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Master thesis

Sarcoptic mange in the Scandinavian wolf population

Master in Applied Ecology

2014

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Abstract

Sarcoptic mange, a parasitic disease caused by the mite *Sarcoptes scabiei,* is epidemic in red foxes *Vulpes vulpes* and reported on wolves *Canis lupus* in Scandinavia. The small, inbred wolf population of Scandinavia might be particularly susceptible to sarcoptic mange. I address the questions of how this parasite is distributed and transmitted within the recovering wolf population.

A total of 198 serum samples, collected over 15 years from 141 captured Scandinavian wolves were tested for antibodies against *S. scabiei* by enzyme- linked immunosorbent assay (ELISA). This data was correlated with ecological data on the individual, pack and population scale. Additionally, necropsy reports of 269 wolves were used in the study. I tested whether individual, intrinsic characteristics such as body condition, age, inbreeding coefficient, and reproductive state or extrinsic density factors such as wolf pack density, pack size and habitat productivity fit the given data better by using generalized linear regression models selected by AICc.

Among the captured wolves, I found a temporal declining mean seroprevalence of 11.1% (SE = 2.5%), and a higher probability of finding seropositive wolves in the southern part of their distribution. Females had a lower probability than males of contracting mange, and the sex difference decreased with increasing pack size. No wolf pup (N=56) was seropositive at capture. Additionally, I report recovery from mange on adult wolves. Mange-caused mortality was limited, and starvation was the most common cause of death reported from necropsy.

I conclude that sarcoptic mange has a small potential negative effect on the recovery of the Scandinavian wolf population. More support for intrinsic than wolf density factors and a heterogenic distribution of mangy animals on pack scale, suggest low probability of wolf-to-wolf transmission of *S. scabiei* in Scandinavia.

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Introduction

Sarcoptic mange is an epizootic, wide spread skin disease of canids and over one hundred other mammalian species, caused by the mite *Sarcoptes scabiei* (Davis et al. 2001; Pence & Ueckermann 2002). The mite, burrowing through the stratum corneum while consuming living cells, causes the host to mount a specific immunological response (Falk 1980; Arlian *et al.* 1994; Bornstein, Zakrisson & Thebo 1995). Enzyme- linked immunosorbent assay (ELISA), testing the presence of antibodies against specific proteins of a sought agent, is considered as a useful tool for diagnosis of sarcoptic mange in canids (Bornstein *et al.* 2006). Clinical signs, triggered by a hypersensitive response are pruritus, hyperkeratosis, alopecia, hyperpigmentation and dermal inflammation, potentially effecting the entire body (Pence & Ueckermann 2002). Wolves *Canis lupus* and other canids hosting sarcoptes mites, react with intense scratching and biting and often become debilitated and emaciated due to secondary bacterial infections and difficulties catching its natural prey (Davis *et al.* 2001; Wobeser 2006). Severe mange infections cause high mortality rates, often due to indirect factors, including starvation and hyperthermia in winter (Kreeger 2003). However recovery from even severe sarcoptic mange on wolves is reported (Jimenez *et al.* 2010; Almberg *et al.* 2012) and observed mortality rates are inconsistent, ranging from minor in Yellowstone and Spain (Oleaga *et al.* 2011; Almberg *et al.* 2012) up to about 30% of the overall wolf mortality in Michigan and a 12% population size drop in Wisconsin (Schultz, Wiedenhoeft & Wydeven 2003; Wydeven *et al.* 2004; Jimenez *et al.* 2010). Wolf packs with mangy members tend to be smaller or have reduced annual growth rates and the total extinction of single packs has been observed (Schultz, Wiedenhoeft & Wydeven 2003; Almberg *et al.* 2012). In Wisconsin, pup mortality increased when mange was present (Jimenez *et al.* 2010) and during predator control programs in Alberta, Canada in the seventies, the proportion of culled pups was negatively related to the prevalence of mange among all removed animals (Todd, Gunson & Samuel 1980). Sex and age are not reported as factors influencing the prevalence of mange in wolves, but was only analysed in two studies (Todd, Gunson & Samuel 1980; Oleaga *et al.* 2011).

