A R T I C L E   I N F O

Article history:
Received 29 January 2013
Received in revised form 8 August 2013
Accepted 13 August 2013

Keywords:
Hemoplasma
Hemotropic Mycoplasma
Odocoileus virginianus
White-tailed deer
Zoonotic diseases

A B S T R A C T

Globally, hemotropic Mycoplasma spp. are emerging or re-emerging zoonotic pathogens that affect livestock, wildlife, companion animals, and humans, potentially causing serious and economically important disease problems. Little is known about hemotropic Mycoplasma spp. prevalence, host-specificity, or route of transmission in most species, including wildlife. DNA amplification by PCR targeting the 16S rRNA and the RNaseP genes was used to establish the presence and prevalence of hemotropic Mycoplasma spp. in a white-tailed deer (O. virginianus) population in eastern North Carolina. Sixty-five deer (89%) tested positive for hemotropic Mycoplasma spp. where sequence analysis of the 16S rRNA and the RNaseP genes indicated the presence of at least three distinct species. This study represents the first detection of three distinct hemotropic Mycoplasma species in white-tailed deer and the first report of two novel hemotropic Mycoplasma species.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Hemotropic Mycoplasma spp. (hemoplasmas) are cell-wall deficient, obligate epierythrocytic bacteria that infect numerous animal species, including humans. Infections are often chronic and sub-clinical; however, animals can develop hemolytic anemia, particularly when: immuno-suppressed; stressed from poor nutrition, pregnancy or lactation; or concurrently infected with other, more virulent pathogens or more than one Mycoplasma species [1–4]. Globally, Mycoplasma spp. are emerging or re-emerging zoonotic pathogens that affect livestock [5–11], wildlife [12–17], companion animals [1,4,18–22], and humans [23–28], causing potentially serious and economically important diseases problems.

Mycoplasma ovis, a hemoplasma parasite of sheep and goats [7–10], has been associated with lethargy and anemia of varying severities in captive reindeer (Rangifer tarandus) and in farm-raised white-tailed deer (Odocoileus virginianus) [13,16]. A high rate of infection with Mycoplasma ovis – like organisms has been also reported in several other cervids species (Blastocercus dichotomus, Mazama rana, and Mazama americana) raised in captivity [15] as well as in free ranging species (B. dichotomus, Ozotocerus bezoarticus, and O. bezoarticus) [14]. More recently, M. ovis or infection with a Mycoplasma ovis – like organisms has been also described infecting dogs [22] and humans [23,25] highlighting the potential zoonotic potential of this species.

The objective of this study was to determine the molecular prevalence of hemotropic Mycoplasma species in 73
free-ranging white-tailed deer (*O. virginianus*) from a hunting preserve in eastern North Carolina.

2. Materials and methods

2.1. Sample selection

During a population health assessment in July 2008 and March 2009, 73 white-tailed deer (13 males and 60 female) from eastern North Carolina were surveyed for the presence of *Mycoplasma* spp. Hofmann Forest (~34°53′ N, ~77°23′ W), owned and managed by the North Carolina State Natural Resources Foundation, is a 31,565-acre tract managed for lobolly pine production. Hofmann Forest has nine hunt clubs (>400 members) that use hounds to hunt white-tailed deer (estimated population of 2000–5000 deer) [29,30].

2.2. Blood sampling

Deer blood was obtained via cardiac puncture immediately after animals were killed by head-shot with a high-powered rifle. Blood samples were collected using an evacuated tube system (Vacutainer, Beckon, Dickson and Company, Franklin Lakes, NJ, USA) and analyzed for packed cell volume (PCV) in situ and stored at −20 °C until processed.

2.3. Body gross examination and health condition analysis

Gross body condition of each animal, assessed in the field by wildlife biologists and veterinarians, was evaluated on the basis of body-condition indicators (e.g., kidney fat, femur marrow fat) and later by blood analysis (glucose, urea nitrogen, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, calcium, phosphorus, sodium, potassium, chloride, albumin/gLOBulin ratio, BUN/creatinine ratio, globulin, and creatine kinase, as previously described [30]).

