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Anaplasma phagocytophilum infection in moose (*Alces alces*) in Norway

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Abstract

Anaplasma phagocytophilum is a tick-borne bacterium that infects a wide range of animal species. The aim of our study was to investigate the prevalence of *A. phagocytophilum* in Norwegian moose *Alces alces* and to characterize the bacteria by sequencing of partial *msp*4 and 16S rRNA genes. Hunters collected spleen samples from 99 moose of different ages during 2013 and 2014 in two areas: Aust-Agder County (n = 70) where *Ixodes ricinus* ticks are abundant and Oppland County (n = 29) where ticks were either absent, or abundance very low. *A. phagocytophilum* was detected only in moose from the *I. ricinus* - abundant area. The overall prevalence of infection according to 16S rRNA and *msp4* gene-based PCR was 41.4% and 31.4% respectively. Sequence analysis of the partial 16S rRNA and *msp4* gene revealed two and eight different sequence types respectively. Four of eight *msp4* sequence types determined in this study were unique, while others were identical to sequences derived from other ruminants and ticks. The present study indicates that moose could be a potential wildlife reservoir of *A. phagocytophilum* in Norway.

Keywords: *Anaplasma phagocytophilum*; Moose; *Alces alces*; *msp4*; 16S rRNA; Norway

1. Introduction

During the past decades, the distribution and abundance of *Ixodes ricinus* ticks have increased in Europe due to changes in climate and the distribution of tick hosts, particularly roe deer and other cervids, due to changes in land management, and from other anthropogenically induced changes [1]. Norway represents the northern limit in the geographical distribution *I. ricinus*, and Jore et al. [2] indicated an expansion of its range further north and at higher altitudes. This could affect the spread of infections such as tick-born encephalitis, Lyme borreliosis, anaplasmosis, and babesiosis into new territories and increase the risk of tick-borne infections. Hosts of the adult ticks are large vertebrates including wild cervids. This may lead to high density of ticks in the areas with established populations of moose *(Alces alces)*, roe deer *(Capreolus capreolus)* and red deer *(Cervus elaphus)* [3].

Tick-borne fever caused by *Anaplasma phagcytophilum* is a well-known disease of domestic animals in several countries in Europe, Asia, and America [4,5]. The disease is characterized by infected neutrophils, high fever, neutropenia, reduced milk yield, abortion and reduced fertility in domestic sheep [6]. Besides domestic ruminants [7], *Anaplasma phagocytophilum* has been identified in roe deer [8,9], red deer [10], moose [11] and humans [12]. Several studies have reported seroprevalence in moose and other ruminants in Norway [13,14]. Different genetic variants of *A. phagocytophilum* have been described in Norwegian red deer and sheep based on 16S rRNA and *msp4* genes [15]. However, the prevalence and genetic diversity of *A. phagocytophilum* in moose populations based on PCR-based data has not been previously reported.

The aim of our study was to investigate the prevalence of *A. phagocytophilum* in the moose and characterize the bacteria by partial sequencing of the *msp*4 and 16S rRNA genes.

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2. Materials and methods

2.1. Collection of samples

Hunters collected spleen samples from 99 moose of different ages (calves ≤1 year, n = 35; older animals ≥1 year, n = 49 and animals of unknown age, n = 15) from two areas in Norway in 2013 and 2014. All spleens were collected during the moose-hunting season from 25 September - 15 November. Study area 1 (n = 70 animals) was located in eight municipalities in Aust-Agder County in southern Norway and study area 2 (n = 29 animals) in Øystre Slidre municipality, Oppland County, south-central Norway (Fig. 1). Study area 1 was located within the boreonemoral zone characterized by heterogeneous landscapes. The forest here consists of young, medium and old-aged coniferous, deciduous and mixed stands. Area 2, situated at higher altitude and latitude, consists mostly of boreal forest primarily in the younger successional stages [16]. In area 1, *I. ricinus* are abundant during spring-autumn while in area 2 ticks are either absent or rarely observed [2].