Sarcoptes scabiei var. *canis* is able to survive up to 19 days off the host and actively seeks odour and thermal stimuli (Arlian *et al.* 1984a; Arlian *et al.* 1984b). Transmission is occurs through close contact between hosts and is therefore host - density dependent (Pence & Ueckermann 2002). In Yellowstone National Park, the spatio-temporal patterns of mange

infestation on wolves is related to the wolves' connectivity and density, indicating wolf–to-wolf transmission (Almberg *et al.* 2012). In Europe, interspecific transmission from red fox *Vulpes vulpes* is the most likely origin for mange in wolves (Mörner *et al.* 2005; Domínguez *et al.* 2008). In northern Spain, a cycling pattern of mange prevalence in the wolf population is following the red fox prevalence with a one year delay (Oleaga *et al.* 2011). In Scandinavia the wolf was regarded as functionally extinct during the late 1960's, and until 1983, when founders, originated from the Finnish-Russian wolf population, of the present Scandinavian wolf population reproduced for the first time (Wabakken *et al.* 2001; Vilà *et al.* 2003; Liberg *et al.* 2005) During the following 30 years, the recolonizing Scandinavian wolf population increased to an estimated population size of 350 – 410 wolves (Wabakken *et al.* 2013). Until 2013, the population has been founded by five individuals, and severe inbreeding depression has been confirmed on a population scale (smaller litter size; (Liberg *et al.* 2005) with individuals showing congenital defects (Räikkönen *et al.* 2006). Inbreeding has been shown to effect immune responses in birds and insects (Reid *et al.* 2007), as well as to increase susceptibility to ectoparasites due to fitness-related behavioural changes (Luong, Heath & Polak 2007).

Although-, mortality of Scandinavian wolves are mainly human caused (Liberg & Sand 2012), sarcoptic mange may be an important factor among the natural mortality causes (Mörner *et al.* 2005). Sarcoptic mange arrived in the 1970's in Scandinavia with devastating effects on the red fox population (Mörner 1992; Lindström *et al.* 1994). A previous study focusing on imunoglobulin E (IgE) levels tested 57 Scandinavian wolves for sarcoptic mange and 14 individuals were seropositive (Ledin *et al.* 2008). Effects of sarcoptic mange on the Scandinavian wolf population remain unclear but have the potential to influence to the small, inbred population. Here I analyse and present an overview of the mange situation in the Scandinavian wolf population from 1998 to 2013 based on 198 serum samples from live wolves with ELISA for seroprevalence and necropsy reports of 269 dead wolves in Sweden between 2003 and 2013. The main goal of my study was to identify demographic and environmental factors that relate to the probability of finding seropositive samples. Based on the literature and personal observations I predict that: 1) The probability of exposure is density dependent. 2) Individual intrinsic factors such as age and sex play a minor role on the probability of exposure. 3) The transmission of sarcoptic mange is interspecific.

1. Material and Methods

1.1 Study area

The Scandinavian wolf population is located in central Sweden and south-eastern Norway (Figure 1, 59-62°N, 10-15°E) (Wabakken *et al.* 2013). The area is primarily covered by managed forest stands of Norway spruce *Picea abies* and Scots pine *Pinus sylvestris* with wet lands, agricultural areas and settlements covering minor areas, primarily in the southern range of the wolf area (Mattisson *et al.* 2013). The human population density is below 1/km^2 in large areas of the main wolf range (Wabakken *et al.* 2001). The climate is continental with average temperature of 15°C in June and -7°C in January and the area is snow covered from December to March with an average depth of 30 – 60 cm (Swedish national atlas 1991; Statistics Norway 2003). The main prey species are moose *Alces alces* and in the southern range of the wolf area, also roe deer *Capreolus capreolus* (Sand *et al.* 2008; Zimmermann 2014). Red deer *Cervus elaphus*, semi - domesticated or wild rein - deer *Rangifer tarandus tarandus* and domestic sheep *Ovis aries* are other available prey species. Beaver *Castor fiber*, badger *Meles meles*, red fox Vulpes vulpes, mountain and European hares *Lepus timidus, Lepus europeus* as well as black grouse *Lyrurus tetrix* and capercaillie *Tetrao urogallus* are smaller prey species also consumed by wolves (Sand *et al.* 2008; Zimmermann 2014) . Known hosts of Sarcoptes mites in the area are red fox *Vulpes vulpes*, lynx *Lynx lynx*, wild boar *Sus scrofa* and domestic dogs *Canis lupus familiaris* (Mörner 1992; Ryser-Degiorgis *et al.* 2005).

