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Förekomst av infektion med *Anaplasma phagocytophilum* hos älgpopulationen (*Alces alces*) på Öland

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**Evidence of *Anaplasma phagocytophilum*
infection in an island population of
Scandinavian moose (*Alces alces*) in the
South-eastern Sweden**

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Abstract

In the second half of the 1990's the moose population on the island Öland declined drastically and the hunters decided to completely stop hunting for moose for a number of years. When the hunting commenced the hunters found the slaughter weights to be lower than expected and the number of calves were also low. The hunters then contacted the Swedish National Veterinary Institute who then initiated a project which this report is a part of.

Samples from a total of 40 moose was collected, 32 out of 33 sampled moose were positive for antibodies against *Anaplasma phagocytophilum* and 4 out of 36 samples were positive by PCR for *Anaplasma phagocytophilum*. Out of these three were calves and one was a female yearling.

Reported slaughter weights for calves were low and the number of observed calves per adult female were half compared to the mainland. This survey indicates that infection with *Anaplasma phagocytophilum* constitutes a contributing cause for the low number of observed calves in the moose population on the island of Öland.

Sammanfattning

Under senare delen av 90-talet minskade älgstammen på Öland drastiskt och jägarna valde då gemensamt att helt avstå jakt under ett antal år. När jakten återupptogs upplevde jägarna att kalvarna var få till antalet och hade låga slaktvikter. De kontaktade då SVA som inledde ett projekt med omfattande provtagning. Detta arbete är en del av detta projekt.

Prover från totalt 40 älgar samlades in, 32 av 33 provtagna älgar visade på antikroppar mot *Anaplasma phagocytophilum* medan 4 av 36 provtagna var PCR-positiva för *Anaplasma phagocytophilum*. Av dessa var tre kalvar och en var en kviga.

De slaktvikter som rapporterades in för kalv visade på låga siffror och antalet observerade kalvar per vuxet hondjur var hälften av vad som observerats på fastlandet. Denna undersökning pekar på att infektion med kan vara en bidragande orsak till det låga antalet observerade kalvar hos älgpopulationen på Öland.

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INTRODUCTION

The island of Öland is located off the south east coast of Sweden and covers 1 342 km². See fig 1. The moose (*Alces alces*) population on the island reached its peak in the mid-eighties, and has since declined. In the late 1990's, the number of culled calves per culled adult declined rapidly, and in the hunting seasons of 1999-2000 and 2000-2001, less than 10% of culled moose were calves. (Swedish Association for Hunting and Wildlife Management 2009) In 2002, the hunters on Öland, decided to stop culling moose for some time to give the population, which was in a poor state at the time, a chance to recover. The main objective for the pause in moose culling was to increase the mean age of the adult moose population. The majority of the bulls observed on the island were young, had small antlers, and with this a questionable ability to cover females. In the fall of 2006, the hunters started culling moose again but now limiting the hunting to just calves. No more than 16 calves were to be culled.

Fig 1. Map of Sweden, Öland highlighted in black.



During the hunting season a total of 11 calves were culled and the hunters claimed to have observed fewer calves than expected. In addition, several adult moose were observed, which gave a low number of calves per observed female. The hunters claimed that the slaughter weights of the calves culled were lower than expected. In addition, calves observed before the pause in hunting had been notably larger. Unfortunately, no data of the individual slaughter weights were recorded.

In the summer of 2005, a moose calf was submitted to the National Veterinary Institute in Uppsala for a post-mortem examination. The report stated infection with *Anaplasma phagocytophilum* (*A. phagocytophilum*) (Ågren 2005) the same agent that causes Tick-borne fever (Foggie 1951).

After the poor result of the hunting season of 2006, the hunters on Öland became very concerned and contacted the National Veterinary Institute (SVA), to ask for help to find a reason for the low numbers of calves, and the apparent low slaughter weights.

