Infections with *Bartonella* spp. have been recognized as emerging zoonotic diseases in humans. Large knowledge gaps exist, however, relating to reservoirs, vectors, and transmission of these bacteria. We describe identification by culture, PCR, and housekeeping gene sequencing of *Bartonella* spp. in fed, wingless deer keds (*Lipoptena cervi*), deer ked pupae, and blood samples collected from moose, *Alces alces*, sampled within the deer ked distribution range in Norway. Direct sequencing from moose blood sampled in a deer ked-free area also indicated *Bartonella* infection but at a much lower prevalence. The sequencing data suggested the presence of mixed infections involving two species of *Bartonella* within the deer ked range, while moose outside the range appeared to be infected with a single species. *Bartonella* were not detected or cultured from unfed winged deer keds. The results may indicate that long-term bacteremia in the moose represents a reservoir of infection and that *L. cervi* acts as a vector for the spread of infection of *Bartonella* spp. Further research is needed to evaluate the role of *L. cervi* in the transmission of *Bartonella* to animals and humans and the possible pathogenicity of these bacteria for humans and animals.

**MATERIALS AND METHODS**

**Collection of material.** A total of 41 moose (*Alces alces*) were sampled within the distribution range of deer ked in southeastern Norway. The studied material comprised 11 carcasses submitted for necropsy in conjunction with an outbreak of deer ked-associated alopecia (thoroughly described by Madslien and coauthors [17]) and free-ranging, presumably healthy moose chemically immobilized in association with radio-collaring. Wingless (fed) deer ked imagines and pupae were collected from the carcasses. Samples were taken from the liver, spleen, and (if available) blood at necropsy, while blood samples were taken from all live animals (Table 1). In addition, blood samples taken from 28 free-ranging, presumably healthy moose immobilized in association with radio-collaring in the Stor-Elvdal area (approximately 38 km north of the recognized deer ked distribution front) were included in the analyses. The collection also included winged deer keds (i.e., imagines that have not yet fed), caught in two localities: (i) Østfold, within a well-established deer ked area, and (ii)...
Akershus, at the deer ked expansion front (see Välimäki [18] for a more thorough description). The deer keds were captured as they settled on a study person walking slowly through a forested area. Blood samples from live animals were collected into EDTA plastic tubes (Becton, Dickinson, Franklin Lakes, NJ) and frozen at −80°C together with the other sampled materials on arrival in the laboratory.

**Bacterial culture.** Culture was attempted from samples obtained from necropsied deer carcasses, live moose, and winged unfed deer ked imagines as mentioned above by using Columbia agar medium with 5% horse blood (CA) and incubated for a total of 6 weeks at 37°C. Deer ked imagines and pupae were previously surface sterilized in 70% ethanol. While culture was attempted on all tissue samples and imagines as mentioned above by using Columbia agar medium with 5% horse blood (CA) and incubated for a total of 6 weeks with 5% CO2 at 37°C. Thereafter, total genomic DNA was purified using the Qiagen QIAamp DNA minikit (Qiagen) according to the manufacturer’s protocol. DNA templates from tissues and blood samples were either obtained directly using the same kit as mentioned above or from cultured isolates by boiling cell suspensions in a phosphate buffer for 10 min at 95°C.

**PCR detection of Bartonella DNA.** DNA extracts from the individual samples were subjected to three different PCR strategies for the detection of *Bartonella*. Quantitative PCR (qPCR) and seminested PCR were initially used to screen the samples for *Bartonella* DNA, based on previously described protocols (19, 20). Both assays detect a region in the *gltA* gene. For further analysis of positive samples, conventional PCR was used to produce amplicons from five housekeeping genes (*gltA*, *rpoB*, *ftsZ*, *rbcL*, and *groEL*) as described previously (21). Primers for *ribC* and *groEL* were, however, redesigned to amplify a wider range of *Bartonella* species. All primers were purchased from Invitrogen, and their respective sequences are listed in Table 2. Amplifications were performed in 25-μl reaction mixtures containing 1X Taq buffer, 0.4 μM forward and reverse primers, 0.2 mM deoxynucleoside triphosphate (dNTP) mix, 1.5 mM MgCl2, 1 U Taq DNA polymerase, and 2 to 3 μl DNA template. Each PCR was carried out under the same culturing conditions as described above.

