Sheep as Sentinels for the Geographic Distribution of Anaplasma phagocytophilum in Denmark?

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Publication date: 2017

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Citation for published version (APA):
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Introduction

*Anaplasma phagocytophilum* can cause Human Granulocytic Anaplasmosis (HGA). *A. phagocytophilum* is known to be widely distributed in Danish *Ixodes ricinus* ticks, and in different mammal species such as humans, roe deer, sheep, horses, cats and dogs (1-5).

Free ranging roe deer are considered a good sentinel for *A. phagocytophilum* infection with a seroprevalence as high as 95.6% and a PCR positivity of 42.6% (3).

Since sheep are much easier to handle than roe deer, this study was done to clarify, if sheep can be used as sentinels for *A. phagocytophilum* in Denmark. The objective of this study was to clarify if sheep can be used as sentinels for monitoring the geographical distribution of *Anaplasma phagocytophilum* in Denmark.

Method

**Sheep:** Blood was sampled from 315 clinically healthy sheep from May to June 2014. Twenty-four sheep farms distributed in DK were included (Figure 1). Age, sex, breed, grazing habitat and estimated tick exposure were noted. The plasma were tested for the presence of *A. phagocytophilum* IgG antibodies using a modified commercial indirect immunofluorescence assay (Focus Diagnostics, California, USA), replacing the conjugate with diluted (1:10) FITC-Labeled Antibody to Sheep IgG (H+L) (SeraCare, KPL Antibodies and Reagents, Gaithersburg, USA).

The plasma and buffy coat fractions were tested with a real-time PCR assay specific to *A. phagocytophilum* (6).

**Roe deer:** The plasma and buffy coat fractions from 180 roe deer, killed in the hunting season of 2013-2014, were tested for the presence of *A. phagocytophilum* DNA by real-time PCR.

Samples with no signal in the *A. phagocytophilum* PCR assay were excluded, if the internal control was negative.

Results

- For results of PCR and serology look to table 1 and 2, and figure 1.
- The bacterial load as estimated by the *A. phagocytophilum* DNA level was highest in the buffy coat fraction compared to the plasma fraction.

### Table 1: The percentage of sheep and roe deer samples positive for *A. phagocytophilum* DNA or IgG antibodies determined by real-time PCR or an indirect immunofluorescence assay, respectively.

<table>
<thead>
<tr>
<th>Sentinel</th>
<th>PCR (Buffy coat)</th>
<th>PCR (Plasma)</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>14% (45/315)</td>
<td>7% (22/315)</td>
<td>47.6% (150/315)</td>
</tr>
<tr>
<td>Roe deer</td>
<td>93.3% (169/180)</td>
<td>Not determined</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

### Table 2: The percentage of sheep samples in nature reserves or grasslands positive for *A. phagocytophilum* DNA or IgG antibodies determined by real-time PCR or an indirect immunofluorescence assay, respectively.

<table>
<thead>
<tr>
<th>Sentinel</th>
<th>Habitat</th>
<th>PCR (Buffycoat + Plasma)</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>Nature reserves</td>
<td>21.1% (42/199)</td>
<td>60.3% (120/199)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Grasslands – permanent or crop rotation</td>
<td>1.8% (3/166)</td>
<td>25.9% (30/116)</td>
</tr>
</tbody>
</table>

Conclusion

- Roe deer serves as the best sentinel of *A. phagocytophilum* when using real-time PCR.
- The prevalence of *A. phagocytophilum* DNA in sheep grazing nature reserves might be higher, if sampling is done in August-October.
- Using the buffy coat fraction increases the sensitivity of the *A. phagocytophilum* real-time PCR assay.
- The percentage of roe deer positive for *A. phagocytophilum* DNA has increased from 43% (3) to 94% within the last decade. It is unknown if this tendency is reflected in humans.

Support: The work was funded by grants from TS. Juri Fönden, Læge Else Poulserens Mindelegat, the Region of Southern Denmark, Odense University Hospital and the University of Southern Denmark.

REFERENCES