Literature review

Tick-borne fever, (TBF) is a well known vector-borne disease in veterinary medicine primarily affecting cattle, sheep, horses and goats. TBF was first described in cattle and sheep in 1932 in Scotland (Gordon et al., 1932). The common tick (*Ixodes ricinus*), was in 1933 identified as the main vector for TBF (MacLeod 1933). In 1951, Foggie determined that the causative agent for TBF was a rickettsiae then called *Rickettsia phagocytophila*. (Foggie 1951) In humans, the disease is known as human granulocytic anaplasmosis (HGA), which is characterized by acute onset of fever accompanied by headache, myalgia and pancytopenia. In bloodsmears granulocytic cytoplasmic morulae is seen and the infective agent was in 1994, through PCR analysis, shown to have close resemblance to bacteria then called *Ehrlichia phagocytophila* and *Ehrlichia equi*. (Chen et al., 1994)

Ehrlichia equi, the human granulocytic anaplasmosis agent (HGA agent), and *Ehrlichia phagocytophila*, are all rickettsiae. Rickettsiae are obligate intracellular bacteria that infect neutrophils. These antigens is today categorised as one species - *Anaplasma phagocytophilum* (*A. phagocytophilum*) (Dumler et al., 2001).

A study in the United Kingdom suggests the roe deer (*Capreolus capreolus*) as a reservoir and a natural host for *A. phagocytophilum*. (Alberdi et al., 2000). Other studies suggest that small rodents such as bank voles (*Clethrionomys glareolus*) and yellow-necked mouse (*Apodemus flavicollis*) are acting as reservoirs in the U.K. and mainland Europe. (Liz et al., 2000, Ogden et al., 1998)

In Norway a moose calf that had died of a *Klebsiella pneumoniae* septicaemia was found to be heavily infested with *I. ricinus* ticks and positive for *E. phagocytophila* in both blood smears and through PCR examination. (Jenkins et al., 2001) In the literature this was the first report of TBF recorded in moose.

A recent Norwegian study showed that lambs infected with *A. phagocytophilum* had a significantly reduced growth compared with uninfected lambs when kept on a pasture with a low number of ticks and where no cases of TBF had been reported. (Stuen et al., 2002)

In other animals there are reports of various clinical signs related to infection with *A. phagocytophilum*. In the early 1960's a team of researchers in Scotland reported a high prevalence of abortion in beef cattle heifers let out in calf on a tick infested pasture. All heifers were in the last trimester of pregnancy when they aborted. A high prevalence of stillbirths and weak borne calves was also noted. (Wilson J. C. et al., 1964)

It has been shown that *A. phagocytophilum* acts as an immunosuppressant were the effects of a secondary infection with another infectious agent are aggravated when occurring simultaneously with *A. phagocytophilum*. Clear examples of this has been infection with *Pasturella haemolytica* causing severe pneumonia and septicaemia and co-infection with *Staphylococcus aureus* giving rise to the common tick pyemia seen in sheep flocks in the hill pastures of the United Kingdom. (McEwen 1947, Brodie et al., 1986)

Study aim

The observed number of calves in the fall in the moose population on Öland is low according to the hunters and I intend to see if this statement is true and if so, find the reason for this decline. My hypothesis is that infection with *A. phagocytophilum* in moose cows can reduce fertility and/or cause abortions. In addition, newborn calves infected soon after birth can presumably die acutely, or recover with a loss of growth as a consequence.

The aim of this study was to investigate to which extent the moose population is, or have been infected with *A. phagocytophilum*.

MATERIALS AND METHODS

Hunters observations

Hunters throughout Sweden are yearly encouraged to report their observations of moose during the first 7 days of hunting every hunting season. At the same time the number of participants in the hunt and how many hours they spent out hunting is noted. These hunter observations is considered a good surveillance technique for discovering trends in the moose population and for monitoring reproduction over time (Ericsson & Wallin 1999).

Sampling

The hunting parties participating to the study cover almost 900 km² of the island (Johansson 2007. Öland has some urban and suburban areas which are not suitable for moose hunting. Taking this in to account almost all of Öland is covered by the hunting parties contributing to this study.