**DNA extraction.** Prior to DNA extraction, deer ked imagines and pupae were surface disinfected by immersion in 0.5% hypochlorite (5 min) and 70% ethanol (5 min), followed by three rinses in sterile water. These were transferred into a sterile 2-ml microcentrifuge tube containing one 3-mm tungsten carbide bead (Qiagen) and 0.5 g 0.1-mm glass beads. These were transferred into a sterile 2-ml microcentrifuge tube containing one 3-mm tungsten carbide bead (Qiagen) and 0.5 g 0.1-mm glass beads and 0.5 g 0.1-mm glass beads. These were transferred into a sterile 2-ml microcentrifuge tube containing one 3-mm tungsten carbide bead (Qiagen) and 0.5 g 0.1-mm glass beads.

**Table 2: Primers and probe used in this study**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward sequence (5′→3′)</th>
<th>Reverse sequence (5′→3′)</th>
<th>Probe sequence (5′→3′)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>gltA</td>
<td>GGGGACCACTCATGGTGGA</td>
<td>AATGCAAAAGAAGACGTAAC</td>
<td>NA</td>
<td>~350</td>
<td>22</td>
</tr>
<tr>
<td>gltAa</td>
<td>GGGGACCACTCATGGTGGA</td>
<td>CTGAGTCAAAATTTTATA</td>
<td>NA</td>
<td>~143</td>
<td>19</td>
</tr>
<tr>
<td>gltAb</td>
<td>GTTATCTATTGACGCAA</td>
<td>CCAAAACCATAGG</td>
<td>NA</td>
<td>~685</td>
<td>20</td>
</tr>
<tr>
<td>rpoB</td>
<td>GACAGATGGCAGCATGCTTTTC</td>
<td>CGCATATAGTCGATTTGCC</td>
<td>NA</td>
<td>~401/387c</td>
<td>21</td>
</tr>
<tr>
<td>groEL</td>
<td>ATGGACAAGTTGACC</td>
<td>TTCCACACCAGCAAAA</td>
<td>NA</td>
<td>~720</td>
<td>This study</td>
</tr>
<tr>
<td>ribC</td>
<td>TAACCGGATATTTGAG</td>
<td>GCCACAGAGATTGCA</td>
<td>NA</td>
<td>~588</td>
<td>This study</td>
</tr>
<tr>
<td>ftsZ</td>
<td>CATATTGCTTTTTCATTAG</td>
<td>TTTTGCAGAGATCCTG</td>
<td>NA</td>
<td>~515</td>
<td>21</td>
</tr>
</tbody>
</table>

a qPCR.
b Seminested PCR.
c Seminested PCR product.
d Dually labeled oligonucleotide probe with 5′-6-carboxyfluorescein and 3′-black hole quencher 1. NA, not applicable.
out in a PTC-100 programmable thermal controller (MJ Research Inc., Watertown, MA) with the following thermal cycling conditions: initial denaturation cycle at 95°C for 3 min; followed by 35 cycles of amplification at 95°C for 10 s, 55 or 60°C (depending on the gene) for 10 s, and 72°C for 60 s; and a final extension at 72°C for 10 min. With the exception of groEL (annealing at 60°C), all other genes were amplified at annealing temperature of 55°C. PCR products were identified by electrophoresis. In all analyses, positive and negative controls were included within each PCR assay.

**Multilocus sequence analysis (MLSA) and phylogeny of Bartonella DNA.** PCR products of the five examined housekeeping genes were purified with the Nucleocur purification kit (Macherey-Nagel, Düren, Germany), according to the manufacturer’s protocol and sequenced in both directions on an automatic DNA sequencer (ABI 3130 Genetic Analyzer; Applied Biosystems, Foster City, CA). Sequencing reactions were performed in a PTC-100 programmable thermal cycler using the amplicon target PCR primers at a concentration of 2.5 μM. Cycling conditions for the sequencing reactions were as described by Platt and coauthors (23).

Raw chromatograms (both directions) were assembled, inspected visually for errors, and edited using Sequencher 4.5 (Gene Codes, Ann Arbor, MI). Consensus individual gene sequences aligned in ClustalX 2.1 (24) were compared to reference sequences obtained from the GenBank database.

Phylogenies for individual genes generated using a neighbor-joining algorithm with the Kimura 2 parameter model (1,000 bootstrap replicates) in PAUP 4.0b10 (25) provided congruent tree topologies, and so the sequences were concatenated and reanalyzed using a maximum likelihood algorithm in PhyML (26) with a GTR substitution model (estimated using jModelTest [27]) implemented through the University of Oslo bioportal (www.uio.no/biportal/).

