Introduction: *Ehrlichia chaffeensis* is an emerging zoonotic tick-borne rickettsial pathogen that has been detected in a wide range of vertebrate hosts, including ruminants, canids and primates. Although white-tailed deer (*Odocoileus virginianus*) are considered the primary reservoir of *E. chaffeensis*, this pathogen was also reported in other naturally infected cervids, including Korean spotted or sika deer (*Cervus nippon*) and Brazilian marsh deer (*Blastocerus dichotomus*).

Case presentation: A captive adult bull elk (*Cervus elaphus*) from east-central Missouri was submitted for post-mortem analysis. The elk was in poor body condition with easily palpable ribs and vertebral spinal processes. Excoriations were noted on the occipital region of the head and on the left scapula, which had moderate amounts of maggots within the lesions. Large numbers of ticks were scattered over the body. Novel and established PCR assays were used to detect *E. chaffeensis* in blood and spleen samples from this elk, but the pathogen was not detected in *Dermacentor albipictus* ticks collected at necropsy. Portions of several gene sequences were analysed from the infecting agent.

Conclusion: To the best of our knowledge this is the first report of *E. chaffeensis* infection in an elk. It was not determined whether the pathogen contributed to cause of death. Notably, the pathogen was not detected in *D. albipictus* ticks collected from the elk.

Keywords: *Dermacentor albipictus*; *Ehrlichia chaffeensis*; elk; PCR-based diagnostic tests; tick-borne pathogen.
the lesions. It was also noted that large numbers of ticks were scattered over the body. No other gross lesions were noted. On microscopic examination, the skin had a severe ulcerative dermatitis with large numbers of eosinophils, neutrophils, macrophages and mast cells in the superficial and mid-dermis, especially around blood vessels and adnexal structures. A thick serocellular crust with bacterial colonies, often cocci, was also noted. No apparent aetiology was present in the skin sections examined. No significant histopathologic changes were noted in the liver, kidney, lung, heart, spleen or testicle.

The elk was a captive animal from east-central Missouri, where *E. chaffeensis* is endemic. As ticks were found parasitizing the animal, blood was tested for ehrlichial infection, and blood, spleen and ticks collected from the elk were subsequently sent to our laboratory for tick identification and for more specific PCR-based assays for *Ehrlichia* spp.

**Investigations**

**Tick identification**

To substantiate speciation based on external morphology, molecular techniques were used to confirm the identity of the ticks collected from the elk. The 18S rRNA internal transcribed spacer-2 (ITS-2) sequence has been used to characterize tick species, including those of the genus *Dermacentor* (Dergousoff & Chilton, 2007; Shone et al., 2006). Tick samples collected from the elk and *Dermacentor variabilis* controls were amplified with PCR using primers DermITS2-F (GTGGGTCCGTCGACTCGTT) and DermITS2-R (TC-GCCCAACAGGCGCTACT) as previously described (Dergousoff & Chilton, 2007; Shone et al., 2006). These PCR products were subjected to agarose electrophoresis and sequence analysis.

**PCR assays for *E. chaffeensis***

Heparinized elk blood (1.0 ml) was centrifuged at 1200 g for 30 min at 4 °C, and buffy coats (approximately 200 μl) were manually transferred to 1.5 ml microcentrifuge tubes for nucleic acid isolation and PCR analysis. Spleen samples were stored at −80 °C prior to DNA extraction. Ticks were stored in a humidity chamber for 112 days at room temperature and 95 to 100 % relative humidity, with a 12 h:12 h (light:dark) photoperiod. A dichotomous key was used to identify four adult male and two adult female ticks, which were then bisected along the sagittal plane, pooled and placed on dry ice for template isolation. Nucleic acids were extracted from buffy coat and tick samples with the High Pure Viral Nucleic Acid kit (Roche) as previously described (McClure et al., 2009). Spleen samples were thawed on ice and nucleic acids were isolated from 10 mg portions with the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer’s instructions. Six PCR assays were used to test these samples for the presence of *E. chaffeensis*, and each sample was tested a minimum of two times with each PCR assay.

