pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 36(5) : 245-247 (2019)
http://dx.doi.org/10.17555/jvc.2019.10.36.5.245

Journal of Veterinary Clinics
www.ksvc.or.kr

PCR-Based Determination of the Prevalence of Common Venereal Bacterial Pathogens in Breeding Thoroughbreds of South Korea

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(Received: March 18, 2019 / Accepted: September 03, 2019)

Abstract : *Taylorella equigenitalis* (*T. equigenitalis*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) are sexually transmittable bacteria known to cause venereal diseases (VD) in horses. *T. equigenitalis* causes contagious equine metritis (CEM), which is a considerable concern for equine breeding industry. *K. pneumoniae* and *P. aeruginosa* may cause endometritis and infertility in susceptible mares. The purpose of this study was to investigate the prevalence of these bacteria among breeding Thoroughbreds in South Korea. External genital swabs were collected from 178 breeding Thoroughbreds, including 11 stallions and 167 mares. The samples were tested using a commercial multiplex real-time PCR kit. *T. equigenitalis*, *P. aeruginosa*, and *K. pneumoniae* were present in 5.6%, 7.3%, and 5.6% of tested Thoroughbreds, respectively. The results highlight the need for regular testing of South Korean Thoroughbreds, particularly those used for breeding, for these bacteria. The regular pre-breeding test for these bacteria will prevent health complications for the horse and financial losses for the owner as a result of VD.

Key words **:** *Taylorella equigenitalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, venereal disease, Thoroughbred.

Introduction

Contagious equine metritis caused by *Taylorella equigenitalis* (*T. equigenitalis*) and urogenital tract bacterial infections caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are common venereal transmitted diseases of horses, generally leading to reduced fertility in susceptible mares (6,10,13). *T. equigenitalis* is closely adapted the equine genital tract, and is highly contagious and primary venereal transmissible bacterial agent in horses (1,11). *T. equigenitalis* infected stallions generally remain asymptomatic carriers, which persists as a commensal on external genitalia (10). Infected mares can result in clinical signs including endometritis, cervicitis, vaginitis and infertility or may become asymptomatic carriers (10,13). Although the infected mare is asymptomatic, the mare can continue an infectious carrier for several months (11). Therefore asymptomatic carriers make CEM challenging to detect and control (11). *P. aeruginosa* and *K. peumoniae* inhabit the external genital of the stallion and asymptomatic in the stallion (8,11). Infection may occur in stallions from indiscriminate washing of the penis with soap, antibiotic treatment, or breeding with an infected mare (4,11). These organisms can induce endometritis with reduced fertility in susceptible mares with weak uterine clearance (11). These venereal diseases have significant economic impact resulting from the costs associated with veterinary treatment and loss of future foal sale if the mare remains infertile (2,4).

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Genital swabs collected annually to screen for *T. equigenitalis*, *P. aeruginosa*, and *K. pneumoniae* are used to test for presence of VD (1,9,10,15). Annual pre-breeding testing is recommended by the Horserace Betting Levy Board's code of practice for the control of equine venereal diseases, in the Ireland, United Kingdom, France, Germany, and Italy (9). Conventional culture for the detection of *T. equigenitalis* and *P. aeruginosa* has the disadvantage due to long culture time up to 6 days and insensitivity, leading possibly to false-negative results (6,10). PCR based detection assays has advantages on conventional culture method, particularly the speed and specificity, as well as the ability to detect the agent in horses under antibiotics treatment (6). There is a commercial multiplex real-time PCR kit for the detection of these pathogens available in the market at present.

This study was conducted to investigate the prevalence of *T. equigenitalis*, *P. aeruginosa*, and *K. peumoniae* in South Korean breeding Thoroughbred's using a commercial multiplex real-time PCR kit and to prevent the potential loss in equine breeding practice in South Korea due to theses bacterial venereal infection.