1.2 Serum collection

A total of 198 serum samples from 141 individual free-ranging wolves out of 54 different packs in Sweden and Norway were analysed. The animals were immobilized by darting from a helicopter during winter between 1998 and 2013 as part of ongoing research (http://skandulv.nina.no/) following standard capture procedures (Arnemo, Evans & Fahlman 2012). The captured animals were tagged with ear tags or microchips and the identity further confirmed by DNA analysis. Forty individuals were recaptured to change radio collars or for translocations (28 wolves were recaptured once, 7 twice and 4 three times). In average, 9% (2-18%) of the estimated maximum Scandinavian wolf population was captured each year.

Blood was drawn from the cephalic vein using 8 ml sterile, evacuated serum-seperator tubes with gel and clot activator (Venosafe^{TM}, Terumo Europe N.V, Leuven, Belgium). Serum was separated by centrifugation at 1500 g for 15 minutes the same day and kept frozen until shipment to the laboratory (Department of Parasitology, National Veterinary Institute (SVA), Uppsala, Sweden).

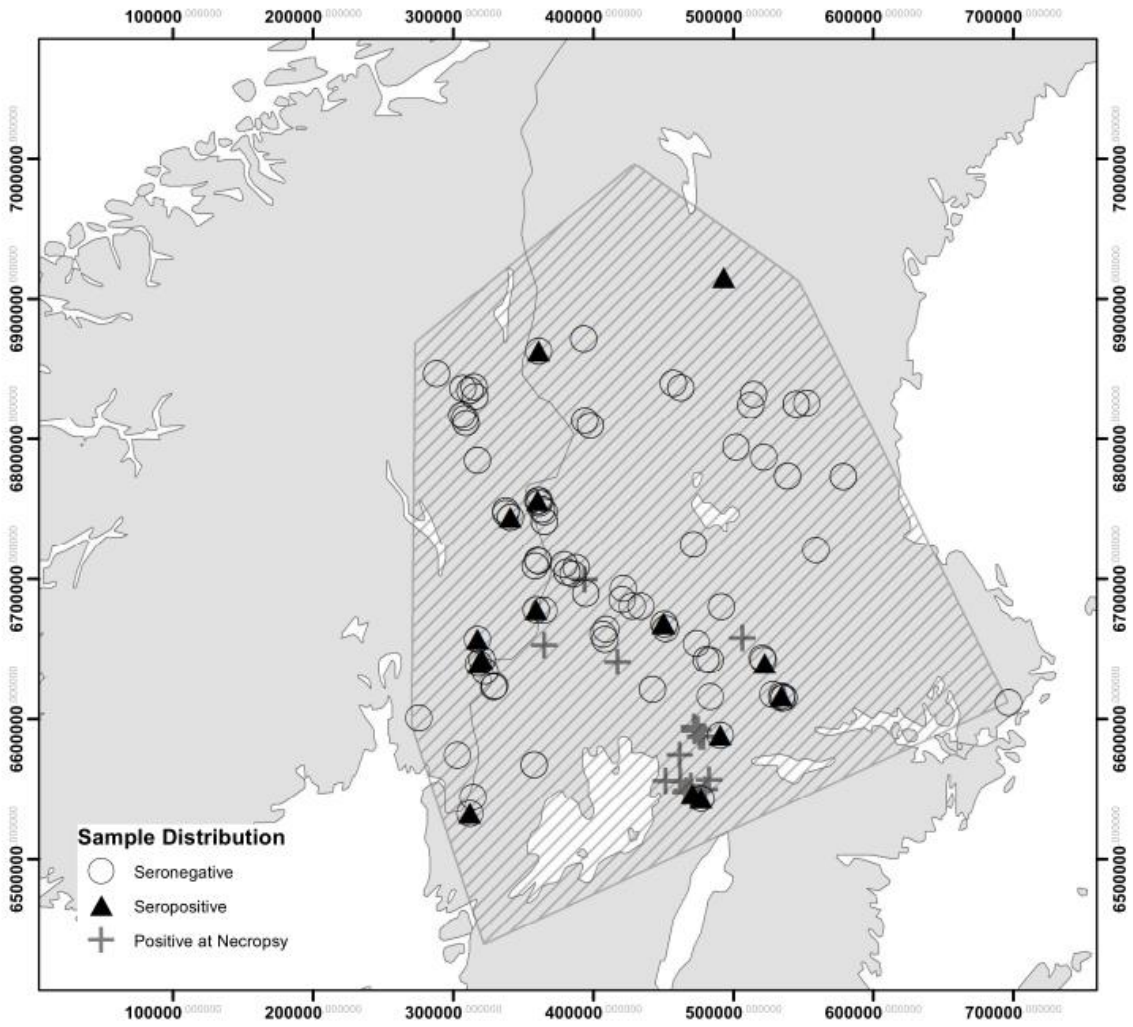


Figure 1: The area covered by the reproducing Scandinavian wolf population with pack centre points of captured territorial wolves or pups displayed as seropositive (N=20) or negative (N=178) according to the ELISA and the location of collected wolves with clinical signs at necropsy (N=21). Due to data collection of several animals at the same location, symbols might be hidden.

1.3 Serology

Serum samples were analysed by ELISA using a crude (whole mite extract) antigen according to Bornstein and Zakrisson (1993) and a recombinant major sarcoptes mite antigen (MSA-1) (Ljunggren *et al.