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Publication date:
2015

Document version
Accepted author manuscript

Citation for published version (APA):

Andersen, N. S., Hansen, S. G., Moestrup Jensen, P., Thamsborg, S. M., & Skarphédinson, S. (2015). Seroprevalence of *Anaplasma phagocytophilum* in Danish Sheep. Poster session presented at International Conference on Lyme Borreliosis and other Tick-Borne Diseases, Wien, Austria.

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Seroprevalence of *Anaplasma phagocytophilum* in Danish Sheep

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Objective

The objective of this study was to determine the seroprevalence of *Anaplasma phagocytophilum* within the Danish sheep population and to evaluate possible risk factors.

Introduction

Anaplasma phagocytophilum can cause Tick-borne fever (TBF) in ruminants. *A. phagocytophilum* reduces the profitability of the livestock industry due to direct disease costs related to mortality, arthritis, and abortion, and indirect losses caused by immunosuppression, loss in milk-yield and infertility in rams. In Norway, Denmark's northern neighbour, financial losses were estimated to be more than 3 million Euro annually due to indirect losses from TBF infections in sheep (1).

A. phagocytophilum is known to be widely distributed in Danish *Ixodes ricinus* ticks (2). Studies have demonstrated *A. phagocytophilum* in different mammal species such as humans, roe deer, horses, cats and dogs (3-6). TBF is not a condition given much attention in Denmark and only one study has shown the occurrence of the bacterium in sheep therefore its geographical distribution is presently unknown (7).

Method

Blood was sampled from 406 clinically healthy older sheep encompassing 26 sheep farms (Figure 1). Age, sex, breed and grazing habitat were noted. The samples were all tested for the presence of *A. phagocytophilum* antibodies using a modified commercial indirect immunofluorescence assay test – IFA (Focus Diagnostics, California, USA), replacing the conjugate with diluted (1:10) FITC-Labeled Antibody To Sheep IgG (H+L) produced in Rabbit (SeraCare, KPL Antibodies and Reagents, Gaithersburg, USA) (Figure 2). Potential risk factors were evaluated for their statistical association with *A. phagocytophilum* prevalence in sheep by logistic regression using R.