2.4. DNA testing

All blood samples were analyzed for the presence of hemotropic *Mycoplasma* DNA by PCR targeting a 1380 bp region of the 16S rRNA, and a 165 bp region of the RNAse P gene as previously described [23,31]. DNA from 200 micro-liter of blood–EDTA from each white-tailed deer was extracted using a Qiamp DNA Mini Kit (QIAGEN Inc., Valencia, CA). After extraction following the manufacture protocol, DNA concentration and quality was measured using absorbance ratio between 260/280 nm (Nanodrop, Thermo Scientific, USA). Amplification of hemotropic *Mycoplasma* 16S rRNA was performed using two sets of oligonucleotides HemMycop165s: 5′ GYTATCMATAAY-ACATGCAAGTCGARCG-3′ and HemMycop165sP30s: 5′-CTCCACCACTTGTGAGTCCCCTC 3′ and HemMycop165sP30s: 5′ GCCCATATTCTCTACCCGAAGACGACT 3′ and HemMycop165s-1420as: 5′ GTTGACGGCGGGTGCTGAAGACGACT 3′. Sequence derived from amplicons obtained from each primer set (with an overlap of 600 bp to assure consistency in species identification from each sample) was aligned and edited using AlignX (Vector NTI suite 11.5.1). Similarly, amplification of *Mycoplasma* RNAseP gene was performed using oligonucleotides HemMyco RNAseP30s: 5′ GAT KGT GYG AGY ATA TAA AAA AAT AAR CTC RAC 3′ and HemoMycop RNAseP200as: 5′ GGGC TTT ACC CCG TTT CAC 3′ as forward and reverse primers, respectively. Amplification was performed in a 25-μl final volume reaction containing 12.5 μl of Tak-Ex® Premix (Fisher Scientific), 0.25 μl of 30 μM of each forward and reverse primer (IDT® DNA Technology), 8 μl of molecular grade water, and 5 μl of DNA from each sample tested. PCR negative controls were prepared using 5 μl of DNA from blood of a healthy dog and positive controls were prepared using 5 μl of DNA from dog blood infected with *Mycoplasma hematoparvum*. Conventional PCR was performed in an Eppendorf Mastercycler Eppgradient® under the following conditions: a single hot-start cycle at 95 °C for 2 min followed by 55 cycles of: denaturing at 94 °C for 15 s, annealing at 68 °C (16S rRNA) or 59 °C (RNAseP) for 15 s, and extension at 72 °C for 18 s. Amplification was completed by an additional cycle at 72 °C for 30 s, and products were analyzed by 2% agarose gel electrophoresis with detection using ethidium bromide under ultraviolet light.

