Variations in Equid SLC11A1 (NRAMP1) Genes and Associations with Rhodococcus equi Pneumonia in Horses

Natalie D. Halbert, Noah D. Cohen, Nathan M. Slovis, Jay Faircloth, and Ronald J. Martens

Rhodococcus equi is an important intracellular pathogen of horses, most commonly causing chronic, suppurative bronchopneumonia in foals. Although most foals likely are exposed to environmental R equi within the first few days of life, only some develop R equi pneumonia, and the basis of differences in susceptibility among foals currently is unknown. In this study, we investigated solute carrier family 11 member 1 (SLC11A1) gene sequences in the 5’ untranslated region, exon 1, and a portion of intron 1 for variations in 3 equid species (horse, donkey, zebra) and compared variants within 3 independent horse breeding farms for associations with R equi pneumonia by use of an age-matched case-control design. Seven novel variants in the 5’ untranslated region were identified as specific for one or both of the non-horse equid species sampled. In addition, a single novel horse variant in the 5’ untranslated region, -57CT, was identified in 4 breeds. The -57CT variant was found on 2 of the 3 farms with endemic R equi pneumonia, representing 2 different horse breeds. Significant allelic and genotypic associations with susceptibility to R equi pneumonia were observed for the -57CT variant in foals from these farms. Although the functional impact of this novel variant remains to be determined, this study represents an important step in our understanding of natural resistance to R equi foal pneumonia and other intracellular bacterial diseases affecting equids.

Key words: Foal disease; Intracellular bacteria; Natural resistance; NRAMP.

Diseases caused by intracellular bacteria, such as Rhodococcus equi, are an important threat to companion and wildlife species, despite widespread use of antimicrobials and vaccinations. R equi causes chronic, suppurative bronchopneumonia in foals and represents a considerable burden to the equine industry.1,2 The insidious nature of R equi foal pneumonia often results in delays in diagnosis and treatment of the disease, and leads to higher rates of morbidity and mortality.3 Effective vaccines are not available for the disease, and administration of hyperimmune plasma (the only proven preventative measure4) is costly, labor intensive, and not completely effective.5 Development of R equi pneumonia is influenced by host,6 pathogen,7 and environmental8-10 factors. Most foals are likely exposed to R equi within the first few days of life11 but only some foals from farms with a history of the disease actually develop R equi pneumonia. Differences among foals reared in the same environment that eventually develop R equi pneumonia compared with those that do not are poorly understood.1,12 Several foals from an individual mare diagnosed with R equi pneumonia have been observed anecdotally,13 and the incubation period among foals with R equi pneumonia follows a log-normal distribution (Startwell’s model),14 suggesting a genetic etiology, perinatal infection, or both.

Innate resistance to intracellular bacterial infections is well studied in mammals, and, in the past decade, many studies have begun to unravel deoxyribonucleic acid (DNA) mutations, leading to changes in cellular processes that ultimately contribute to an individual’s ability to resist infection. Expression of the solute carrier family 11 member 1 (SLC11A1; formerly NRAMP1) gene has been shown to confer innate resistance to certain bacterial infections, and mutations in the SLC11A1 gene have been associated with many infectious and autoimmune diseases in humans, mice, and cattle.14-18 Several factors indicate this gene is a good candidate for involvement in the immune response, and possibly natural resistance, to R equi pneumonia. The SLC11A1 gene encodes a divalent ion transporter protein expressed on lysosomal, endosomal, and phagosomal membranes of macrophages and neutrophils.19 R equi survives and replicates in host macrophages20 and inhibits the phagosome-lysosome fusion process21-23 in which SLC11A1 likely is involved.24 In addition, mutations in the SLC11A1 gene have been shown to impair the ability of murine macrophages to restrict intracellular replication of certain intracellular bacteria.25 Finally, iron is crucial for the growth and survival of R equi,26 and SLC11A1 is believed to mediate pathogen resistance by influencing intracellular concentrations of iron,27 either through sequestration of essential metal ions from bacteria within the phagosome or through delivery of divalent ions into the phagosome and generation of hydroxyl radicals via the Fenton/Haaber-Weiss reaction.19