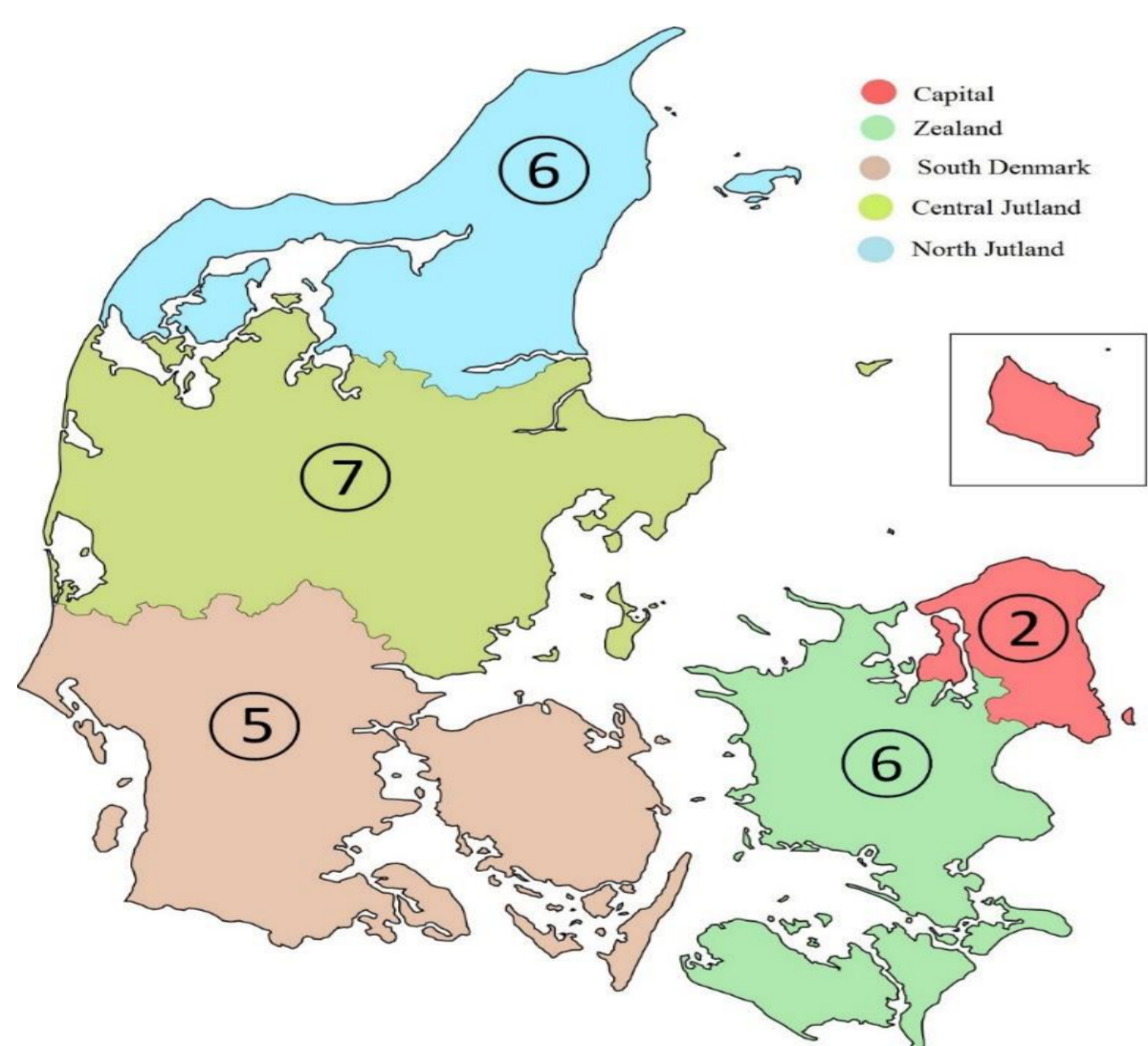


Figure 1: The map shows the number of sheep farms sampled within each region of Denmark