Materials and Methods

Genital swabs were collected using sterile nylon swabs (Noblebio, Republic of Korea) and a routine clinical examination of external genitalia was performed on 178 Thoroughbreds of a breeding stock in South Korea (age range, 4-24 years). Penile swabs (n = 11) were taken from the surface of the penis, fossa glandis, and urethral sinus of stallions that had been sedated. Vulvovaginal swabs (n = 167) were taken from the clitoral fossa and sinuses of mares. Immediately

Table 1. The percentage of sampled South Korean Thoroughbreds testing positive for the bacteria *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* as detected by a commercial multiplex real-time PCR kit using genital swabs

	Number of Thoroughbreds	*T. equigenitalis*	*K. pneumoniae*	*P. aeruginosa*
Stallions	11	2 (18.2%)	1 (9.1%)	2 (18.2%)
Mares	167	8 (4.8%)	9 (5.4%)	11 (6.6%)
Total	178	10 (5.6%)	10 (5.6%)	13 (7.3%)

Table 2. The summary of the bacteria *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* detected in genital swabs sampled from breeding Thoroughbreds in South Korea using a commercial multiplex real-time PCR kit

	Number of Thoroughbreds	Infected horses	Non-infected horses
Stallions	11	3[a] (27.3%)	8 (72.7%)
Mares	167	27[b] (16.2%)	140 (83.8%)
Total	178	30 (16.9%)	148 (83.1%)

[a]*T. equigenitalis* was positive from 1 stallion, both *K. pneumoniae* and *P. aeruginosa* were positive in 1 stallion, and both *T. equigenitalis* and *P. aeruginosa* was positive in 1 stallion.
[b]*T. equigenitalis* was positive in 8 mares, *K. pneumonia* was positive in 8 mares, *P. aeruginosa* was positive in 10 mares, and both *K. pneumoniae* and *P. aeruginosa* were positive in 1 mare.

after collection the swabs were stored in sterile tubes at −20°C. DNA was extracted from the swabs using the cador Pathogen 96 QIAcube HT Kit (Qiagen, Germany) with the QIAcube HT system (Qiagen, Germany) according to the manufacturer's instructions and stored at −20°C. Extracted nucleic acids were then amplified and probed for *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* with the cador TKP PCR reagent kit (Qiagen, Germany) according to the manufacturer's instructions. Briefly, a 5 μL sample of extracted DNA was added to a cador TKP PCR reaction tube containing 15 μL of TKP primer/probes mixture and 5 μL of 5X pathogen master mix. Denaturation took place at 95°C for 5 minutes, followed by 40 cycles of PCR for the detection of fluorescence signals (15 seconds at 95°C, 45 seconds at 60°C, and 20 seconds at 72°C). Positive control and internal control, which were supplied with cador TKP PCR reagent kit (Qiagen, Germany), were used and nuclease-free water (Qiagen, Germany) functioned as a negative control. The real-time PCR was performed by a QuantStudio 5 Real-Time PCR instrument (Applied Biosystems, USA).

Results

T. equigenitalis was detected in 2 out of 11 stallions (18.2%) and 8 out of 167 mares (4.8%); *K. pneumoniae* was detected in 1 out of 11 stallions (9.1%) and 9 out of 167 mares (5.4%); and *P. aeruginosa* was detected in 2 out of 11 stallions (18.2%) and 11 out of 167 mares (6.6%) (Table 1). One stallion and one mare were positive for both *K. peumoniae* and *P. aeruginosa*. Another stallion was positive for both *T. equigenitalis* and *P. aeruginosa* (Table 2). The external genital of infected Thoroughbreds showed no visual clinical signs like vaginal discharge or pus.

Discussion

T. equigenitalis, *K. pneumoniae*, and *P. aeruginosa* are bacteria associated with VD in horses often presenting as infectious endometritis and infertility in susceptible mares (4,6,10,13,16). Infection with these bacteria results in economic losses to the equine breeding industry (14). These bacteria are generally asymptomatic in stallions, while infected mares may or may not be symptomatic (12). Symptomatic mares present clinically with endometritis, cervicitis, and vaginitis with discharges (1,11). Although the mare may become asymptomatic carriers, the mare still remain capable of transmitting infection (1,11). *T. equigenitalis* is highly contagious resulting in CEM and has been detected worldwide prompting many countries to introduce strict regulations regarding testing and reporting (7,12). In North America, *T. equigenitalis* is a reportable disease; in most European countries, and South Korea it is a notifiable disease (2,5). In the United Kingdom (UK) the Horserace Betting Levy Board code of practice for CEM includes testing for *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* prior to breeding (7,15). Breeding can only occur if the test results are negative (15). This code of practice is commonly followed in France, Germany, Ireland, Italy and the UK (15). In South Korea, annual nationwide testing for *T. equigenitalis* has occurred since a 2015 outbreak of CEM (3). However, testing for *K. pneumoniae* and *P. aeruginosa* has not introduced.

