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

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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 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 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>PCR (Buffycat + Plasma)</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>Human resource reserves</td>
<td>21.1% (42/199)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Grasslands – permanent or crop rotation</td>
<td>1.8% (3/166)</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.

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REFERENCES