Journal of Equine Veterinary Science 47 (2016) 42–46

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

First Isolation of *Taylorella equigenitalis* From Thoroughbred Horses in South Korea

Hye-Young Jeoung [a], Ki-Eun Lee [a], Sun-Joo Yang [b], Taemok Park [b], Sang Kyu Lee [b], Ji-Hye Lee [a], Sung-Hee Kim [a], Byounghan Kim [a], Yong-Joo Kim [a], Jee-Yong Park [a,*]

[a] *Animal and Plant Quarantine Agency, Anyang, Gyeonggi-do, Republic of Korea*
[b] *Equine Hospital, Korea Racing, Authority, Gwacheon, Republic of Korea*

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form 25 July 2016
Accepted 26 July 2016
Available online 4 August 2016

Keywords:
Taylorella equigenitalis
Stallion
Mare
Contagious equine metritis

ABSTRACT

Contagious equine metritis (CEM) is a highly contagious venereal disease of the equid species caused by the bacterium *Taylorella equigenitalis*. CEM has been reported from at least 30 countries across the world but in Asia, Japan has been the only country that had previously reported CEM, which was successfully eradicated in 2010. Since then, there had been no reports of CEM in Asia, but during the course of this study to test Thoroughbred horses in South Korea for the possible presence of CEM, a total of five strains of *T. equigenitalis* were isolated from four stallions and one mare which had shown no clinical signs indicative of the disease. The isolated bacteria were coccoid rod-shaped, gram negative, and were positive for oxidase, catalase and phosphatase tests. Sequencing of the 16S rDNA gene and phylogenetic analysis confirmed that the five isolates were *T. equigenitalis*, which showed high-sequence identity (99%–100%) with other *T. equigenitalis* strains. All Korean isolates were sensitive to streptomycin. This study describes the first isolation and characterization of *T. equigenitalis* in Thoroughbred horses raised in South Korea, representing the reintroduction of CEM into Asia.

1. Introduction

Contagious equine metritis (CEM) is a highly contagious venereal disease of horses associated with temporary infertility and is a serious threat to the equine industry due to its potential for devastating negative effect on equine reproductive efficiency. CEM can also cause further economic losses due to movement restrictions and loss of international trade [1,2]. For these reasons, CEM remains a high priority disease for many countries with a developed equine industry, and is included in the list of diseases with importance to international trade by the World Organization for Animal Health (OIE).

CEM is caused by the bacterium, *Taylorella equigenitalis*, which is classified as a nonmotile small rod or pleomorphic coccobacillus that is gram negative and microaerophilic. CEM was first reported as an outbreak of acute metritis during the breeding season in England and Ireland in 1977 [3,4], which has since been detected in at least 30 countries across several continents including Europe, North and South America, Australia, and South Africa [4–9]. In Asia, Japan is the only country that had occurrences of CEM, which was first detected in 1980 [8] but has since been successfully eradicated in 2010. CEM is usually transmitted directly by sexual contact with infected stallions during breeding but can also be transmitted indirectly by artificial insemination with contaminated semen and fomites such as breeding phantoms and veterinary instruments. Whereas stallions are typically asymptomatic carriers, 30%–40% of mares develop acute endometritis, cervicities, or vaginitis that usually results in temporary infertility and in rare cases, abortion. A proportion of mares may become

The authors declare that they have no competing interests.

* Corresponding author at: Dr. Jee-Yong Park, Animal and Plant Quarantine Agency, Anyang, Gyeonggi-do, 430-824, Republic of Korea.
E-mail address: parkjyyunesy@korea.kr (J.-Y. Park).

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2016.07.022

long-term symptomless carriers and together with infected stallions, can also spread infection during mating [10,11]. According to the OIE, effective vaccines are not yet available, and prior infection is not fully protective. Therefore, the principle tool for control of CEM is identification of infected horses through testing for *T. equigenitalis* on swab samples collected from the reproductive tract by bacterial culture or molecular tests and prevention of transmission by prohibiting the use of positive horses for breeding [12–14]. For international trade, traditional bacteriology based on isolation with agent identification by morphologic biochemical criteria from multiple swabs of the predilection sites of the external genitalia is currently established as the gold standard method [15]. South Korea had been considered to be historically free from CEM, and a study conducted to test high-value Thoroughbreds for CEM, which was expected to provide evidence that would support this initial view, resulted in the surprising discovery of CEM-infected horses. This study describes the first identification and characterization of *T. equigenitalis* isolated from Thoroughbred horses raised in South Korea, which also represents the reintroduction of CEM into Asia.