* 2006). In each series, samples were tested in duplicates and positive and negative control samples from dogs were included. Optical density (OD) was measured at 450nm with a multiscan EX (Thermo Labsystems, Vantaa, Finland). In order to get an OD value relative to the positive control, mean OD values for each duplicate were calculated and the mean OD of a blank (PBS-T instead of a sample) subtracted. For valid results, positive control OD values must be between 0.8 and 1.6 for the crude antigen and for the MSA-1 antigen between 0.7 and 2.0. OD values of the samples are divided by the OD value of the positive control. Positive results should reach >0.3 for the crude antigen and >0.5 for the MSA-1, relative to the positive control. Doubtful results are defined as 0.2-0.299 by the crude antigen and 0.35-0.499 by the MSA-1 antigen, OD values below that are considered to be negative. Sensitivity and specificity for the test are 92 and 96%, respectively, in dogs with acute sarcoptic mange and 95% and 98%, in chronic cases (Bornstein, Thebo & Zakrisson 1996). The same test has been successfully used in red foxes (Bornstein, Zakrisson & Thebo 1995) with sensitivity and specificity of 95.4% and 100%, respectively (Bornstein *et al.* 2006). Experimentally measured seroconversion on dogs and red foxes takes up 1 – 5 weeks post infection and 1 – 3 weeks from onset of clinical signs (Bornstein & Zakrisson 1993; Bornstein, Zakrisson & Thebo 1995) and persists 1 to > 4.5 months after successful treatment in dogs (Lower *et al.* 2001).

All doubtful and seropositive samples were tested by Western blot as described previously by Bornstein, Zakrisson and Thebo (1995). The sarcoptes proteins in the crude antigen extract are separated by their atomic weight (kDa) on a nitrocellulose membrane. The samples are exposed to the proteins. When positive, the antibodies in the samples bind with the protein at 164 kDa or 147 kDa and 105 kDa. By binding a fluorescent substrate to the antibodies and exposing it to a film, if present the fixed antibodies are visible as a bare on the film.

