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.; Skarphedinson, Sigurdur

Publication date:
2017

Document license
Unspecified

Citation for published version (APA):

Terms of use
This work is brought to you by the University of Southern Denmark through the SDU Research Portal. Unless otherwise specified it has been shared according to the terms for self-archiving.
If no other license is stated, these terms apply:
• You may download this work for personal use only.
• You may not further distribute the material or use it for any profit-making activity or commercial gain.
• You may freely distribute the URL identifying this open access version.
If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 10. Jan. 2020
Sheep as Sentinels for the Geographic Distribution of *Anaplasma phagocytophilum* in Denmark?

Larsen SL, Andersen NS, Hansen SG, Skov MN, Jensen PM, Kemp M, Jensen TG, Thamsborg SM, Skarpödinssson S

1. CCEVI - Clinical Center for Emerging and Vectorborne Infections, 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 95.8% 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.

**Sentinel**

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

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.

**Sentinel**

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

**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.

**REFERENCES**


**Support:** The work was funded by grants from T5, Juri Fonden, Læge Else Poulsen Mindelegat, the Region of Southern Denmark, Odense University Hospital and the University of Southern Denmark.

**CCEVI – Clinical Center for Emerging and Vectorborne Infections**