In horses, these bacteria are isolated commonly from the external genitalia swabs in most venereal transmission cases (1). The clitoral fossa and sinuses are common reservoirs of these bacteria which are introduced into the uterus during coitus (2,4). In stallions, these bacteria inhabit the external genitalia including the urethral fossa, urethra, and penile sheath (10,15). Diagnosis of infection is usually achieved by culturing genital swabs, however, this method has the limitations of long wait times and the possibility of false negatives (6,10). PCR on the other hand is a fast, sensitive method and also able to identify carriers (4,6,10). In this study, *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* were detected simultaneously using a commercial multiplex real-time PCR assay. The Thoroughbreds testing positive in this study represented no clinical signs and seems to be asymptomatic carriers. Prevalence of *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* in breeding Thoroughbreds ranged from 5.6% to 7.3% (Table 1). Although the number of the stallions are relatively limited, the positive rate for these bacteria in stallions are higher than the mares': at lowest 9.1% versus 4.8% and at highest 18.2% versus 6.6%. Evidence may suggest that these bacteria were transmitted from the infective mares to stallions during covering and inhabited and persisted in the

stallions.

The prevalence of VD in breeding Thoroughbreds caused by *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* in South Korea was revealed firstly by this study using a multiplex real-time PCR kit. Because of the risk of infertility of infected mares, and economic losses for the owner and industry several countries have strict codes and practices in regard to regular pre-breeding VD (7,15). The data shows regular pre-breeding testing for VD in stallions and mares needed to prevent the spread of VD in South Korea. The regular pre-breeding testing for VD and covering in only negative horses are recommended and will help to prevent infertility in mares caused by infection and the accompanying financial impact for owners in South Korea.

References

1. Blanchard TL, Kenney RM, Timoney PJ. Venereal disease. Vet Clin North Am Equine Pract 1992; 8: 191-203.
2. Causey RC. Uterine therapy for mares with bacterial infections. In: Current Therapy in Equine Reproduction. St. Louis: Saunders Elsevier. 2007: 105-107.
3. Jeong HY, Lee SK, Park JY, Kim HJ, Yang SJ, Lee SK, Ko JA, Yang SK, Park C, Kim SH, Kang HE, Choi JG. Status of Taylorella equigenitalis Infection in Thoroughbred Horses in the Republic of Korea and the Molecular Characterization of the Korean Taylorella equigenitalis Isolates. J Equi Vet Sci 2018; 69: 102-107.
4. Metcalf ES. Venereal disease. In: Equine Reproduction, 2nd edn. Chichester: Wiley. 2011.
5. Ministry of Agriculture, Food and Rural Affairs website. Act on the prevention of contagious animal diseasess. Available at: http://www.mafra.go.kr. Accessed Mar 05, 2019.
6. Moore JE, Buckley TC, Millar BC, Gibson P, Cannon G, Egan C, Cosgrove H, Stanbridge S, Anzai T, Matsuda M, Murphy PG. Molecular surveillance of the incidence of Taylorella equigenitalis and Pseudomonas aeruginosa from horses in Ireland by sequence-specific PCR. Equine Vet J 2001; 33: 319-22.
7. Morel MCGD. Infertility. In: Equine Reproductive Physiology, Breeding and Stud Management. Ipswich: Farming Press Books 1993: 386-392.
8. Pasolini MP, Prete CD, Fabbri S, Auletta L. Endometritis and infertility in the mare - the challenge in equine Breeding Industry-a review. In: Genital Infections and Infertility. London: IntechOpen. 2016: 290.
9. Ricketts S, Troedsson MHT. Fertility expectations and management for optimal fertility. In: Current Therapy in Equine Reproduction. St. Louis: Saunders Elsevier. 2007: 54-55.
10. Samper JC, Tibary A. Disease transmission in horses. Theriogenology 2006; 66: 551-559.
11. Satué K, Gardon JC. Infection and Infertility in Mares. In: Genital Infections and Infertility. London: IntechOpen. 2016: 257-283.
12. Snider TA. Reproductive Disorders in Horses. Vet Clin North Am Equine Pract 2015; 31: 389-405.
13. Tibary A, Pearson LK, Fite CL. Reproductive tract infections. In: Equine infectious diseases, 2nd edn. St. Louis: Saunders. 2013: 104-106.
14. Troedsson MHT. Endometritis. In: Equine Reproduction, 2nd edn. Chichester: Wiley. 2011: 2609-2613.
15. Horseracing Betting Betting Levyboard website. Codes of Practice 2019. Availabe at: https://codes.hblb.org.uk. Accessed Mar 05, 2019.
16. Wilson D. Endometritis, bacterial and fungal. In: Clinical veterinary advisor : the horse. St. Louis: Saunders. 2010: 166-168.