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

Larsen SL1,2, Andersen NS1,2, Hansen SG3, Skov MN1,2, Jensen PM4, Kemp M1,2, Jensen TG1,2, Thamsborg SM3, Skarpheðinsson S1,5

1: CCEVI - Clinical Center for Emerging and Vectorborne Infectious, Odense University Hospital, Denmark.
2: Department of Clinical Microbiology, Odense University Hospital, Denmark.
3: Veterinary Parasitology, Department of Veterinary Disease Biology, University of Copenhagen.
4: Department of Plant- and Environmental Sciences, University of Copenhagen.
5: Department of Infectious Medicine, Odense University Hospital.

**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 of *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 habit 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>PCR (Buffycat + Plasma)</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>21.1% (42/199)</td>
<td>60.3% (120/199)</td>
</tr>
<tr>
<td>Sheep</td>
<td>1.8% (3/166)</td>
<td>25.9% (30/116)</td>
</tr>
</tbody>
</table>

**Figure 1:** The map shows the habitat and the locations of the sheep farms, and the areas in which the roe deer are sampled. The boxes indicate the number of sheep positive for *A. phagocytophilum* DNA of all tested in the particular farm.

**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. Jüni Fonden, Læge Else Poulsens Mindelegat, the Region of Southern Denmark, Odense University Hospital and the University of Southern Denmark.

**References**