The hunting season on Öland started on November 2, 2007. Well in advance personnel from SVA had given instructions to the shooters on how to collect samples and had also distributed the necessary equipment for sampling. All shoot moose during the whole shooting season were sampled. Furthermore, personnel from SVA were present during the first days of the shooting season to assist in the collection of samples.

Forty moose were sampled in this study. Thirty-one samples came from moose culled during the hunting season of 2007, and nine samples came from animals that had died in traffic accidents, or culled for other reasons. Samples were collected between the June 13, 2007 and July 29, 2008. Since animals sampled were shot during the traditional shooting season, animals shot and sampled were taken at random from the population.

Moose blood was collected in serum and EDTA tubes (BD Vacutainer Systems, Plymouth, UK), a palm sized portion of the spleen was taken and, when present, ticks were collected from the skin. Slaughter weights were recorded and reported by the hunters.

Serology

Sera were used to analyse the presence of antibodies against *A. phagocytophilum*. An immunofluorescence assay (IFA) was used. Two-fold dilutions of fresh sera were added to slides precoated with *E. equi* antigen (Cappel; Organon Teknika, West Chester, Pa. USA). To visualize the bound antibodies, a fluorescein isothiocyanate (FITC)-conjugated rabbit anti-horse immunoglobuline was added and slides were examined by fluorescence microscopy. An IFA titer $\geq 1:40$ was considered positive. The test is accredited according to the standard EN ISO/IEC 17025 with inclusion of positive and negative controls. The limit for seropositivity was originally set by calibrating the test according to results obtained from external laboratories. (Franzén et al 2005)

PCR

Materials from the spleens collected were used as a source for DNA for PCR analysis. For the PCR, the primers used were GER3(TAGATCCTTCTTAACGGAAGGGCG) and GER4 (AAGTGCCCGGCTTAACCCGCTGGC) (Thermo Scientific Biopolymers Webshop, Waltham, Mass. USA). Amplification of Ap 16S rRNA was carried out in a 50 μ L reaction mixture of 50mM KCl, 10mM Tris-HCl (pH 8.3), 4mM MgCl₂, 0.2mM each of deoxynucleoside triphosphates, 0.25 mM of each primer and 1U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA. USA) and 10 μ L of extracted DNA as a template. The reaction mixture was subjected to amplification reaction was started by a heating step at 95°C for 8 minutes. A touchdown protocol followed, where each cycle involved heating to 95°C for 15 seconds, cooling to 68°C for 30 seconds and heating again to 72°C for 1 minute, the annealing temperature was decreased to 58°C over 20 cycles. After the touch-down cycles, an additional 40 cycles of 95°C for 15 seconds, 55°C for 30 seconds and 72°C for 1 minute were performed. In each PCR run a non template control, with the DNA template substituted with water in the reaction mixture, an *A. phagocytophilum* positive blood control sample and an *A. phagocytophilum* negative blood control sample. Amplicons were visualized on 1.5% agarose gels with 100-bp ladders as molecular weight markers. (Franzén et al 2005)

RESULTS

The hunter observations during the first days of the hunting season on Öland show that the reported number of calves per observed adult female is 0,329 on the northern half of the island and 0,437 on the southern part. This is to be compared with the mainland and the eight most southern counties of Sweden where the number of calves per adult female ranged between 0,667 and 0,817. (See Table 1.) Approx 85% of the hunting parties handed in their reports. (Swedish Association for Hunting and Wildlife Management 2009)

Table 1. Number of calves observed per adult female during the first 7 days of the moose hunting season of 2007 in the southern half of Sweden.

