Veterinary Microbiology 160 (2012) 435–442

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Prevalence and persistence of *Taylorella asinigenitalis* in male donkeys

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ARTICLE INFO

Article history:
Received 22 March 2012
Received in revised form 13 June 2012
Accepted 15 June 2012

Keywords:
Taylorella asinigenitalis
Donkeys
Normal flora
CEM

ABSTRACT

This study was undertaken to investigate the prevalence of *Taylorella asinigenitalis* in a subset of the donkey population of Michigan and in other equids on farms on which the organism was identified. Other aims were to further characterize the carrier state in terms of persistence and preferred sites of colonization of *T. asinigenitalis* in the male donkey as well as determine the genotype of any isolates of the organism. Initial testing of 43 donkeys and 1 mule turned up 4 (9.3%) donkeys culture positive for *T. asinigenitalis*. The 4 culture-positive donkeys resided on 2 farms accommodating a collective total of 89 equids, of which 23 (25.8%) were confirmed positive for *T. asinigenitalis*. The positive equid population on the 2 farms comprised 14 (67%) of 21 gelded donkeys, 8 (36.4%) of 22 intact male donkeys, and 1 (25%) of 4 gelded horses. *T. asinigenitalis* was not isolated from 27 female donkeys, 11 female horses, 2 female mules, 1 male horse, or 1 male mule resident on these premises. Isolations of the bacterium were obtained from a number of male donkeys whenever they were sampled over a span of 33 months; preferential sites of isolation were the urethral fossa (fossa glandis), dorsal diverticulum of the urethral sinus, and terminal urethra. Isolates of *T. asinigenitalis* from the 23 culture-positive equids comprised 2 genotypes, one identical to the type strain isolated in California in 1997, and the other identical to 2 strains isolated from donkey jacks in Kentucky in 1998.

1. Introduction

In late October 1997, a *Taylorella equigenitalis*-like bacterium was recovered from a Mammoth donkey jack in California (Anonymous, 1998) and in early January 1998, a similar bacterium was recovered from 2 male donkeys, 7 nurse mares and 1 stallion in Kentucky (Jang et al., 2001; Meade et al., 2010). Based on DNA–DNA hybridization studies, these 3 isolates were not *T. equigenitalis*; they were a new species in the genus *Taylorella* named *Taylorella asinigenitalis* (Jang et al., 2001).

Since originally reported, *T. asinigenitalis* has been recovered from equids in Europe and the USA. The organism was isolated from a 3-year-old stallion in Sweden (Båverud et al., 2006), that previously shared paddock space with a donkey jack in Belgium. In a second report (Franco et al., 2009) *T. asinigenitalis* was recovered from 2 donkey jacks in Apulia, Italy. They were at the same facility for over 3 years and were previously culture negative for *Taylorella* species.

Breuil et al. (2011) published on the phenotypic and 16S ribosomal RNA gene diversity of 43 strains of *T. asinigenitalis* isolated from 22 donkey jacks, 2 stallions and 1 mare in France between 1995 and 2009. Based on sequence

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http://dx.doi.org/10.1016/j.vetmic.2012.06.016

analysis, strains fell into 2 clusters which seemed to correlate with their respective geographic origins.

Recent reports from the US confirmed isolation of *T. asinigenitalis* from a mare in Oklahoma (Reilly et al., 2012) and a miniature donkey in Kentucky (E.S. Ford, personal communication).

The source of *T. asinigenitalis* has not been identified in any of the published reports (Anonymous, 2000; Jang et al., 2001; Katz et al., 2000; Meade et al., 2010), and very little is known about the epidemiology of *T. asinigenitalis*, particularly its persistence in equid populations. Accordingly, the following study was undertaken to determine: (1) the prevalence of *T. asinigenitalis* in a subset of the donkey population in Michigan presented for castration, (2) the prevalence of *T. asinigenitalis* in all equids on farms with a known culture-positive donkey jack, (3) the duration of persistence of *T. asinigenitalis* in equids, principally donkey jacks, (4) preferential sites of persistence of *T. asinigenitalis*, and (5) whether multiple genotypes of *T. asinigenitalis* are present and co-circulate on the same premises.