**Nucleotide sequence accession numbers.** Sequences obtained during the present study have been submitted to GenBank under the following accession numbers: gltA, JN990623 to JN990630; rpoB, JN990603 to JN990615; groEL, JN990631 to JN990639; ribC, JN990640 to JN990650; and ftsZ, JN990616 to JN990622.

### RESULTS

**Bacterial culture.** *Bartonella* spp. (as identified by subsequent MLSA) were successfully cultured from a single pooled sample of 10 pools of wingless/fed deer ked imagines and from 17 of 29 blood samples from live moose within the deer ked distribution range. No *Bartonella* spp. were cultured from deer ked pupae, winged/unfedimagines, moose tissue or blood from carcasses, or blood samples from live moose outside the deer ked distribution range (Table 1). Culture from necropsy samples was generally severely compromised by nonspecific bacterial contamination. With the exception of a single carcass, deer ked imagines and pupae were identified on all animals sampled within the deer ked distribution range but not on animals immobilized in the ked-free area (Stor-Elvdal).

**Prevalence of Bartonella infection.** On qPCR screening, positive signals were obtained from 8 of 10 pools of wingless/fed deer kedimagines and 1 of 10 pools of pupae. The cycle threshold (C<sub>T</sub>) values, representing the number of genome copies in the sample, were in the range of 20 to 30, indicative of a heavy *Bartonella* presence. In addition, weak positive signals (C<sub>T</sub> > 30) were identified from 2 of 10 pools of wingless/fed imagines and 4 of 10 pools of pupae examined, while the winged/unfed flies were negative for *Bartonella* DNA (Table 1). Although some inhibition was apparent, all tissues from the carcasses were also negative. An overall prevalence of 87% was detected directly from blood and blood-cultured samples from 30 individual moose within the deer ked range. Of these, 17 were positive by qPCR, and the remaining were detected on seminested PCR amplification and by culture. Generally, qPCR gave weak signals from blood (C<sub>T</sub> > 36.9), and some culture-positive samples were negative by qPCR. Positive but generally weak PCR signals (C<sub>T</sub> > 36.4) were also obtained from 10 of 28 blood samples from live moose in the presumed ked-free area, resulting in 36% prevalence (Table 1). The identity of these positive samples was confirmed by sequencing.

**Multilocus sequence analysis.** Good-quality sequences for all 5 genes were not obtained from all individual samples tested. However, a total of 16 (gltA), 17 (rpoB), 17 (groEL), 18 (ribC), and 15 (ftsZ) sequences were obtained from 7 wingless/fed imagine deer ked pools, 4 cultured bacterial isolates, and 10 moose blood extracts from within and outside the deer ked zone. Sequence chromatograms retrieved from some deer ked imagines and blood samples originating within the deer ked zone suggested the existence of a mixed infection of related *Bartonella*, as a small number of ambiguous bases were consistently identified in most genes, while all sequences from cultured isolates were identical with no ambiguous bases. Interestingly, most of the ambiguous bases observed in this study were at positions which are diagnostic for species identification in other ruminant-infecting *Bartonella* spp. (Table 3). Sequences obtained from moose blood sampled outside the deer ked zone displayed a very low level of ambiguity (Table 3). For phylogenetic placement, representative sequences obtained directly from blood and blood cultured isolates displaying no ambiguity in nucleotide sequence were used. The consensus maximum likelihood phylogeny based on concatenated sequences from all 5 studied genes resulted in two *Bartonella* lineages (Fig. 1; see also Fig. S1 in the supplemental material). Lineage I was confined within the deer ked zone and clustered closely with *Bartonella chomelii, B. schoenbuchensis,* and *Bartonella capreoli,* all infectious bacteria of ruminants within a single clade of *Bartonella.* Identity levels between examined samples and the type sequences of ruminant bartonellae deposited in GenBank were different for each of the 5 loci (see Table S1 in the supplemental material), and it proved difficult to subscribe the bacterial isolate concerned or other sequences generated to an individual *Bartonella* species. On the other hand, lineage II was identified both inside (in 10 of 26 infected moose) and outside (in 10 of 10 infected moose) the deer ked zone. Although the lineage II sequences were almost identical, sequences retrieved from samples originating outside the zone displayed very few ambiguous bases compared to those originating within the zone. As lineage II sequences displayed only limited identity to lineage I isolates/strains (approximately 95% at ftsZ, 97% at rpoB, 96% at gltA, 92% at ribC, and 97% at groEL) and to other sequences in GenBank (see Table S1), it should be considered a genetically distinct, previously undescribed clade of ruminant-infecting *Bartonella.*