For a more detailed description of ELISA and WB see Appendix.

1.4 Necropsy reports

During the study period, wolves shot or found dead in Sweden were delivered to SVA or to the Swedish Museum of Natural History for necropsy. At SVA, serology as described above or microscopy was done if clinical signs of sarcoptic mange were detected. Since 2003, all dead wolves were delivered for necropsy at SVA. I compared observed seroprevalence from captured Swedish wolves including border packs from 2003 to 2013 with the necropsy data. Detailed data from Norwegian necropsy reports were not available.

1.5 Intrinsic data

Captured animals were sexed (104 males, 94 females) and body mass and body measurements were noted. As a proxy for body condition used in the statistical analysis, I divided body mass by body length (nose to base of the tail). Age of the animals at capture ranges from 7 months up to > 10 years and was estimated by growth zone on the radius and ulna on pups and tooth wear in adults (Gipson *et al.* 2000). Age was adjusted if DNA analysis could prove the year of birth or by counting cementum layers in teeth of dead wolves. The sampled animals were grouped into offspring pack members, territorial breeders, territorial non breeders and non-breeding dispersers according to the monitoring reports, age and retrieved collar data. I included the inbreeding coefficient (f), as the probability of two homologous genes in an individual being identical by descent (Keller & Waller 2002). The method used to estimate the inbreeding coefficient is published in Liberg *et al.* (2005).

1.6 Population data

The annual monitoring of the Scandinavian wolf population for the entire study area was based on snow tracking, DNA-analyses of scats and radio collar data, and published in annual reports e.g.(Wabakken *et al.* 2013). The intense effort carried out by management and research institutions, gives a total number of packs and estimates of pack- and population size. For statistical analysis in this study, the mean pack size was used until 2011, when the Swedish monitoring system changed to only confirm reproduction and minimum pack size. Pack size was used as one of three wolf density proxies and does not represent the litter size but number of wolves per territory. It ranges from two to ten and includes non-breeding, territorial pairs. In Scandinavia, wolf packs with offspring older than 2 years and both adults present have not been observed.

Mattisson *et al.* (2013) calculated the local density of packs by number of pack centre points within a 40 km radius around each pack centre point. I calculated the mean Euclidean distance from each pack centre point to the next three neighbouring centre points using the spatstat package (Baddeley & Turner 2005) in R 3.0.3 (R Core Team 2014). The centre points of territories are calculated from radio collar locations and are also used for analysing the spatial distribution of the serum samples. Mattisson *et al.* (2013) showed that the home range size of Scandinavian wolves increased with latitude, independently to available prey biomass. They linked it to an environmental gradient from mosaic with open cultivated fields to a homogenous boreal forest, increasing snow depth, landscape roughness and could correlate it to roe deer density. I used latitude as a general bio density and habitat productivity proxy. Location data was projected in WGS 84 UTM 33N.

1.7 Model selection

In logistic regression models I related sex, body condition, age, pack size, latitude, inbreeding coefficient, pack density within 40 km and Euclidean distance to the closest three neighbours to the probability to find a seropositive serum when capturing wolves. The response variable was a binary term with 1 for seropositive and 0 for seronegative samples. All analysis were done using statistical extensions available in R 3.0.3 (R Core Team 2014). Due to recaptures of individuals and territories I initially included animal and territory ID as nested random factors in the models. However, during the modelling process, I decided to simplify the

models and to run them without the random factors. This decision was based on 1) convergence problems; 2) the assumption that the time interval between capture events was long enough for individuals to change between seropositive and negative (Bornstein & Zakrisson 1993; Sarasa *et al.* 2010); and 3) high variation of mange positive animals within packs in my, as well as in other studies (Almberg *et al.* 2012), (Schultz, Wiedenhoeft & Wydeven 2003), (Oleaga *et al.* 2011) personal communication with Emily Almberg (Department of Biology, Pennsylvania State University), and Ilka Reinhardt (Büro Lupus, Germany). Continuous variables were tested for variance inflation (vif) (Fox & Weisberg 2011), and the models were tested for outliers (cooks distance) and over-dispersion (Zuur *et al.* 2009). Model selection was based on comparing biological meaningful combinations of predictors with AICc and cut-off delta AICc = 4 (Mazerolle 2011). Observations with lacking data were omitted from the model selection.

