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The effect of lamb age to a natural *Anaplasma phagocytophilum* infection

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ABSTRACT

Tick-borne fever (TBF) is caused by the bacterium *Anaplasma phagocytophilum* that is transmitted by the tick *Ixodes ricinus*, which is a major challenge in sheep farming along the coast of south-western Norway. Few efficient and sustainable preventive measures are available, but older lambs seem to be more susceptible than younger lambs to an *A. phagocytophilum* infection. A field experiment was carried out in 2008 and 2009 on two sheep farms with the breed Norwegian White Sheep (NWS). Three treatment groups of lambs on each farm and year were established: 1; lambs ≥3 weeks old when turned out to pasture and born in early spring, 2; lambs ≤1 week old when turned out to pasture and born in late spring, 3; lambs ≥3 weeks old when turned out to pasture and born in late spring. The study included a total of 337 lambs distributed to treatment group 1, 2 and 3 with 116, 111 and 110 lambs respectively. Recordings of tick-counts, rectal temperature, clinical symptoms and mortality together with weight, blood serology and blood smears were used to analyze the effect of age of lambs to a natural *A. phagocytohpilum* infection. Gompertz weight curve parameters were estimated for all lambs and were used to compare weight gain in lambs between the treatment groups. There were observations of tick-bites, clinical disease (including fever) and mortality, but no significant effect of lamb age to a natural *A. phagocytophilum* infection was detected. However, lambs infected with *A. phagocytophilum* in group 2 had higher ($P < 0.05$) maximum spring growth rate (358 g/day) than infected lambs in group 1 (334 g/day) and group 3 (310 g/day). Further, lambs not infected with *A. phagocytophilum* showed no weight differences ($P < 0.05$) between treatment group 2, compared to groups 1 and 3. Pasturing of ≤1 week old lambs on tick-infested pastures in tick endemic areas, can therefore be recommended to prevent weight loss due to an *A. phagocytophilum* infection. Note should however be taken on annual and seasonal variations in tick activity relative to lambing, different genetic variants of *A. phagocytophilum* involved and turnout time as these factors probably will influence the effect of pasturing young lambs.

1. Introduction

A main scourge in Norwegian sheep farming is tick-borne fever (TBF) caused by the bacterium *Anaplasma phagocytophilum* that is transmitted by the tick *Ixodes ricinus* (Stuen, 2003). The normal distribution area of *I. ricinus* ticks is the coastal areas of Norway from the southeast to latitudes of approximately 69° (Jore et al., 2011; Mehl,

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1983). *A. phagocytophilum* infected lambs are commonly found in tick endemic areas (Stuen and Bergstrom, 2001a). The tick population is expected to increase influenced by climate change (i.e. warmer winter climate), changes in land use (i.e. bush encroachment) and an increase in the deer population (Jaenson and Lindgren, 2011; Jore et al., 2011; Gray et al., 2009; Stafford, 1994).

The main consequence of an *A. phagocytophilum* infection in sheep is the ensuing immunosuppression that may lead to secondary infections. Direct loss of more than 30% of lambs in one flock due to *A. phagocytophilum* and secondary infections has been observed (Stuen and Kjølleberg, 2000). Indirect loss, measured as reduced weight gain in infected lambs compared to non-infected lambs, has also been observed (Stuen et al., 2002; Grøva et al., 2011). Impaired growth as a consequence of experimental infection with *A. phagocytophilum* has been shown to last for several months after the primary infection (Stuen et al., 1992). Several genetic variants of *A. phagocytophilum* have been observed and these variants may cause different clinical symptoms with varying hematological and serological responses (Stuen et al., 2003; Granquist et al., 2010). However, it is mainly the primary *A. phagocytophilum* infection in sheep that is associated with disease problems (Øverås, 1962; Woldehiwet, 1983).

There are few efficient preventive measures against tick infestation and TBF. General advice is to clear vegetation, drain land, treat lambs regularly with acaricides and infect lambs early (i.e. when they are young) (Stuen et al., 1992; Sonenshine, 1993; Vatn et al., 2008). However, there is concern about lack of efficient and feasible preventive measures against TBF among sheep farmers in areas where *I. ricinus* is abundant.

Experimental infection studies have shown that clinical response to *A. phagocytophilum* is less severe in young lambs compared to older lambs (Stuen et al., 1992; Stuen, 1993). This difference in response is explained by innate resistance to *A. phagocytophilum* infection (Stuen et al., 1992). When re-infected, the clinical response and symptoms are normally less severe than after primary infections (Øverås, 1962; Stuen et al., 2003). However, this may depend on the genetic variant of *A. phagocytophilum* involved (Stuen et al., 2003). Thus, it may be hypothesized that lambs turned out to tick-infested pastures within the first week after birth will perform better than lambs being more than 3 weeks old at turn out.