2.2. Molecular detection of A. phagocytophilum

DNA from the spleens was extracted with the Genomic DNA Purification Kit (Thermo Fisher Scientific Baltics, Lithuania) according to the manufacturers' instructions. *A. phagocytophilum* was detected using nested-PCR to specifically amplify 381 bp fragment of the *msp4* gene and 546 bp fragment of the 16S rRNA gene [17,18]. A positive DNA sample for *A. phagocytophilum* (JN181111) was included as a positive control in each PCR. Negative controls (distilled water) were added to the first PCR after every five experimental samples. To prevent false-positive results, separate rooms were used for DNA extraction, PCR mix preparation and PCR reactions.

2.3. Sequencing and phylogenetic analysis

PCR products were separated by electrophoresis on 1.5% agarose gel and visualized under ultraviolet light. Positive samples from gel were purified using GeneJET Gel Extraction Kit (Thermo Fisher Scientific Baltics, Lithuania). The *msp4*

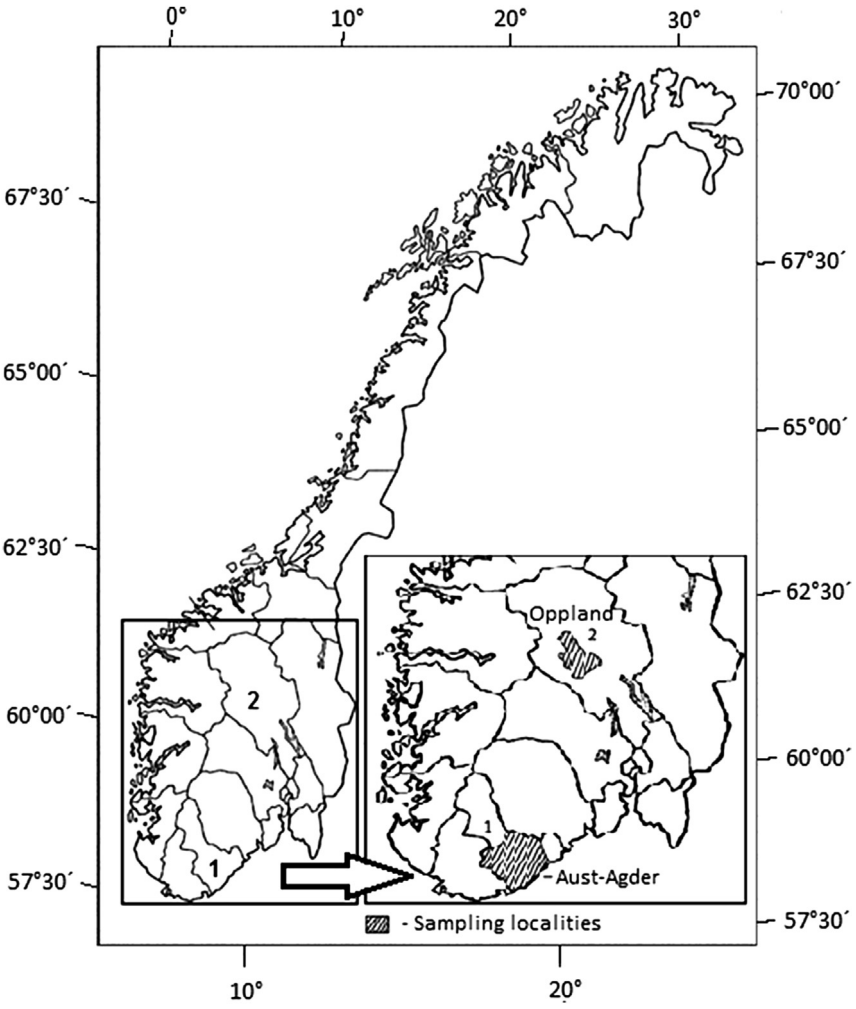

Fig. 1. Samples collecting places. Study area 1 was located in 8 municipalities (Grimstad, Arendal, Vegårdshei, Evje og Hornes, Tvedestrand, Froland, Lillesand, Birkenes) in Aust-Agder County and study area 2 in Øystre Slidre municipality, Oppland County.