According to my hypothesis I *à priori* formulated two main models:

Intrinsic model: The probability to find a seropositive serum sample can be explained by individual based intrinsic factors body condition, age, inbreeding coefficient and the interaction of sex and pack size, assuming more physical contact of the reproducing females with their pups, as well as a potential effect on pup survival by seropositive females. Other interactions were not included in the model because they lacked biological relevance.

Density model: The probability to find a positive serum sample can be explained by density factors including distance to neighbours, number of packs within a 40 km radius, pack size, and latitude. The two predictors distance to the closest three neighbours and number of pack centre points within 40 km were conceptually confounded, and I kept the first of these two because it did not contain zero values and showed a more even distribution of observations along the x-axis.

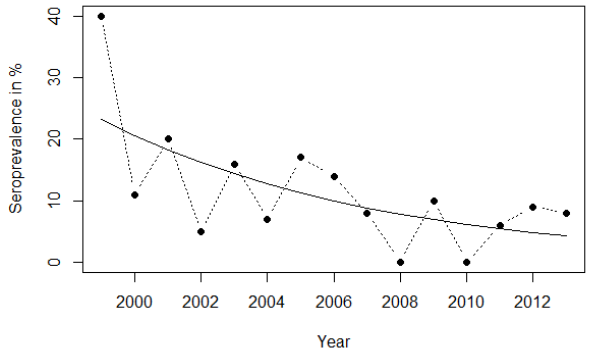
Based on the main models I selected by parsimony for each group of variables the best submodel (lowest AICc, no cut off) and combined the variables in a combined model. The combined model was included in the selection.

2. Results

2.1 ELISA

Twenty (10.1 %) out of the 198 samples and 17 (12.1 %) out of the 141 individuals were tested seropositive. Mean annual seroprevalence of the sampled serum was 11.3% (SE 2.5%), ranging from 40% in 1999 (N = 5) to zero in 2008 (N = 7) and 2010 (N = 15). All wolf pups (N = 56, 7 to 10 months old) were seronegative and did not show any clinical signs at capture. The seroprevalence of the tested sera decreased with time ($\chi_{1,13}$ = 43.31; p < 0.01) but no significant cycling pattern was observed (Figure 2). Of the 20 seropositive samples at capture, clinical signs were mentioned on the capture forms for seven wolves (35 %). Normal fur is reported for five (25%) sampled wolves and no information is available for eight animals (40%). Of the 178 seronegative samples two wolves (1.1%) showed clinical signs at capture.

a



b

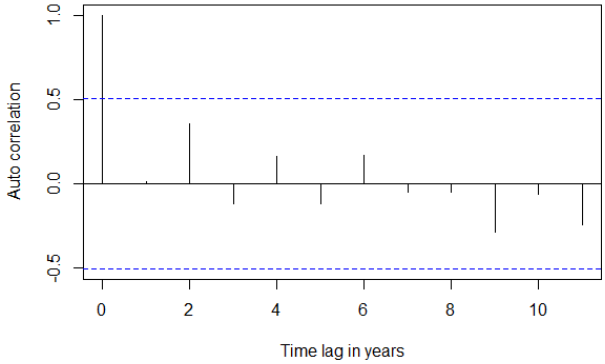


Figure 2a: Seroprevalence in the captured wolves (N = 198 samples) during the study period (1999-2013). Black dots show observed prevalence in % and the black line shows the prediction of a GLM.
Figure 2b: No significance in cycling patterns of seroprevalence of captured wolves. Auto correlation function of not de-trended seroprevalence. The dashed line displays the 95% c.i. and must be reached to prove a significant cycling pattern.