The objective of the present study was to reveal possible effects on performance when turning young lambs, i.e. ≤1 week old compared to older lambs i.e. ≥3 weeks old, on to tick-infested pastures.

2. Methods

2.1. Study groups and treatment

A field study was conducted in 2008 and 2009 on two sheep farms in tick endemic areas where ticks and losses to TBF have previously been observed. The farms were located on the south west coast of Norway in Sandnes municipality (58°53′ N, 6°0′E) (farm A) and on the west coast of Norway in Tingvoll municipality (62°60′N, 8°15′E) (farm B). Spring pastures in this study were permanently fenced pastures close to the farm dominated by open grassland, including areas with bush vegetation, all within an altitudinal range of 0–100 m above sea level (masl). Lambs were expected to be exposed to ticks at turnout on these farms. In each year and on each farm the following three treatment groups were established with respect to age of lambs at time of pasturing and time of birth:

- 1: Lambs ≥3 weeks old when turned out to pasture and born in early spring.
- 2: Lambs ≤1 week old when turned out to pasture and born in late spring.
- 3: Lambs ≥3 weeks old when turned out to pasture and born in late spring.

The number of lambs included in the study in 2008 and 2009, per treatment group 1, 2 and 3 presented in parentheses respectively, was 88 (30, 30, 28) and 90 (30, 30, 30) on farm A and 77 (30, 23, 24) and 82 (26, 28, 28) on farm B (Table 1). The total number of lambs in treatment group 1, 2 and 3 was 116, 111 and 110 respectively (Table 1).

To ensure equal conditions for tick infestation, treatment group 1 and 2 were turned on to pasture at the same point of time. Consequently, these treatment groups differed in time of birth. In order to correct for a possible effect of time of birth we established treatment group 3, allowing for comparison between different times of birth (early and late spring). All lambs in this study were born in spring between 31st of March and 8th of May. The mean time of birth for lambs born in early spring in 2008 and 2009 was 7th of April (±1.6 days) and 8th of April (±5.0 days), respectively. The mean time of birth for lambs born in late spring in 2008 and 2009 was 19th of April (±4.6 days) and 20th of April (±7.4 days), respectively. The mean number of grazing days (SD) on spring pasture for treatment group 1, 2 and 3 was 38 (±6), 43 (±6) and 32 (±5) respectively.

The number of lambs per ewe was assessed by embryo screening to assist selection and allocation of ewes to the treatment groups. The three treatment groups were made as equal as possible with respect to age and number of lambs per ewe.

The ewes in this study were fed differently after lambing; ewes in treatment group 2 were fed indoors for about 1 week before they were turned out on spring pasture, while ewes in treatment group 1 and 3 were fed indoors for about 3 weeks. Indoor feeding consisted of hay, silage and concentrates.

All lambs were treated against coccidian infection (Baycox Sheep vet., Bayer Animal Health GmbH) 1 week after pasturing. Gastrointestinal (GI) parasites in lambs were regularly monitored by fecal egg counts. Preventive treatment against GI parasites (Valbazen vet., Pfizer) was conducted on all ewes before lambing and regularly every 4 weeks on lambs on pasture during the spring grazing period. Lambs were not treated with acaricides in this study. Permission from the Norwegian Animal Research Authority was obtained.

2.2. Recordings and blood sampling

Recordings of tick-bites, clinical signs of disease (including measurement of rectal temperature), mortality and weight were conducted every second week, making a total of four to five observations per lamb during the spring grazing period. In addition, mortality and weaning weight were recorded at the end of the summer grazing period (average age 136 days ± 18)

Tick-bites were registered by observing the head, flank and abdomen for attached ticks. In the statistical analyses it was treated as a binary observation of presence of ticks or not. Clinical signs of disease were registered by a veterinarian. The registration of rectal temperature was used as a binary observation of fever (≥40.5 °C) or no fever (<40.5 °C) in the statistical analysis. The observation of other clinical signs of disease apart from fever was used as binary observation of other clinical signs of disease or no disease. Mortality was registered both at the end of the spring grazing period and at the end of the summer grazing period. In the statistical analysis, the observation of mortality was used as a binary observation of dead or alive. A routine autopsy mortem was conducted on dead lambs that were retrieved.

Whole blood samples of all lambs were collected three times; during the first week after birth, at 8 weeks of age (average 56 days (±7)) and at 4.5 months of age (average 136 days (±18)). Hereafter, the antibodies detected at each sampling time are referred to as maternal, spring and autumn antibodies, respectively.

EDTA blood was sampled from all lambs one time at 8 weeks of age for blood smear preparation. In addition, whole blood and EDTA blood was sampled from all lambs with a temperature ≥41 °C and/or showing