The first assay was based on a hypothetical outer membrane protein gene (*homp*) sequence that is unique to the *E. chaffeensis* genome sequence, which was used to design and optimize a novel fluorogenic real-time PCR assay (Stoffel et al., 2014). This *E. chaffeensis*-specific assay used the forward primer ECH144762S, reverse primer ECH144994A and the fluorogenic probe ECH144816S (Table 1). Templates (2.5 μl) were assayed in 25 μl reactions containing 1 × PCR buffer, 2 mM MgCl₂, 0.06 U μl⁻¹ Platinum Taq DNA polymerase, 200 μM dNTP mix (Invitrogen), 1 % (v/v) DMSO, 1.8 μM each of primers ECH144762S and ECH144994A and 0.25 μM of ECH144816S probe. These assays were run in an ABI 7300 real-time thermocycler (Applied Biosystems) at 95 °C for 2 min followed by 60 cycles of 95 °C for 15 s and 61 °C for 1 min. PCR-positive samples were also subjected to 1.5 % agarose electrophoresis with 1 × TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) and ethidium bromide (0.5 μg ml⁻¹).

A conventional PCR assay based on the P28 outer membrane protein gene of *E. chaffeensis* was also used to assay the same samples (Wagner et al., 2004). Templates (2.5 μl) were assayed in 25 μl reactions containing 1 × PCR buffer, 2 mM MgCl₂, and 200 μM dNTP mix, 0.06 U μl⁻¹ Platinum Taq DNA polymerase, 8 % DMSO, and 1 μM each of primers ECH181-101 and ECH360-341. These assays were run in an ABI 2720 thermocycler at 93 °C for 2 min, followed by 95 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 30 s, followed by a final extension step of 72 °C for 7 min. These PCR products were electrophoresed as described above.

A 16S rRNA gene (16S rDNA)-based real-time PCR assay for *E. chaffeensis* was also used to assay buffy coat and spleen samples (Loftis et al., 2003). Templates (2.5 μl) were assayed in 25 μl reactions containing 1 × PCR buffer, 2 mM MgCl₂, 0.06 U μl⁻¹ Platinum Taq DNA polymerase, 200 μM dNTP mix (Invitrogen), 0.2 μM forward primer ECH16S-17, 0.8 μM reverse primer ECH16S-97 and 0.1 μM fluorogenic probe, ECH16S-38 (Table 1). These reactions were run in an ABI 7300 real-time thermocycler at 95 °C for 2 min followed by 60 cycles of 95 °C for 15 s and 61 °C for 1 min.

A trp32-based PCR was also used with nested primer sets FB5A and FB3A or FB5 and FB3 (Table 1) (Paddock & Childs, 2003). Templates (2.5 μl) were assayed in 25 μl reactions containing 1 × PCR buffer, 2 mM MgCl₂, 0.06 U μl⁻¹ Platinum Taq DNA polymerase, 200 μM dNTP mix (Invitrogen) and 0.5 μM of each primer. PCR reactions were run in an ABI 2720 thermocycler with 3 cycles of 94 °C for 1 min, 52 °C for 2 min and 70 °C for 1.5 min, followed by 37 cycles of 88 °C for 1 min, 55 °C for 1.5 min and 70 °C for 1.5 min, with a final extension step of 68 °C for 5 min. These PCR products were also electrophoresed as described above.
Amplicon sequence analysis

Amplicons were excised from agarose gels and DNA extracted using the Wizard SV Gel and PCR Clean-Up System (Promega). Nucleotide sequences were determined in both directions with the same forward and reverse primers used for the respective PCR assays, with a 3730 96-capillary DNA Analyzer (Applied Biosystems), at the University of Missouri DNA Core Facility. Regions of local similarity between nucleotide sequences were assessed with the BLAST program (National Center for Biotechnology Information, Bethesda, MD) against nucleotide collections using default parameters. Sequence alignments were produced with CLUSTAL W2 using default nucleotide parameters (Chenna et al., 2003).

Diagnosis

Tick identification

Using morphologic characteristics of individual ticks, a dichotomous key was used to identify the ticks removed from the elk (Fig. S1, available in the online Supplementary Material). For each tick examined, the basis capitulum was visible from the dorsal surface, and the palps were approximately the same length as the basis capitulum, with second palpal segments of equal width and length. The spiracular plates displayed morphologic variation ranging from round to showing a dorsal prolongation. Ornate scuta were present on all of the ticks. Based on these characteristics, the four male and two female ticks were tentatively identified as D. albipictus. The ITS-2 sequences amplified from these ticks with primers DermITS-F and DermITS-R (Fig. S2; Genbank accession no. KP236454) were 100 % identical to the nucleotide sequence reported for D. albipictus (Dergousoff & Chilton, 2007; Shone et al., 2006).