The structure of the SLC11A1 gene in horses is similar to that in other mammals, including 15 exons corresponding to a putative protein of 544 amino acids.28 To date, single nucleotide polymorphisms (SNPs) have been identified in the horse SLC11A1 intron 529,30 and 5’ untranslated region (UTR).28 To our knowledge, SLC11A1 polymorphisms have not been investigated in non-horse equid species to date. In this study, we used direct genomic sequencing to compare SNPs in the SLC11A1 5’UTR, exon 1, and a portion of

From the Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A & M University, College Station, TX (Halbert, Cohen, Martens), Hagyard Equine Medical Institute, Lexington, KY (Slovis), and Breeding Center, Al-Marah Arabians, Tucson, AZ (Faircloth).

Funding for this study was provided by the Link Equine Research Endowment at Texas A&M University.

Reprint requests: N.D. Halbert, PhD, Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4475; e-mail: nhalbert@cvm.tamu.edu.

Copyright © 2006 by the American College of Veterinary Internal Medicine

0891-6640/06/2004-0026/S3.00/0
Table 1. List of equids sampled; approximately equal numbers of individuals affected with *Rhodococcus equi* pneumonia as foals and age-matched controls were sampled from each endemic farm.

<table>
<thead>
<tr>
<th>Species</th>
<th>Breed</th>
<th>Farm</th>
<th>Disease Class</th>
<th>Sampled (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Equus caballus</em></td>
<td>Andalusian</td>
<td>Various</td>
<td>Affected</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Arabian*</td>
<td>A</td>
<td>Control</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Arabian*</td>
<td>B</td>
<td>Affected</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Hanoverian</td>
<td>Various</td>
<td>Control</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Quarter Horse</td>
<td>Various</td>
<td>Affected</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Thoroughbred*</td>
<td>C</td>
<td>Control</td>
<td>13</td>
</tr>
</tbody>
</table>

*E asinus*  9  
*E burchellii*  10  

*Some individuals within each of these farms (but not between) are related. All other breed representatives are unrelated based on 5-generation pedigrees (Andalusians, Hanoverians, Quarter Horses) or known histories (donkeys, zebras).

Intron 1 among 3 equine species: *Equus caballus* (domestic horse), *E asinus* (donkey), and *E burchellii* (zebra). In addition, we used a case-control study design to examine associations of these SNPs with resistance or susceptibility to *R equi* pneumonia among 3 horse farms.  

Materials and Methods

Hair or blood samples were collected from 103 equids, including 84 horses (5 breeds), 9 donkeys, and 10 zebras (Table 1). Sampled donkeys included 5 miniatures, 2 standards, and 2 mammoths. We attempted to collect samples from unrelated individuals of each breed or species by use of either 5-generation pedigree information (horses) or known histories of individuals (donkeys and zebras).

Samples were collected from approximately equal numbers of individuals affected with *R equi* pneumonia as foals and age-matched (by birth month or year) controls from 3 different US breeding farms (Table 1, farms A–C) in which *R equi* infections were endemic (ie, a history of *R equi* foal pneumonia in at least 3 of the past 5 years based on culture of *R equi* from either tracheobronchial aspirate [TBA] fluid or postmortem lung samples from foals). Farm A is a Crabbet Arabian breeding farm located in Arizona. Whole blood samples from farm A were collected from foals born in 2003 and 2004. A diagnosis of *R equi* pneumonia at Farm A was determined presumptively on the basis of farm history (ie, foals with microbiologically confirmed *R equi* pneumonia in previous years), clinical signs of pneumonia (including fever, increased rate or effort of breathing, nasal discharge, or cough) and either neutrophilia (>8,500 neutrophils/μL) or hyperfibrinogenemia (>400 mg/dL) detected by complete blood count. If a foal responded dramatically to treatment within a few days, *R equi* was not considered to be the causative agent. Age-matched controls were selected from those foals that either did not have clinical signs of pneumonia or did not have neutrophilia or hyperfibrinogenemia and recovered from mild respiratory infection without treatment. All individuals sampled from Farm A were related by a shared stallion or grand-stallion; 3 stallions produced 8 of the 12 sampled individuals.