Area	No of calves observed per adult female
Borgholm (Northern Öland)	0,33
Mörbylånga (Southern Öland)	0,44
Skåne county	0,70
Mainland Kalmar county	0,76
Blekinge county	0,71
Halland county	0,67
Kronoberg county	0,82
Västra götlands county	0,78
Östergötland county	0,77
Jönköping county	0,76

Out of the 40 moose sampled in the study 23 were cows or heifers, five were bulls and 12 were calves. (See table 2 and 3)

Table 2. Number of individuals sampled, their sex and results for serology and PCR

	No of ind.	Spleen samp.	Blood samp.	PCR pos/neg	Serology pos/neg
Calves	12	10	9	2/8	8/1*
Females	4	4	4	1/4	4/0
Males	8	6	5	1/5	4/1*
Yearlings	11	11	10	1/10	10/0
Females	11	11	10	1/10	10/0
Males	0	0	0	0	0
Adults	17	17	14	0/17	14/0
Females	10	10	8	0/10	8/0
Males	7	7	6	0/7	6/0

* A male calf found dead in June 2007, just a few weeks old. PCR negative.

Out of the 40 samples 31 were complete with both blood samples and spleen which facilitated the opportunity for examine both the serology for antibodies against *A. phagocytophilum* and the presence of *A. phagocytophilum* with a PCR-analysis. For the remaining nine moose either blood or spleen were sampled.

The 31 complete samples came from 19 cows, four bulls and eight calves. The non-complete samples were from four cows, one bull and four calves.

Apart from a newly born calf found dead on June 13, all sampled moose (n=33) had antibodies against *A. phagocytophilum*. Out of the 38 moose were spleen was collected a total of four samples were PCR positive.

Slaughter weights are seen in Table 3. The mean weight for calves, cows and bulls was 54,8kg, 162,3kg and 230kg, respectively.

Table 3. Slaughter weights recorded and reported by the hunters for moose culled during the hunting season. Weight reported as carcass (skinned body without guts, head, and lower legs).

	No of individuals	Range (kg)	Median weight (kg)	Average weight (kg)
Calves	6	44-77	51,5	54,8
Females	2	55-61	58	58
Males	4	44-77	46	53,3
Adults	22	112-240	170	168,4
Females	20	112-212	169	162,3
Yearlings	11	112-185	140	146,4
Older	9	145-240	185	181,7
Males	2	220-240	230	230

DISCUSSION

The background for this study is that the hunters have identified a problem in the moose population and has then made contact with the researchers. This enabled me and the researchers to sample all culled moose out of an isolated island population.

All sampled moose, apart from a young calf found dead in June, had developed antibodies against *A. phagocytophilum* (Table 2.). This indicates that the exposure to of *A. phagocytophilum* in the population is very high. In 2002, Stuen et al reported variations in seroprevalence among moose, red deer (*Cervus elaphus atlanticus*) and roe deer (*Capreolus capreolus*) in different areas of Norway. There, 43% of moose, 55% of red deer and 96% of roe deer were seropositive for *A. phagocytophilum* antibodies and this indicates that moose is less prone to attract *A. phagocytophilum* infection, compared to roe and red deer. Since nearly 100% of the moose sampled in this study were seropositive for *A. phagocytophilum* it is likely that the bacteria is well spread among ticks on Öland. This can possibly result in a high incidence of disease among other animals, wild and domestic, and in humans. Looking at data on the number of treatments for TBF in cattle on Öland shows that this done yearly. The total number of recorded treatments though, is to low to draw any real conclusions. (Animal health statistics 2007 Swedish Board of Agriculture)

The low presence of PCR-positive animals in the adult population indicates that moose effectively can eliminate *A. phagocytophilum*. However, 25 % of the calves and one yearling female were positive for *A. phagocytophilum*. The yearling female was killed in traffic in early June and one of the positive calves

was found dead at early age in late June. June and July is one of two main periods when ticks are active and can cause infections (Stuen et al. 2002). The second period is in August. The two PCR-positive animals could have been infected or re-infected a short time before they died, and not eradicated the bacteria. The two other calves that tested positive for *A. phagocytophilum* in November were positive more than three months after the ticks' second main period of infection. This indicates that infection can persist in individual calves. It is also possible that infection persists in individual adult animals as well, but presumably not as long as in calves. Adults could have eliminated the bacteria at the time of the hunting season. To my knowledge, there are no studies that describe how long the elimination time is for moose, nor for how it can differ between individuals. Due to variations in blood quality and sampling, no exact titers of antibodies were performed. Description of titers could have facilitated an estimate on when the infection was present in the blood. After experimental infection with *A. phagocytophilum* one white-tailed deer was positive 66 days post infection when PCR analysis was performed on post-mortem tissues from femoral bone marrow. (Tate et al. 2005) The above mentioned study indicates that infection can persist in single individuals for longer periods of time and this study supports that this is probably the case for moose as well.

