An Outbreak of Contagious Equine Metritis (CEM) from a Stud Farm in Iran: The First Report of *Taylorella equigenitalis* Isolation from Iran

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Introduction

The only true venereal sexually transmissible disease in horses is known as contagious equine metritis (CEM). CEM in horses is caused by the bacterium *Taylorella equigenitalis*, a micro-aerophilic, non-motile, small Gram-negative rod or pleomorphic coccobacillus. CEM, a venereally transmitted and highly contagious disease, has been diagnosed in many countries. Clinical signs of CEM, which develop in only 30–40% of mares served by an infected stallion, may include vaginal discharge, infertility and early abortion. Asymptomatic mares are the most likely reservoir of infection (5). Most CEM affected mares recover spontaneously from this condition, but a small proportion become carrier of CEMO (2).

The transmission of CEM is mainly affected through coitus, although other routes can also contribute to spread of the disease (7). Infected stallions are asymptomatic and act as the principal source of infection as they mate with numerous mares and the carrier status may persist for many months or even years (8). They harbour the organism in the urethral fossa, the urethra or the sheath. Diagnosis of CEM is achieved by culturing the organism from these sites as well as from semen (4, 9, 10). The recommended transport medium is Amies supplemented with charcoal. Swabs are plated on Columbia blood–chocolate agar at 37°C and 7% CO₂. Because of the slow growth of *T. equigenitalis*, the possibility of false negative results is relatively high. Another test that is commonly used is the polymerase chain reaction (PCR) assay, which appears to be very sensitive.

There was some doubt about existing the disease in some stud farms in Iran based on clinical features of most repeat breeder mares with shortened luteal phase, some showing vulvar discharge. The aim of this study was to find the cause of subfertility in the mares in a stud farm in Tehran, Iran, in which we suspected to *T. equigenitalis* involvement. For the first step, was decided to confirm the presence of *T. equigenitalis* in mares and stallions by taking samples from clitorial sinus and clitoral fossa of carrier mares and the urethral fossa of carrier stallions.

Materials and Methods

Thirty mares of 4 to 15 years of age were randomly selected from a total population of 85 mares in a stud farm in Tehran suspected to have a venereal disease. Genital swabs were taken from clitorial sinus and clitoral fossa of each mare, two swabs from each site (clitorial sinus and fossa), one for culture and another for direct PCR. Based on positive result of culture or PCR, a genital swab was taken from stallions’ penis that had covered these positive mares. Swabs were taken for culture and culture PCR were transported to the laboratory in 5 ml Amies transport media with charcoal and swabs taken for direct PCR were transported in 1 ml LB Broth medium in 4°C. We also recorded the smegma volume of each mare as 1+ (it means that smegma could not be seen on the swab) and 2+ (it means that smegma could be seen on the swab) to evaluate if there is any relationship between smegma volume and the bacterium isolation (Graph 1).

Results and Discussion

*T. equigenitalis* was isolated from 6 mares, whereas one of the samples was positive in culture. From 3 stallions which had covered the infected mares, all of them were positive for *T. equigenitalis* in PCR assay.

Among 21 (1+) and 9 (2+) specimens tested from clitorial fossa, *T. equigenitalis* was detected in 4 (19%) and 2 (22.2%) specimens respectively. Also among 19 (1+) and 11 (2+) specimens tested from clitorial sinus, this pathogen was detected in 5 (26.3%) and 1 (9.1%) specimens respectively. Q square analysis showed that there is not any significant difference between (1+) and (2+) specimens in both clitorial sinus and clitorial fossa. In the present investigation *T. equigenitalis* was isolated for the first time in Iran from the genital tract of mares and stallions with a natural infection. The results of our study demonstrated that the sensitivity of the PCR assay is superior to that of culture as previously shown (1, 6). The PCR assays enable us to detect bacterial concentrations as low as 10CFU. In practice, 10CFU would probably be concealed by the genital flora and therefore, remain undetectable by bacteriological procedures (3).
The lower degree of sensitivity of the culture method and culture-PCR assay can partially be explained by treatment with antibiotics, which resulted in the killing or inactivation of *T. equigenitalis* while its DNA remained and thus detectable in direct PCR assay. We also found that the most of the mares had low volume of smegma at the sampling procedure time. The results of this study shows that we should not pay attention to the volume of the sample on the swab taken from clitoral sinus and fossa, even low volume of smegma may harbor this pathogen. There is not any report about this issue in the literature to be compared. All three stallions which covered the positive mares in a stud farm were also detected to be positive by direct-PCR. They can be the main source of contamination occurred in that stud farm. In conclusion: This study indicated that *Taylorella equigenitalis* could be the main cause of subfertility in a stud farm in Tehran, Iran, which harbored in clitoral area and penis of mares and stallions respectively. Another research is needed to specify the role of this pathogen in mare endometritis and repeat breeding in that farm.

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**References**