Table 1

Sequence homologies on the 16S rRNA gene (partial sequence) (Genbank accession numbers in parenthesis).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mycoplasma spp. deer</th>
<th>Mycoplasma spp. deer</th>
<th>Mycoplasma spp. deer</th>
<th>Mycoplasma wenyonii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USA (FJ824847)</td>
<td>Brazil (HQ634379)</td>
<td>Japan (AB558899)</td>
<td>(EU367964)</td>
</tr>
<tr>
<td>10</td>
<td>1056/1060 (99.6%)</td>
<td>1037/1060 (97.8%)</td>
<td>1045/1060 (98.6%)</td>
<td>1017/1060 (95.9%)</td>
</tr>
<tr>
<td>28</td>
<td>1056/1060 (99.6%)</td>
<td>1037/1060 (97.8%)</td>
<td>1045/1060 (98.6%)</td>
<td>1017/1060 (95.9%)</td>
</tr>
<tr>
<td>61</td>
<td>1056/1060 (99.6%)</td>
<td>1037/1060 (97.8%)</td>
<td>1045/1060 (98.6%)</td>
<td>1017/1060 (95.9%)</td>
</tr>
<tr>
<td>65</td>
<td>1056/1060 (99.6%)</td>
<td>1037/1060 (97.8%)</td>
<td>1045/1060 (98.6%)</td>
<td>1017/1060 (95.9%)</td>
</tr>
<tr>
<td>66</td>
<td>1056/1060 (99.6%)</td>
<td>1037/1060 (97.8%)</td>
<td>1045/1060 (98.6%)</td>
<td>1017/1060 (95.9%)</td>
</tr>
<tr>
<td>7</td>
<td>1225/1268 (96.6%)</td>
<td>1243/1268 (98.0%)</td>
<td>1221/1268 (96.8%)</td>
<td>1212/1268 (95.6%)</td>
</tr>
<tr>
<td>46</td>
<td>1225/1268 (96.6%)</td>
<td>1243/1268 (98.0%)</td>
<td>1221/1268 (96.8%)</td>
<td>1212/1268 (95.6%)</td>
</tr>
<tr>
<td>47</td>
<td>1225/1268 (96.6%)</td>
<td>1243/1268 (98.0%)</td>
<td>1221/1268 (96.8%)</td>
<td>1212/1268 (95.6%)</td>
</tr>
<tr>
<td>63</td>
<td>1225/1268 (96.6%)</td>
<td>1243/1268 (98.0%)</td>
<td>1221/1268 (96.8%)</td>
<td>1212/1268 (95.6%)</td>
</tr>
<tr>
<td>70</td>
<td>1225/1268 (96.6%)</td>
<td>1243/1268 (98.0%)</td>
<td>1221/1268 (96.8%)</td>
<td>1212/1268 (95.6%)</td>
</tr>
<tr>
<td>6</td>
<td>1091/1134 (96.2%)</td>
<td>1111/1134 (97.8%)</td>
<td>1088/1134 (95.9%)</td>
<td>1076/1134 (94.9%)</td>
</tr>
<tr>
<td>74</td>
<td>1091/1134 (96.2%)</td>
<td>1111/1134 (97.8%)</td>
<td>1088/1134 (95.9%)</td>
<td>1076/1134 (94.9%)</td>
</tr>
</tbody>
</table>
Table 2
Sequence homologies in a 102 bp partial region of the RNaseP gene (Genbank accession numbers in parenthesis).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Candidatus M. haemocereae* (AB561882)</th>
<th>Mycoplasma wenyonii (EU078610)</th>
<th>Mycoplasma ovis (EU078612)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>94/102 (92.1%)</td>
<td>90/102 (88.2%)</td>
<td>92/102 (90.2%)</td>
</tr>
<tr>
<td>28</td>
<td>94/102 (92.1%)</td>
<td>90/102 (88.2%)</td>
<td>92/102 (90.2%)</td>
</tr>
<tr>
<td>61</td>
<td>94/102 (92.1%)</td>
<td>90/102 (88.2%)</td>
<td>92/102 (90.2%)</td>
</tr>
<tr>
<td>65</td>
<td>94/102 (92.1%)</td>
<td>90/102 (88.2%)</td>
<td>92/102 (90.2%)</td>
</tr>
<tr>
<td>66</td>
<td>94/102 (92.1%)</td>
<td>90/102 (88.2%)</td>
<td>92/102 (90.2%)</td>
</tr>
<tr>
<td>7</td>
<td>97/102 (95.0%)</td>
<td>90/102 (88.2%)</td>
<td>93/102 (91.2%)</td>
</tr>
<tr>
<td>46</td>
<td>97/102 (95.0%)</td>
<td>90/102 (88.2%)</td>
<td>93/102 (91.2%)</td>
</tr>
<tr>
<td>47</td>
<td>97/102 (95.0%)</td>
<td>90/102 (88.2%)</td>
<td>93/102 (91.2%)</td>
</tr>
<tr>
<td>63</td>
<td>97/102 (95.0%)</td>
<td>90/102 (88.2%)</td>
<td>93/102 (91.2%)</td>
</tr>
<tr>
<td>70</td>
<td>97/102 (95.0%)</td>
<td>90/102 (88.2%)</td>
<td>93/102 (91.2%)</td>
</tr>
<tr>
<td>6</td>
<td>90/102 (88.2%)</td>
<td>91/102 (89.2%)</td>
<td>90/102 (88.2%)</td>
</tr>
<tr>
<td>74</td>
<td>90/102 (88.2%)</td>
<td>91/102 (89.2%)</td>
<td>90/102 (88.2%)</td>
</tr>
</tbody>
</table>

Sequence homology analysis of 12 randomly selected positive samples (Table 1) suggested the presence of three distinct hemotropic Mycoplasma groups (Fig. 1): Group A with a 99.6% match with a Mycoplasma sp. recently described in a sick, farm-reared fawn [13] from rural Indiana, USA (Genbank FJ824847); Group B, matching 96.6% with Genbank FJ824847 sequence, 98% with a Mycoplasma sp. described in marsh deer (Blastoceros dichotomus) from Brazil (HQ634379), 96.8% with a Mycoplasma sp. described in sika deer (Cervus nippon) from Japan (AB558899), and 95.3% with M. wenyonii (EU367964) detected in cattle; and Group C matched only 96.2% with Genbank FJ824847 and 97.8% with a Mycoplasma sp. described in marsh deer (Blastoceros dichotomus) from Brazil (HQ634379).