Farm B is an Egyptian Arabian farm in Texas with a history of *R equi* foal pneumonia. Whole blood samples from farm B were collected from foals between 3 and 24 weeks of age born in 2000 in conjunction with a previous study. A foal was considered to have *R equi* pneumonia if it had clinical signs of pneumonia (including fever, increased respiratory rate or effort, nasal discharge, or cough) and if *R equi* was isolated from TBA fluid or postmortem lung specimen, or if the foal had clinical signs of pneumonia and at least 2 of the following: multifocal pulmonary opacities on thoracic radiographs, ultrasonographic evidence of pulmonary abscesses, or gram-positive intraacellular coccobacilli evident during cytologic evaluation of a TBA specimen. Foals that did not meet these criteria were selected as age-matched controls. Eighteen of the 22 sampled individuals from this farm were produced from 3 stallions, whereas 3 individuals shared a grand-stallion, and 1 individual was related to others through a great-grand-stallion.

Farm C is a Thoroughbred farm in Kentucky with a history of *R equi* foal pneumonia. Mane-hair samples from Farm C were collected from foals born in 2004. All foals born in 2004 were routinely examined by ultrasonography, and the number, the size, and the location (left or right lung lobe) of any areas of consolidation or apparent abscessation were recorded. A foal was considered to have *R equi* pneumonia if it had sonographic evidence of pneumonia and if *R equi* was isolated from TBA fluid. Foals in which no abscesses were detected by ultrasonography and who showed no clinical signs of pneumonia were used as age-matched controls. All individuals sampled from Farm C were related to one another at least through 3rd generation ancestors; 6 sires accounted for 15 of the sampled individuals. None of the individuals shared ancestors for the previous 5 generations among farms A, B, and C.

Genomic DNA was isolated from hair samples with a commercially available DNA extraction solution and from whole-blood samples applied to DNA binding cards according to manufacturer instructions. Previously published primers (HNR2F and I1ENRR) and thermal profiles were used to generate a 239-base pair (bp) polymerase chain reaction (PCR) product. Each 25-μL reaction contained: 5 μL hair DNA solution or one 1.2-mm card punch; 0.4 μM solutions of each primer; 1 U *Taq* DNA polymerase and 1× buffer; 3 mM MgCl₂; 1× PCR-enhancing solution with betaine and 0.25 mM solutions of each dNTP. At least 1 extra well containing all PCR reagents except the DNA template was used in each PCR experiment to detect potential PCR contamination. Each resultant product was purified with a commercially available kit and was bidirectionally sequenced by use of dye terminator technology. Sequences were analyzed with SNP detection software. A representative number of samples was extracted, amplified, and sequenced a second time to confirm initial results. Representative alleles from each farm and each genotypic class with more than one SNP were validated by a 2nd PCR, subcloning with a commercially available kit and subsequent sequencing with the M13R primer.

For each endemic farm, allelic and genotypic distribution differences for each polymorphic site were compared between *R equi* pneumonia affected and control groups by use of the Fisher exact test in a population analysis program with the following Markov chain parameters: 10,000 dememorizations, 200 batches, and 10,000 iterations per batch. In addition, haplotype frequencies were compared between affected and control groups. For most complex heterozygotes, haplotypes were determined directly by allele subcloning. For 5 complex heterozygotes from farm 3 (2 affected and 3 control foals); however, haplotypes were inferred based on the most common alleles observed in the other 23 samples. A variation of the Z_{max} test was used to evaluate associations between specific genotypic and disease classes. Results for all tests were considered statistically significant if *P < .05*.

Results

Excluding primer sequence, a total of 196-bp of sequence were analyzed for each sample, including 171-
Table 2. *SLC11A1* 5′ untranslated region polymorphic sites identified by breed/species. Sites not listed are consistent with GenBank accession AF354446. The most common *Equus caballus* polymorphism is shown in parenthesis. Frequencies of variants (not in parenthesis) are shown for each breed/species and dashes indicate the variant is not found in a given breed/species.