The low number of calves per adult observed (see table 1) indicates reproduction problems, and/or a high mortality among young calves. Since there are no large predators on Öland, high mortality in calves most likely has other causes e.g. infections, parasites, starvation or intoxications. Problems with reproduction can come from infertility or subfertility in females or males, infection, parasites, starvation or intoxications of the female before or during pregnancy.

High calf mortality in a population of moose could come as a result of infection with *A. phagocytophilum* early in life. Moose calves are born in May-June, coinciding with the first active tick-period. Increased calf mortality can hence derive from an acute infection with *A. phagocytophilum* or through weakening of the immunsystem, which may facilitate secondary bacterial, viral, or parasitic infections.

A UK study reported that after having infected eight adult sheep with louping-ill virus alone only three developed signs of the disease and recovered while all of eight adult sheep simultaneously infected with louping-ill virus and a TBF producing strain of *A. phagocytophilum*, (at the time named *Cytoecetes phagocytophila*) died within ten days. (Brodie, T.A. et al., 1986)

In order to determine if calf mortality is increased an inventory of the number of calves born need to be carried out. First, there is a need to investigate whether an increased mortality is present or not. If dead calves are found these need to be sampled and tested for presence of *A. phagocytophilum* as well as other infectious agents as close after death as possible before they are destroyed by heat, flies and scavengers. The moose gives birth in early summer, which could limit an inventory of the number of newborn calves. Subsequently, the possibility of finding suitable sampling material from dead calves is very limited.

Few reliable data for slaughter weights of moose calves can be found from the southern parts of Sweden or from Öland. Two Norwegian (Sæther and Gravem

1988, Moe et al. 2008) and one Swedish study (Hawley et. al. 1983) reporting calf slaughter weights from 16 000 calves shot during normal hunting season between 1970-2004 state a mean slaughter weight of approximately 70kg for males, and 63kg for females. The present study reports a substantially lower mean slaughter weight for males and females (Table 3.) Unfortunately not all slaughter weights for calf were reported by the hunters. Furthermore, the recording of slaughter weights has not been consistent and no data from previous years is available. Hence, it is difficult to draw any real conclusions from the slaughter weight data. The general opinion though among hunters and researchers contributing to and participating in this study is however that calves shot and calves observed, are smaller than expected.

Infection with *A. phagocytophilum* presumably plays a role in the declining number of calves on Öland, but other factors can be involved. A comparison with the moose population on the mainland would be very helpful. Such a study would focus on whether or not the moose on the mainland have been exposed to *A. phagocytophilum* to the same extent as the moose on Öland. At the same time, a comparison of calf slaughter weights between Öland and mainland Sweden would be carried out. Little is known about the effects of infection with *A. phagocytophilum* in moose. The moose is a wild species, and day to day studies with experimental *A. phagocytophilum* infection is difficult to carry out.

Tick-borne fever and the effects of infection with *A. phagocytophilum* has been well studied in domestic animals. In my opinion these studies gives reason to believe that *A. phagocytophilum* probably plays a role and is contributing to the low number of calves and their poor condition. Either through direct effect on the pregnant cow or on the new borne calf or through the immunosuppressant abilities of the organism making way for secondary infections.

The present study shows that there are fewer calves in the moose population on Öland, compared to the rest of the Swedish moose population. All shot moose in this study have been exposed to *A. phagocytophilum*. The slaughter weights indicate that the calves are smaller than in the rest of the Swedish moose population. To determine the role of *A. phagocytophilum* in this decline in calves, more research is needed.

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