### DISCUSSION

The current report describes *Bartonella* infection in moose (*Alces alces*) and deer ked (*Lipoptena cervi*) feeding on this host. Our data indicated a higher prevalence of *Bartonella* DNA in moose within the deer ked zone than in animals outside the zone. Such variation may be due to levels of fly infestation, as there was no indication of deer ked, ticks, or other common cervid-parasitizing hirudinids in the deer ked-free areas. The strong qPCR signals, equating to large numbers of *Bartonella* genomes in all but two pools of wingless/fed deer ked imagines, is consistent with the proliferation of *Bartonella* in the gut of this insect as described by Dehio and coworkers (9). The signal from moose blood was weak but posi-
tive, consistent with low numbers of circulating Bartonella-infected cells. Most interestingly, a strong signal was also obtained from a single pool of pupae and a weaker signal from a further four pools of pupae, suggesting the possibility of vertical transmission. Although this may have represented contamination of the pupae by bacteria from the genital tract of the female after pupal formation (hippoboscid produce fully developed pupae in the female reproductive tract), extensive hypochlorite and ethanol disinfect-

### TABLE 3 Comparison of the sites with ambiguous bases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Gene Position</th>
<th>Inside the ked zone</th>
<th>Outside the ked zone</th>
<th>B. chomelii</th>
<th>B. capreoli</th>
<th>B. schoenbuchensis</th>
<th>B. melophagi</th>
<th>B. bovis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ftsZ</td>
<td>411</td>
<td>A/G (2)</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
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<tr>
<td></td>
<td>432</td>
<td>A/G (2)</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<tr>
<td></td>
<td>453</td>
<td>A</td>
<td>A/G (3)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>489</td>
<td>A/G (5)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>492</td>
<td>A</td>
<td>A/G (3)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>497</td>
<td>G/T (1)</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
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<td>624</td>
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<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>gltA</td>
<td>909</td>
<td>T/C (4)</td>
<td>ND</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>918</td>
<td>T/C (5)</td>
<td>ND</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>936</td>
<td>T/C (3)</td>
<td>ND</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1077</td>
<td>T/C (3)</td>
<td>ND</td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>groEL</td>
<td>861</td>
<td>A/G (4)</td>
<td>ND</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>ribC</td>
<td>147</td>
<td>T/C (3)</td>
<td>ND</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>T/C (2)</td>
<td>ND</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>rpoB</td>
<td>1833</td>
<td>T/C (1)</td>
<td>T/C (2)</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>1854</td>
<td>G/T (3)</td>
<td>G/T (1)</td>
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<td>G</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>G</td>
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<tr>
<td></td>
<td>1894</td>
<td>A/G (4)</td>
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<td>A</td>
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<td>G</td>
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<tr>
<td></td>
<td>1902</td>
<td>T/C (2)</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>1915</td>
<td>A/G (9)</td>
<td>A/G (1)</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>1923</td>
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<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>A</td>
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<tr>
<td></td>
<td>1974</td>
<td>A or G</td>
<td>A/G (2)</td>
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<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>A</td>
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<tr>
<td></td>
<td>2013</td>
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<td>A</td>
<td>A</td>
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<td>A</td>
<td>A</td>
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<tr>
<td></td>
<td>2019</td>
<td>A/G (3)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

*Ambiguous bases within sequenced gene fragments of the examined samples and relevant nucleotides in the type sequences of ruminant-infecting bartonellae. The position in the gene is indicated by nucleotide number and with the number of samples in which they were found. Bold indicates the polymorphic sites of ruminant-infecting Bartonella spp., where the ambiguities in samples from moose occurred. ND, no data.

![FIG 1 Concatenated phylogenetic tree of Bartonella-type isolates and a representative isolate of this study, based on fragments of 3 genes, gltA, rpoB, and ribC (832 bp in total), generated using the maximum likelihood algorithm in PhyML with a GTR substitution model (1,000 replicates; bootstrap values indicated at the nodes).](http://aem.asm.org/Downloaded from http://aem.asm.org/ on October 23, 2017 by guest)
tion would have been expected to denature surviving bacterial DNA on the surface of the cuticle. The most likely explanation is, therefore, that bacteria are transferred from mother to larva in utero, as suggested by Zacharias (28).