<table>
<thead>
<tr>
<th>Nucleotide Variant</th>
<th>Species</th>
<th>Breed</th>
<th>-149 (T)</th>
<th>-148 (G)</th>
<th>-113 (C)</th>
<th>-86 (G)</th>
<th>-67 (G)</th>
<th>-57 (C)</th>
<th>-45 (A)</th>
<th>-40 (G)</th>
<th>-39 (G)</th>
<th>-38 (C)</th>
<th>-10 (T)</th>
<th>-4 (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. caballus</em></td>
<td>Andalusian</td>
<td>0.13</td>
<td>—</td>
<td>0.13</td>
<td>—</td>
<td>—</td>
<td>0.13</td>
<td>0.63/—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arabian*</td>
<td>—</td>
<td>—</td>
<td>0.16</td>
<td>—</td>
<td>—</td>
<td>0.52</td>
<td>0.19/—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hanoverian</td>
<td>—</td>
<td>—</td>
<td>0.25</td>
<td>—</td>
<td>—</td>
<td>0.13</td>
<td>0.63/—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quarter horse</td>
<td>0.10</td>
<td>—</td>
<td>0.20</td>
<td>—</td>
<td>0.10</td>
<td>—</td>
<td>0.80/—</td>
<td>—</td>
<td>0.15</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thoroughbred*</td>
<td>—</td>
<td>—</td>
<td>0.18</td>
<td>—</td>
<td>—</td>
<td>0.07</td>
<td>0.64/—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>E. asinus</em></td>
<td>—</td>
<td>1.00</td>
<td>—</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>—</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>E. burchellii</em></td>
<td>—</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.80/0.20</td>
<td>1.00</td>
<td>1.00</td>
<td>—</td>
<td>0.70</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Some individuals sampled within each of these breeds are related (see text).*

bp of the 5′UTR (-171 to -1), exon 1 (7-bp), and 18-bp of intron 1. As previously observed, no equid variants were identified in exon 1 or intron 1. A total of 12 variants in the 5′UTR region were identified in this study (Table 2), 8 of which were novel. A single novel horse variant, -57T, was identified in Andalusian, Arabian, Hanoverian, and Thoroughbred breeds. Furthermore, 2 novel variants were identified as donkey specific, -86A, and -4T, whereas -45T was zebra specific. Four other variants were found in both donkeys and zebras but have not been identified in horses to date:

-148A, -40C, -39A, and -10C (Table 2).

Two previously identified *SLC11A1* 5′UTR variants (-45A/G, -113C/T) and 1 novel variant (-57C/T) were detected in the 3 farms with endemic *R. equi* pneumonia that were examined. Although the -45 and -113 variants did not show any associations with disease class, the frequency distribution of the -57 variant was significantly different between disease and control groups from Farm B (*P* = .0118) and Farm C (*P* = .0348; Table 3). Farm A was fixed for the C allele at this site such that statistical tests were not possible. The affected group from Farm B had a significant overabundance of TT genotypes (*Z*<sub>max</sub> = .0043), whereas no TT genotypes were found on Farm C. Alternatively, Farm C had a significant overabundance of CC genotypes in the control class (*Z*<sub>max</sub> = .0113), whereas no CC genotypes were found on Farm B (Table 3). Although the overall frequencies of -57 alleles and genotypes were significantly different between Farms B and C (genetic differentiation *P* < .0001; genotypic differentiation *P* < .0001), the overall effects with regard to disease class were similar, with the -57C allele more frequently found in the control groups (Table 3).

Five haplotypes were identified in the 3 farms with endemic *R. equi* pneumonia as follows: Farm A haplotypes 2, 3, and 4; Farm B haplotypes 1, 2, 3, and 4; and Farm C haplotypes 1, 2, 3, 4, and 5 (GenBank accession numbers DQ269030–DQ269034). The haplotypes were represented by the following polymorphisms: (-113, -57, -45; all other sites consistent with GenBank accession AF354446): haplotype 1 (C, T, A), haplotype 2 (C, C, A), haplotype 3 (T, C, A), haplotype 4 (C, C, G), and haplotype 5 (C, T, G). Significant differentiation was not detected within any of these farms between disease class and an individual haplotype (*P* = .2020, .1028, .2886, respectively) or combination of haplotypes (*P* = .2192, .0609, .2120, respectively).