2. Materials and methods

2.1. Sample population

The animals tested consisted of two different populations. The first population consisted of 43 donkeys and 1 male mule that were presented to the College of Veterinary Medicine, Michigan State University (MSU) for castration. Samples were taken from 3 of these 44 animals, even after it was discovered that 3 donkeys had been castrated previously when they arrived at MSU. These 44 equids were part of a joint study with investigators at MSU and the University of Kentucky, Veterinary Diagnostic Laboratory (UKVDL) and the Maxwell H. Gluck Equine Research Center to determine the normal microaerophilic bacterial flora on the external genitalia and in the testes and epididymides of male donkeys (Carleton et al., unpublished data). This population of donkeys was sampled between April 2006 and June 2007. The approximate ages of the 43 donkeys and 1 male mule at the time of initial sampling were 27, 1-year-old or younger, 4, 3-year-olds, and 12, 5–11-year-olds; the mule was 2-years old. Animals submitted for castration represented 12 different owners with premises located throughout Michigan. The castration, blood sampling and swabbing of the external genitalia of the equids included in this study were carried out in accordance with the guidelines of the College of Veterinary Medicine, Michigan State University Institutional Animal Care and Use Committee.

The second population of equids included in this study consisted of donkeys, mules, and horses on each of 2 premises the property of different owners that routinely presented donkeys for castration to the MSU Vet College, some of which subsequently tested culture positive for *T. asinigenitalis*. The first owner managed a total of 64 animals comprising two groups on closely adjacent premises in southern Michigan and referred to as Farm 1. The equid population consisted of 20 male donkeys, 5 gelded donkeys, 24 female donkeys, 1 male mule, 2 female mules, 1 male horse, and 11 female horses. Samples from the equids located on this premises were collected on July 9 or 10 and November 19, 2007. The second owner maintained a premises (Farm 2) in northern Michigan which accommodated 25 equids including 2 male donkeys, 16 gelded donkeys, 3 female donkeys, and 4 gelded horses. All animals on Farm 2 were sampled on August 13 and November 14, 2007. Additional samples were obtained from some of the culture-positive animals on Farm 1 in November 2008; and from animals on Farm 2 in November 2007, August and November 2008, and March 2010. Histories of the culture-positive equids on these two farms are summarized in Tables 1 and 2.

2.2. Sampling procedures

Swabs for the culture of *Taylorella* species and blood for the detection of antibodies against *Taylorella* species were taken from all equids. Swabs (one per site) were taken from the urethral fossa (fossa glandis), dorsal diverticulum of the urethral sinus, terminal urethra, and external surface of the penis/prepuce of the entire or gelded male animals and from the clitoral fossa and clitoral sinuses of females; a swab of the cervix was also obtained from three (2 donkeys and 1 horse) animals on Farm 1. Swabs were placed in individual tubes of Amies transport medium with charcoal (CultureSwab Plus; Becton, Dickinson and Company, Sparks, MD, USA). Testes with attached epididymides from each of the males presented for castration were also submitted for culture. Approximately 20 ml of whole blood was collected by jugular venipuncture from each animal. Each blood sample was allowed to clot at room temperature, the serum drawn off and transferred to a chemically clean screw-capped tube, and stored at −20 °C until tested.

Table 1
History of donkeys on Farm 1 that were culture positive for *Taylorella asinigenitalis*.

Animal	Approx. age July 1, 2007	Origin	Duration on farm July 1, 2007	Intact male	Date castrated
1	10 months	Farm 1	10 months	Yes/No[a]	05/15/07
2	10 months	Farm 1	10 months	Yes/No	05/15/07
3	1 year	Michigan	1 year	Yes	Not applicable
4	4 years	Farm 1	4 years	Yes	Not applicable
5	5 years	Farm 1	5 years	Yes	Not applicable
6	8 years	Farm 1	8 years	Yes	Not applicable
7	2 years	Farm 1	2 years	Yes	Not applicable
8	2 years	Michigan	1 year	No	Unknown
9	Unknown	Unknown	Not applicable[b]	Yes/No	11/20/2007

[a] Yes/No, animal was cultured then castrated.
[b] Donkey 9 arrived on Farm 1 in November, 2007 after he was cultured and then castrated at MSU.