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

Larsen, Sanne Løkkegaard; Andersen, Nanna Skaarup; Hansen, Signe Grave; Skov, Marianne Nielsine; Moestrup Jensen, Per; Kemp, Michael; Jensen, Thøger Gorm; Thamsborg, S. M.; Skarphédinsson, Sigurdur

Publication date: 2017

Citation for published version (APA):
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 SM5, Skarpheidinsson S1,5

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% (5).

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).

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 Anaplasma 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 (Buffycoat + 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>

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

References