**Discussion**

The present study establishes novel horse *SLC11A1* 5′UTR variants and provides the 1st known *SLC11A1* sequences for non-horse equid species (donkey, zebra). *SLC11A1* is important in iron regulation and immune response to mammalian intracellular bacterial infections, and the findings reported here are critical to understanding the role of *SLC11A1* in equine natural resistance to infectious diseases.

Statistically significant associations were observed between a novel SNP within the *SLC11A1* 5′UTR and susceptibility to *R. equi*. The -57C allele was associated with the control class in both farms in which the SNP was detected (Table 3), although Farm C had a significantly higher frequency of the -57C allele than Farm B. The differences in allele frequencies among farms likely are a reflection of breeding history and genetic differences among breeds. Overall, however, both individual alleles and genotype combinations were significantly associated with disease prevalence in the 2 farms studied that contained the -57C/T SNP. The association between this SNP and natural resistance to *R. equi* pneumonia is supported by the fact that none of the horses examined between the 2 farms were related. Moreover, the finding of this SNP and its association with *R. equi* pneumonia in 2 different horse breeds minimizes the chance of spurious association because of relatedness or sire effects. The genotypic phase of the -57C/T polymorphism with respect to the surrounding 5′UTR sequence does not appear important in this association, because significant associations were not observed between identified haplotypes and *R. equi* pneumonia susceptibility. A previous report of 51 foals from a Thoroughbred farm did not find any associations between *SLC11A1* 5′UTR or intron 5 polymorphisms.
and susceptibility to *R. equi* pneumonia,\(^{30}\) but this study used a PCR restriction fragment length polymorphism method permitting detection of only 4 of the variants in the 5′UTR region, not including the -57C/T polymorphism.

The current study was limited to foals from 3 endemic farms because of the sporadic nature of this disease.\(^{1,7}\) Because of the lack of robust and consistent diagnostic criteria on some farms, additional analyses on other farms and in other breeds will be necessary to substantiate these findings. Furthermore, this study was limited by the potential for misclassification of foals with respect to disease status, especially on Farm A, where TBA cultures and thoracic radiographs or ultrasounds were not performed. Any disease misclassification on Farm A did not have an impact on the conclusions of this study, however, because all individuals were fixed for the -57C allele. The diagnostic criteria used among the 3 endemic farms in this study varied because of differences in diagnostic preferences, equipment, and expertise of the farm managers and veterinarians. It is possible that some foals were infected with *R. equi* and recovered spontaneously without showing sufficient clinical signs to meet the study definition of being an affected foal. It is also possible that some foals deemed affected suffered from other lower respiratory tract infections, such as those caused by *Streptococcus spp.*. The impact of this misclassification is unknown, but we believe the chance of such misclassification to be low given the farm histories and extensive diagnostic experience of the attending veterinarians.

The functional effect of the detected -57C/T polymorphism remains to be determined. Several sites influencing transcription are present within the surrounding region in mice,\(^{32}\) mutations in which might lead to differences in transcription activity. Alternatively, the -57C/T polymorphism may have no direct functional effect but may be linked to other *SLC11A1* mutations influencing gene expression, such as in the upstream equine *SLC11A1* promoter region. Different human microsatellite repeat alleles in the *SLC11A1* promoter region regulate expression of the gene and are associated with susceptibility to infectious or autoimmune disease.\(^{33}\) Furthermore, cis-acting sequence elements associated with binding of transcription factors are present in the promoter region, which may regulate expression of *SLC11A1*.\(^{32}\) However, we cannot exclude the possibility that the observed associations are caused by linkage with mutations in nearby genes. Strong linkage disequilibrium has been observed at least 100 kb both upstream and downstream of *SLC11A1* in humans,\(^{34}\) a region in which other potential candidate genes involved in immune response are present.\(^{35}\)

