination either fresh or frozen thawed bovine semen used artificial insemination. The adhesion between trichomonads to the sperm cell occurred either by the flagella or sperm head. Motile parasites were observed during the next 12 h, whereas sperm cells in contact with the parasites rapidly became immotile. In the study, while 36 cows (33.9%) were diagnosed as repeat breeder, 5 cows (13.8%) in this group determined as *T. foetus* positive. The effect of parasite on vaginal epithelial cells, uterus, oviduct cells, oocyte and spermatozoa may be associated with repeat breeder in cows.

A total of 14 cows (8.53%) were found as *T. foetus* positive in the study. This ratio is high. We think it could be due to the result of naturally breeding. Some breeders in Turkey believe that if a cow not become pregnant after three or more artificial insemination, mating with a bull can dissolve this problem, which is more preferred in repeat breeder cows. For that reason control strategies must be focus on identification/elimination of infected animals and use of artificial insemination has resulted in a substantial reduction not only of trichomonosis incidence but also of other venereal infections.

CONCLUSION

There is no effective therapy against bovine trichomoniasis (Carvalho and Gadelha, 2007). For that reason elimination of *T. foetus* infected cows and bulls are important for reduce the economic losses. Especially in the field conditions to diagnose and control of this disease with reduced cost and time is very important. Collection and staining of material and diagnosis in Giemsa stain technique is simply and can be done in a minutes by veterinarian without need the serological or molecular-based analyses. Low cost, obtaining of the results in a short time and practicability are the most important positive aspects of Giemsa staining technique.

To avoid false results the test may be repeated in *T. foetus* negative cows in a one week. Thus, it is generally accepted that one single positive result is sufficient to consider *T. foetus* infected; however at least two or three consecutive negative results are necessary to demonstrate freedom from *T. foetus* infection. Especially before or soon after diagnosis of purchased animal to the herd is very important for the control of disease. If natural mating is using in a herd, all bulls must be controlled for *T. foetus* from preputial washings by same method.

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