2. Material and Methods

2.1. Sampling

Swab specimens for the isolation of *T. equigenitalis* were collected at the start of the breeding season in 2015 from Thoroughbred horses that had been gathered at a breeding facility operated by the Korean Racing Authority in Jeju Island, which is a major region for the breeding of Thoroughbreds in South Korea. A total of 36 Thoroughbred horses, consisting of 11 stallions aged between 6 and 19, and 25 mares between six and sixteen were sampled, none of which showed any clinical signs indicative of CEM infection such as metritis. Swab samples were taken from the urethral orifice, skin of the penis, the prepucial folds of stallions, and from the clitoral sinus and clitoral fossa of mares using Cultureswab Plus Amies Gel with Charcoal (BD, New Jersey). All specimens were placed on ice and transported to the Foreign Animal Disease Division, Animal and Plant Quarantine Agency (QIA) to allow for plating on culture medium within 48 hours.

2.2. Culture for Isolation

Bacterial culture was conducted according to the OIE *Manual of Diagnostic Test and Vaccines for Terrestrial Animals, 2012*. Briefly, each swab specimens were inoculated on to 5% (v/v) heated blood ("chocolate") agar plates with antibiotics, which were produced by heating the liquid medium containing blood at 80°C for 12 minutes and cooled to 45–50°C to add trimethoprim (1 μg/mL), clindamycin (5 μg/mL), and amphotericin B (15 μg/mL). Plates were incubated with 8.8% (v/v) CO_2 in air at 37°C for 6–10 days [13].

2.3. Identification

API ZYM (BioMeruix, Lyon, France) microbial identification strip test kit was used to semiquantify the enzymatic activities of the isolated bacteria. The API ZYM strips were inoculated according to the manufacturer's instructions with two drops of bacterial suspension prepared by suspending the bacterial growth collected from agar plates in distilled water. Tests for catalase and oxidase activity were conducted according to previously published methods [16]. A reference strain of *T. equigenitalis* (ATCC 35865) obtained from the American Type Culture Collection (ATCC, Manassas, VA) was included as control for all culture and identification tests.

2.4. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of the isolated strain was determined by a modified broth microdilution method [17]. *Escherichia coli* ATCC 25922 and *T. equigenitalis* ATCC 35865 were used as control strains. The panel of isolated strains and *T. equigenitalis* ATCC 35865 were incubated with 8.8% (v/v) CO_2 in air at 37°C for 4 days, and *Escherichia coli* ATCC 25922 were incubated at 37°C for 1 day. Minimal inhibitory concentrations (MICs) were determined by broth microdilution using the Sensititre microdilution panel from Thermofisher Inc. (formerly TREK Diagnostic Systems; Cleveland, OH, USA). The antimicrobial agents tested were ampicillin (2–32 μg/mL), amoxicillin/clavulanic acid (2/1–64/32 μg/mL), cefoxitin (1–32 μg/mL), ceftiofur (0.5–8 μg/mL), cephalothin (2–64 μg/mL), chloramphenicol (2–64 μg/mL), ciprofloxacin (0.12–16 μg/mL), colistin (2–32 μg/mL), florfenicol (2–64 μg/mL), gentamicin (1–64 μg/mL), nalidixic acid (2–128 μg/mL), neomycin (2–32 μg/mL), streptomycin (2–128 μg/mL), tetracycline (2–128 μg/mL), and trimethoprim-sulfamethoxazole (0.12/2.38–4/76 μg/mL).

2.5. Sequence Analysis of 16S rDNA Genes

The 16S rDNA genes of isolated strains were sequenced at Cosmogentech Institute (Cosmogentech Co., Seoul, Korea). The sequences of isolates were aligned with those of other *T. equigenitalis* strains that were available at GenBank using the CLUSTAL X alignment program. Phylogenetic analysis was conducted by neighbor-joining methods and performed with the computer program PHYLIP (version 3.572c) based on the formulas of Kimura. The robustness of the phylogenetic analysis was determined by bootstrap analysis with 1000 replications.

3. Results

3.1. Isolation and Characterization of T. equigenitalis

After 72–96 hours of incubation, colonies could be identified which were small, convex, shiny, greyish-white, and smooth. Small coccoid rod-shaped gram-negative bacteria were observed by microscopical analysis. Five strains of *T. equigenitalis* were isolated from four stallions and one mare. The four strains isolated from stallions and one isolate from a mare were named "KJTE 2, 3, 10, 11, and 28", respectively. All five strains were positive for oxidase, catalase and phosphatase tests, and negative for other biochemical tests such as for esterase, glucosidase, and lipase. All the isolates demonstrated same biochemical