The same 12 blood DNA samples were amplified using PCR targeting a 165 bp region of the RNaseP gene. Sequence analysis of the RNaseP gene (Table 2) also identified three clusters that matched the three groups described above (Fig. 2): Group A with 92.1% homology with

3. Results

3.1. Gross examination of animal condition

Gross examination of animal condition post-mortem did not show any signs indicative of clinical abnormalities. Packed cell volumes did not indicate anemia in either collection period. PCV samples averaged 45% (SD = 5.6, n = 28) in July, and PCV average was 53% (SD = 6.6, n = 30) in March [29].

3.2. Blood DNA analysis

A total of 65/73 DNA samples (89%) tested positive for Mycoplasma spp. amplification using primers targeting a region expanding 1300 bp of the 16S rRNA gene.
‘Candidatus Mycoplasma haemocoevae’ (AB561882), 90.2% with M. ovis (EU078612) and 88.2% with M. wenyonii (EU078610); Group B matching 95.0% with ‘Candidatus Mycoplasma haemocoevae’ (AB561882), 91.2% with M. ovis (EU078612), and 88.2% with M. wenyonii (EU078610); and Group C matching 88.2% with ‘Candidatus Mycoplasma haemocoevae’ (AB561882) and M. ovis (EU078612), and 89.2% with M. wenyonii (EU078610).

4. Discussion

Hemotropic Mycoplasma spp. (‘hemoplasmas’, formerly classified as Haemobartonella and Eperythrozoon spp.) appear to have co-evolved with many animals species. The development of molecular assays, primarily targeting the 16S rRNA gene of these microbes, has resulted in the recent recognition of several novel human [23–25,27,32] and animal hemoplasmas [13,21,31,33–43]. DNA screening of 73 white-tailed deer blood samples resulted in the detection of a high prevalence Mycoplasma infection similar to previously reported prevalence in sheep [7] and other deer species [13,14,16] supporting the possibility that ticks or other vectors, injuries related to animal-to-animal contact, and perhaps vertical transmission might play a role in widespread infection of these species [15].

Sequence analysis of randomly selected samples identified three hemotropic Mycoplasma sp. groups: one closely related to a previously reported species (Genbank JF824847) described in a sick fawn [13], and two potentially new hemotropic Mycoplasma species not described or reported to date. The lack of clinical abnormalities coupled with the high prevalence of infection suggests that white-tailed deer may serve as a reservoir for at least three Mycoplasma spp. Additional research on the distribution of these hemotropic Mycoplasma spp. is needed to determine if they are locally confined or have a wider distribution in white-tailed deer populations.

Previous studies have shown that hemotropic Mycoplasma infection in humans are more prevalent in farmers, veterinarians and other individuals who have frequent and close contact with animals and or vectors [23,24,28,44]. In fact, M. ovis has been recently described as the most prevalent species infecting humans that has extensive animal and/or vector contact [23,25] than from people with less exposure. While ticks (i.e. Rhipicephalus sanguineus) have been suggested as vectors for the transmission of hemotropic Mycoplasmas, no experimental vector competence data is available [12,45–48].

The pathogenic potential of many hemotropic Mycoplasmas as a cause of human and animal disease remains to be elucidated as do the mechanisms of intra- and inter- species transmission. The sympatric relationships between deer, hunters, their dogs and arthropod vectors in the shared environment present an opportunity to explore the zoonotic potential of hemotropic Mycoplasma spp. present in white-tailed deer.

Sequence data for the 16S rRNA and the RNAseP gene for the three hemotropic Mycoplasma sp. described in this study are deposited in Genbank database with accession numbers: KC512404; KC512403; KC512402; JQ610626; JQ610627; and JQ610628.

Acknowledgements

This project was funded by the North Carolina State Natural Resources Foundation, Inc., the NCSU Department of Forestry and Environmental Resources, and the NCSU Fisheries, Wildlife, and Conservation Biology Program. The research protocol outlined here was approved by the North Carolina Wildlife Resources Commission and the North Carolina State University (NCSU) Institutional Animal Care and Use Committee (08-082-O). We thank the numerous undergraduate, graduate, and veterinary students for volunteering during the field collection of samples.

References