*R. equi* is similar in genetic content, cell structure, and clinical manifestations of infection to *Mycobacterium tuberculosis*.\(^{36,37}\) In addition, interferon-gamma (IFN-γ), which induces *SLC11A1* gene expression,\(^{25}\) is necessary for macrophage killing of both *R. equi* and mycobacteria.\(^{39}\) Therefore, understanding immune response and natural resistance to *R. equi* infection is a valuable tool for better understanding human infection with *M. tuberculosis*. A previous study indicated that mice functionally devoid of the *SLC11A1* gene (*SLC11A1<sup>D169D169</sup>*)) were not more susceptible to infection with virulent *R. equi* than wild-type mice.\(^{40}\) Similarly, *SLC11A1* expression does not seem to be associated with resistance to *M. tuberculosis* in mice.\(^{41,42}\) However, several studies have indicated that mutations in *SLC11A1* are associated with resistance to *M. tuberculosis* in humans\(^{15}\) and *SLC11A1* antagonizes the ability of *Mycobacterium spp.* to block phagosome maturation in humans, thus inhibiting intracellular survival.\(^{43}\) Analogous to the phenomenon observed in humans and mice with respect to susceptibility to *M. tuberculosis*, the present study indicates that *SLC11A1* polymorphisms may influence susceptibility to *R. equi* foal pneumonia, despite the fact that *SLC11A1* expression is not critical to murine susceptibility to *R. equi* infection.

This study represents the first known report of an association between *SLC11A1* and susceptibility to equine infectious disease, enhancing not only our understanding of innate immunity to *R. equi* foal pneumonia but also enabling additional studies of natural resistance to other intracellular bacterial infections in equids. As is true of most infectious diseases, however, natural resistance to *R. equi* pneumonia almost certainly is a polygenic trait\(^{44}\) in which *SLC11A1* likely plays a role as one of many genes influencing immune response and susceptibility. In fact, the results of this study indicate that the -57 C/T SNP is not the only

---

**Table 3.** *SLC11A1* 5′untranslated region variation at nucleotide -57 and associations with *Rhodococcus equi* pneumonia at 2 endemic horse farms; all individuals examined from farm A were fixed for -57C such that association tests were not possible.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Class</th>
<th>Allelic</th>
<th>Genotypic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>B</td>
<td>Affected</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>C</td>
<td>Affected</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) P-Values are indicated for the Fisher exact test.
polymorphism involved in natural resistance to *R* *equi* pneumonia, because the disease was detected in foals from Farm A that were fixed for the -57C allele (presumptively associated with foals exposed but not infected with *R* *equi* pneumonia based on the data from Farms B and C; Table 3). Two other equine gene regions have been associated with the presence of high numbers of *R* *equi* in TBA fluid, indicating other potentially important foci for future research.

### Footnotes

* QuickExtract DNA Extraction Solution 1.0, Epicentre, Madison, WI
* FTA classic cards, Whatman Biosciences, Clifton, NJ
* *Taq* DNA polymerase in storage buffer B and 1× Mg2+ free buffer, Promega, Madison, WI
* MasterAmp PCR Enhancer, Epicentre, Madison, WI
* QIAquick PCR purification kit, Qiagen, Valencia, CA
* ABI PRISM Big Dye Terminator Cycle Sequencing Kit, Applied Biosystems, Foster City, CA
* SeqScape Software Version 1.1, Applied Biosystems, Foster City, CA
* TOPO TA Cloning Kit, Invitrogen, Carlsbad, CA

### Acknowledgments

The authors thank the following individuals and organizations for assistance with this project: Dr Joe Templeton and Christopher Schutta for use of the SeqScape software; Dr Tex Taylor, Ms Heather Quiram, and Dr Pete and Mrs Cathy Teel for collection of donkey samples; Dr Rick Brennan and Omaha’s Henry Doorly Zoo for collection and provision of zebra samples; the International Andalusian and Lusitano Horse Association, the American Hanoverian Society, the American Quarter Horse Association, Dr Dianne Anderson, and the University of California-Davis Veterinary Genetics Laboratory for provision of hair samples from Andalusians, Hanoverians, and Quarter Horses; and all of the farm owners and managers who generously allowed access to their horses and assisted with sample collection.

### References