Generally, species designation in *Bartonella* has been based on housekeeping gene sequence analysis. La Scola et al. (29) reported that gltA and rpoB were the most appropriate targets for species differentiation in the genus *Bartonella*. Cutoff values of \( \approx 96.0\% \) in gltA and \( \approx 95.4\% \) in rpoB gene sequences were proposed for the designation of novel *Bartonella* species. In the present study, we identified two *Bartonella* lineages, with lineage I showing high similarity to corresponding sequences from *B. chomelii*, *B. schoenbuchensis*, and *B. capreoli*, a clade of species primarily infecting ruminants (8, 30–32). The association between lineage I *Bartonella* infection in unwinged flies and moose exclusively within the deer ked range strongly suggests that deer keds may be potential vectors for the transmission of this *Bartonella* species in the sampled moose population. Lineage II sequences could be clearly differentiated as originating from a distinct *Bartonella* clade based on gltA and other housekeeping genes. Based on the concatenated sequences, sequences from lineage II form a distinct cluster separate from other known *Bartonella* species and therefore appear to constitute a novel, previously undescribed clade. As strains in lineage II were commonly found in the moose population both inside and outside the deer ked range, they may represent a long-standing endemic infection, for which the means of transmission is entirely unknown.

It is unknown whether these two bacterial species are pathogenic in moose. A single report has described *B. bovis*-associated endocarditis in cattle (33), but in another study, no effect of bacteremia was found on milk production or reproduction in cattle (34), suggesting that ruminant-infecting *Bartonella* species are of little clinical importance. In spite of a thorough examination, no pathological lesions associated with *Bartonella* were found in the necropsy cases in our study (17), and no signs of disease were reported in immobilized animals. The observed prevalence of *Bartonella* in moose blood, however, indicates that the detected strains are able to cause a persistent and systemic infection in this cervid host. As chronic, asymptomatic infection with long-term bacteremia is common for *Bartonella* spp. in their reservoir host (35–37), this may suggest that the moose is a primary host. This seems to be comparable with findings in roe deer, where \( \approx 80\% \) of the population was found to be positive for *Bartonella* DNA (8).

Many *Bartonella* spp. are important pathogens causing morbidity or mortality in humans (38–41). While Dehio and coauthors (9) suggested that *B. schoenbuchensis* transmitted with the bites of deer ked may establish a local infection in the skin and thereby contribute to the etiology of deer ked dermatitis, there is limited evidence to support a role for the ruminant-infecting *Bartonella*, such as *B. schoenbuchensis*, *B. capreoli*, *B. melophagi*, or *B. chomelii*, as zoonotic agents. Recently, Maggi and coauthors (42) reported isolation of the closely related “*Candidatus Bartonella melophagia*” from the blood of two diseased women, but no causal relationship between the disease and the infection was proven.

Interestingly, it was suggested that in Sweden, *Bartonella*-induced subacute myocardiitis was the cause of sudden unexpected cardiac death (SUCD) in orienteers (43). PCR amplification of a short fragment of the gltA gene revealed sequences that could be consistent with *B. quintana* or *B. henselae* in samples from five orienteers who had succumbed to SUCD (43). Four of these five cases, as well as two other orienteers with cardiomyopathy and 31.3% of elite orienteers (compared with 6.8% of healthy blood donors), had antibodies against *Bartonellaceae* (43, 44). The specific identity of the *Bartonella* sp. concerned is, however, uncertain, as species determination was based on PCR and sequencing of a short fragment of the gltA gene alone, a gene that is prone to recombination (21). How the Swedish orienteers were exposed to *Bartonella* was not determined, although vector-borne transmission via blood-sucking arthropods was suspected (43). In light of our findings, it is pertinent to mention that the accumulation of SUCD among orienteers coincided with a major increase in the abundance and distribution range of the deer ked in Fennoscandia (2). Given the uncertainty over the identity of the *Bartonella* sp. identified from orienteers, it could be speculated that transmission of *Bartonella* spp. by deer keds, which frequently bite orienteers, may be one of the factors behind the observed high seroprevalence and disease among these sportsmen.

In conclusion, the presented findings show the presence of a potential vector-borne pathogen within a prevalent reservoir host (the moose) and a prevalent and geographically invasive vector which frequently attacks humans and other animals. *Bartonella* infection in the Norwegian moose population involves at least two different clades of *Bartonella*, one potentially transmitted by *Lipoptena*, the other almost certainly not. The high prevalence of infections both inside and outside the deer ked distribution range may suggest transmission of *Bartonella* by different vectors. This warrants further research on Lipoptena cervi and *Bartonella* spp. (45).

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