PCR assays for E. chaffeensis

Blood samples tested at the MU VMDL with a generic Ehrlichia assay were PCR-positive, but the species of pathogen was not determined. Therefore, a novel homp-based PCR assay designed for detection of E. chaffeensis was used to test buffy coat samples from the elk. Elk buffy coat samples tested PCR-positive for E. chaffeensis, which was confirmed by approximately 99.5 % identity between the elk-derived sequence and the target sequence from the E. chaffeensis (Arkansas strain) genome (Fig. S3).

Genetic diversity of p28 has been used to differentiate human isolates (Wagner et al., 2004; Yu et al., 1999). Therefore elk buffy coat samples were assayed with an E. chaffeensis p28-based PCR assay to further characterize the E. chaffeensis strain present. In two trials, one of two and one of four replicates, respectively, were amplified with this assay. There was a substantial difference (87 % identity) between the two p28 sequences generated from the elk.

### Table 1. Sequence analyses of amplicons generated from different E. chaffeensis-specific PCR assays

<table>
<thead>
<tr>
<th>Gene targeted</th>
<th>Primer and probe sequences</th>
<th>Amplicon size (bp)</th>
<th>Genbank accession no.</th>
<th>Closest matching strain</th>
<th>Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>homp</td>
<td>ECH1474-625 (ACCCTGCAACACCTACAAC), ECH14499A (CCACCTACAGCACCTAAAGCA) and the fluorogenic probe ECH141481S-FAM (FAM-TCTGTAACACCTATAGCATCTGCAACGC-BHQ)</td>
<td>233</td>
<td>KP236455</td>
<td>Ark</td>
<td>99.48</td>
</tr>
<tr>
<td>p28</td>
<td>ECH81-101 (AGCAGGTAGTGGTATTAACGG) and ECH360-341 (AGATACTTCAAGCTCTATTC)</td>
<td>279</td>
<td>KP236456</td>
<td>V2</td>
<td>86.98</td>
</tr>
<tr>
<td>trp32</td>
<td>FB5 (AAATAGGGTATAAATATGTCAC) and FB3 (GCCTAATTCAGATAAACTAAC) or FB5A (GTGACATCTTAGTTTAATAGAAC) and FB3A (AAGACTGAAACGTTATAGAG)</td>
<td>849</td>
<td>KP236457</td>
<td>81</td>
<td>NA</td>
</tr>
<tr>
<td>16S rDNA</td>
<td>ECH16S-17 (GGGTCGCATCTAGATGATTACG) and ECH16S-97 (GGGTCGCATCTAGATGATTACG)</td>
<td>81</td>
<td>KP236458</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Not applicable.
buffy coat samples compared with the closest match, the V2 strain of *E. chaffeensis*. Interestingly, there were also mismatched nucleotides at the 5′ end of the forward primer, which partially explained the relatively low efficiency of this assay and the lack of amplification from all of the replicates tested (Fig. S4).

The 16S rDNA sequence is 100% identical among known US isolates of *E. chaffeensis* (Yu et al., 2006); so these elk samples were further examined with a 16S rDNA-based real-time PCR assay for *E. chaffeensis* (Loftis et al., 2003). This assay uses a fluorogenic probe that is specific for *E. chaffeensis* and that is not expected to detect *Ehrlichia muris, Ehrlichia canis, Ehrlichia ewingii, Anaplasma phagocytophilum, Neorickettsia sennetsu* or *Rickettsia prowazekii* DNA. All buffy coat samples and one of two spleen samples were PCR-positive with the 16S rDNA-based assay (Fig. S5).

**Fig. 1.** PCR products from elk tissue and tick samples amplified with the *E. chaffeensis trp32*-based assays. Elk buffy coat biological replicates (BC1–6), spleen biological replicates (S1–2), pooled tick sample (T), no-template controls (N), and *E. chaffeensis* Arkansas strain-infected DH82 samples were assayed and electrophoresed with a 100 bp molecular mass marker (M). (a) Trials 1 and 2 utilizing primers FB5A and FB3A. (b) Trials 1 and 2 utilizing primers FB5 and FB3.
E. chaffeensis trp32 was also shown to exhibit inter-strain variation; so nested trp32-based primer sets were used to assay the elk- and tick-derived templates. These primer sets are commonly used for nested PCR, but the analytical sensitivity was sufficient to use these assays individually in this study. Templates were assayed from six different elk buffy coats, two spleen samples and the pooled tick sample. In the first trial, primers FB5A and FB3A amplified target