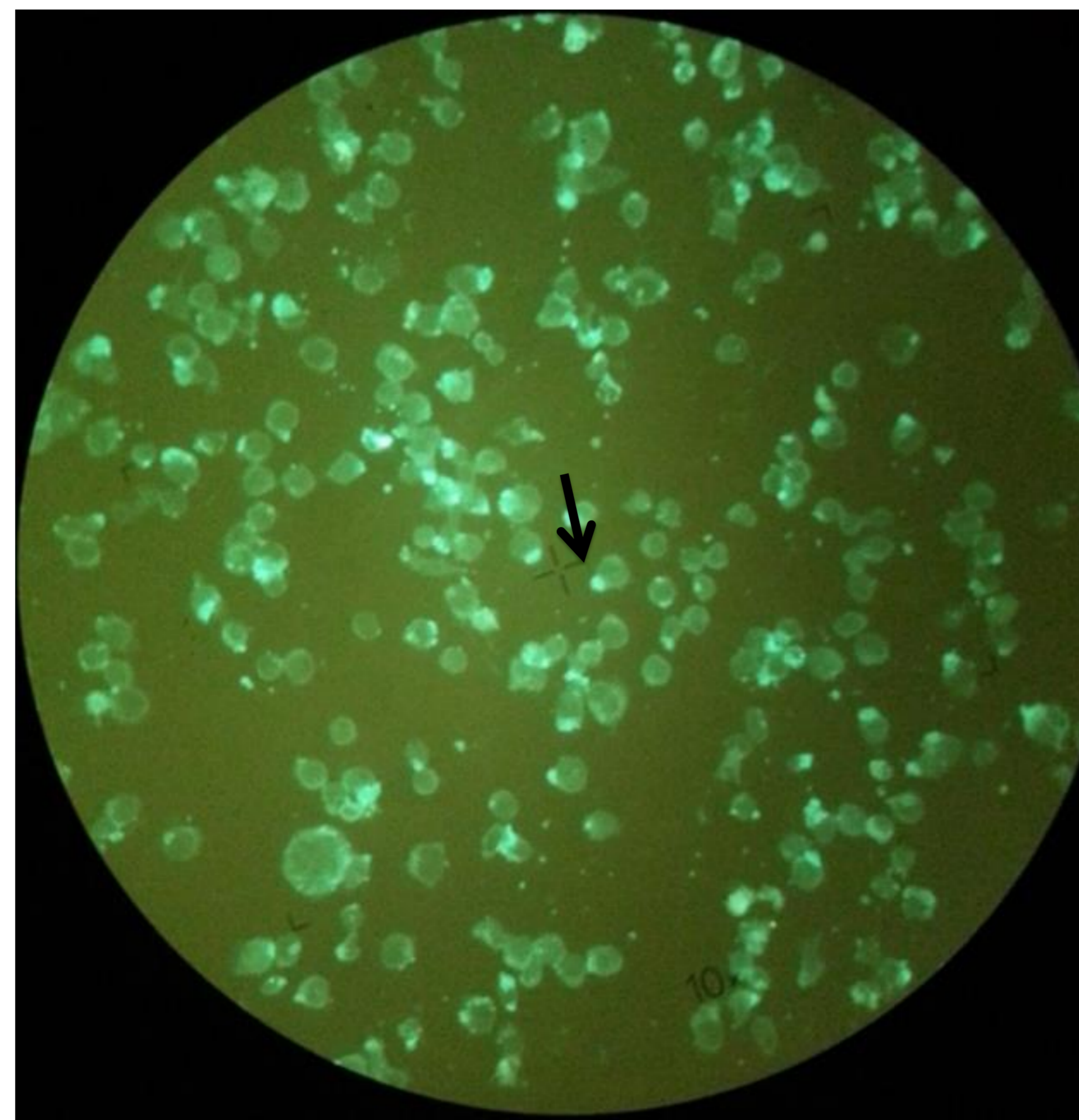


Figure 2: Sheep serum positive for *A. phagocytophilum* IgG anti-bodies on an IFA slide. Apple-green fluorescence of *Anaplasma* inclusion bodies/morula are located focally in the cytoplasm of the granulocytes (arrow).

Results

A total of 205 blood samples were screened positive for IgG antibodies against *A. phagocytophilum*, giving an overall seroprevalence of 50.5% (205/406) in sheep across all regions throughout the country, Table 1. The risk factors habitat and age had significant association with *A. phagocytophilum* prevalence as more infections were seen in sheep grazing nature reserves (mixed vegetation) and in sheep 4 years or older, Table 2. This corresponds well with other studies. The habitat is essential for the survival of ticks and older sheep are more likely to have been exposed to ticks.

Risk factors	Level	P-value	Odds ratio	95%-confidence interval
Habitat	Nature reserves, predominantly dry (heather or moorlands)	0.011	30.24	3.23 – 283.47
	Nature reserves, predominantly wet (marsh, riparian, meadows etc.)		19.04	1.47 – 246.72
	Grasslands – permanent or within crop rotation		1	
Age	1 year	0.035	1.14	0.34 – 3.84
	2-3 years		1	
	≥ 4 years		2.64	1.20 – 5.79

Table 2: The association between *A. phagocytophilum* prevalence (positive/negative by IFA) in the Danish sheep population and the risk factors habitat and age which has significant bearing on individual animal level.

Age	No. of sheep (n)	No. of seropositive sheep (n)	Seroprevalence within an age group (%)
1 year	95	43	45
2-3 year	150	76	51
≥ 4 years	161	86	53
All age groups	406	205	51

Table 1: Shows the number of sheep (n), the number of *A. phagocytophilum* seropositive sheep and the prevalence percentage of *A. phagocytophilum* within each age group.

Conclusions

This is the first study that demonstrates that antibodies against *A. phagocytophilum* is common in Danish sheep. The unawareness of TBF in the sheep industry may lead to indirect losses due to, immunosuppression, arthritis, loss in milk-yield, abortion and infertility in rams, or to direct losses in term of mortality.

Many Danish sheep are used on nature reserves to keep the vegetation down. However, in order to keep vegetation down to a point where it is a hindrance for tick survival, one would need enough sheep to accomplish this. If not the sheep can serve as a driver of the tick population and hence tickborne pathogens such as *A. phagocytophilum*. This could have implications on the health of both humans and animals in Denmark. The extent of this is still unknown.

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SUPPORT:

The work was founded by grants from Odense University Hospital, Denmark.



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