![Alignment of elk buffy coat-derived (Elk) and E. chaffeensis Arkansas strain-infected DH82 cell (Ark) amplicon sequences generated with E. chaffeensis trp32 primer sets. Nucleotide discrepancies between the sequences are denoted by asterisks.](http://jmmcr.sgmjournals.org)
sequences from three of the six buffy coats and one of the two spleen samples, but the pooled tick sample was PCR-negative (Fig. 1). Similar results were observed in the second trial, except an additional buffy coat sample tested PCR-positive. Primers FB5 and FB3 amplified all six buffy coats and both spleen replicates in the first trial, but one spleen sample in the second trial, and none of the pooled tick samples in either trial. With the exception of a single nucleotide, the amplicon sequence derived from the elk samples was nearly 100% identical to that of the E. chaffeensis Arkansas strain (Fig. 2).

Discussion

Multiple locus sequence analysis was used to confirm that E. chaffeensis was consistently detected in elk buffy coat and spleen samples through PCR assays based on four different gene sequences (Table 1). E. chaffeensis has been detected in tissues from other cervids, including blood, bone marrow, inguinal lymph nodes, scapular lymph nodes and spleen from white-tailed deer (Arens et al., 2003; Paddock & Childs, 2003), and blood from Korean spotted deer, also known as sika deer, and Brazilian marsh deer (Kawahara et al., 2009; Lee et al., 2009; Machado et al., 2006).

A definitive cause of death was not determined for the elk, but E. chaffeensis is not thought to cause clinical disease in white-tailed deer (Paddock & Childs, 2003; Varela-Stokes, 2007). Anorexia and depression, signs of ehrlichiosis in other species (Goodman et al., 2003; Harrus & Waner, 2011; Stich et al., 2008), were observed in sika deer in which E. chaffeensis was detected; however, these deer were co-infected with Anaplasma bovis.

Studies examining elk for the presence of ehrlichial pathogens are limited. Elk from California tested PCR-positive for an Ehrlichia-like species that was not E. chaffeensis (Foley et al., 1998). However, human monocytic ehrlichiosis is considered relatively rare in California, and A. americanum, the primary vector of E. chaffeensis in the USA, is not established in California. Therefore it is not surprising that E. chaffeensis was not highly prevalent among wildlife hosts in that state (Paddock & Childs, 2003). Conversely, human ehrlichiosis is highly prevalent in Missouri, where A. americanum and white-tailed deer are indigenous and where E. chaffeensis has been detected in both of these reservoir populations.

Genetic diversity has been demonstrated among p28 and trp32 sequences from different E. chaffeensis strains. In the present study, the regions of p28 and trp32 amplified from elk tissues varied from those of reported strains. The trp32 sequence was similar to that of the Arkansas strain sequence with one mismatch. Conversely, considerable variation was observed between the p28 amplicon and sequences reported from other E. chaffeensis strains, with most similarity to the E. chaffeensis V2 strain.

Ticks collected from the elk were identified using morphologic characteristics and molecular techniques. As previously reported, the spiracular plates of D. albipictus displayed some morphologic variation ranging from round to dorsally elongated (Leo et al., 2010). Interestingly, E. chaffeensis was not detected in D. albipictus collected from the infected elk described in this report, even though this tick is a member of the same ixodid tribe (i.e. the Rhipicephalinae) as other tick species known to acquire E. chaffeensis under natural and experimental conditions (delos Santos et al., 2007; Roland et al., 1998; Stoffel et al., 2014). Furthermore, the atypical one-host feeding behaviour of D. albipictus means that it is almost certain that these adult-stage ticks fed on the elk, both as larvae and as nymphs.

We speculate that a different tick species transmitted E. chaffeensis to the elk, probably A. americanum. It seems unlikely that D. albipictus would be an important vector or reservoir for E. chaffeensis. Nevertheless, our study indicates that elk are susceptible to the infection and suggests that it would be prudent to investigate possible epidemiologic ramifications of this observation.

In conclusion, to the best of our knowledge, this is the first demonstration of a natural E. chaffeensis infection of an elk. We do not claim that this infection contributed to death because a definitive cause of death was not ascertained. Moreover, testing of a single elk at necropsy does not demonstrate susceptibility of this host species to the infection or the disease; further studies are required in order to investigate elk populations for infection and exposure to E. chaffeensis as well as to evaluate the pathogenicity of E. chaffeensis in elk.

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References