2.2 Recaptures

Among the 40 recaptured individuals, eight were tested seropositive at least once. Six were seropositive at first capture and four of them seronegative at recapture one year later (M-0506, M-0606, M-0611, M-0918). M-0105 was seropositive at first and at recapture two years later. M-1114, a Finnish/Russian immigrant which was translocated within Sweden four times, was seropositive at first capture, seronegative nine and eleven months later, and seropositive at fourth capture two years after first capture. M-1114 did not show clinical signs at any of the captures but was treated against parasites due to management requirements at the first capture. During the study period no other wolf received anti - parasite treatment.

2.3 Territory

A sampling event per territory is defined as one or more wolves captured per winter within a pack, including recaptures. In 61 of 95 sampling events multiple wolves were captured. In 13 events at least one individual was seropositive, but at least one seronegative individual got captured as well. In two family groups both the adult male and female were tested seropositive but their captured pups (two each) were seronegative at time of capture (Hagfors and Hasselfors). In eight territories, positive individuals were recaptured and six tested seronegative at recapture. In the Mangen territory, both the adult male and female were captured in 2003 and 2004, the male tested twice seropositive and the female twice negative. Hasselfors is described below.

2.4 Necropsy

The average annual prevalence of mange (individuals with clinical signs are analysed by ELISA N=15, microscopy N=5 or both N=2) among dead wolves collected in Sweden between 2003 and 2013 was 4.5 % (SE = 1.3%, N = 269). In the same period seroprevalence of mange among captured wolves in Sweden (including Norwegian-Swedish cross - border packs) was 7.6% (SE = 2.5%, N = 112 samples). The annual prevalence did not differ significantly between these two samples (Wilcoxon rank sum Test, $N_{Necropsy} = 11$, $N_{Captures} = 11$, $W = 46.5$). No sarcoptes mites could be isolated from wolves in Scandinavia. At necropsy eight (38.1 %) out of 21 wolves with clinical signs were pups, all six to nine months old and all were euthanized (6) or died of starvation (2) due to severe sarcoptic mange. Two were

from the Hasselfors pack collected in October 2002 and six were from the Loka pack collected in December 2012 and January 2013. The alpha male from the Loka pack was euthanized as well and the female was not found any more by DNA. The adult pair (female M-0104, male M-0105) and two pups (M-0103, M-0106) of the total eight Hasselfors wolves were captured in February 2001. The pair was seropositive and the pups seronegative at capture. M-0106 was euthanized five months later in August 2001, M-0103 in December 2001 and the alpha female was found dead due to starvation in March 2002. M-0103 and M-0104 were seropositive at necropsy, M-0106 was not tested with ELISA but showed as the others sever clinical signs of sarcoptic mange. Unmarked pups were observed with clinical signs but could not be detected by DNA later. The alpha male (M-0105) stayed in the territory, reproduced successfully in 2002 and tested seropositive when it was recaptured in 2003. M-0105 died in 2006, remains were found approximately one year post mortem. Of the 21 mange positive wolves at necropsy, five were shot during licence hunt or removed to prevent predation on livestock, ten were euthanized due to mange caused emaciated status and one was euthanized after a traffic accident. In total five wolves were collected dead, four died due to mange related starvation and one after a traffic accident. Between 2003 and 2013, 4% of the total mortality reported to SVA in Sweden was caused by mange.

2.5 Probability of seropositive samples

The full model including all variables was not overdispersed (residuals/degree of freedom < 1) and continuous variables not confounded (VIF <2). According to cooks distance no outliers were in the data set. Because all of the 56 pups were seronegative I excluded them from the statistical analysis and continued on with the samples from the adult wolves. Annual mean seroprevalence of the adult population was 19.9 % (SE 6.5 %). Further omitted are 15 serum samples taken from adult individuals with unclear information about the status of the territory, from dispersing wolves or from immigrants from the Finnish / Russian population captured for translocation. In 20 cases, body mass, body length or both were not available so Body Condition could not be calculated, and f was missing for 13 samples. Totally 5 (10.4%) of the 48 excluded samples from adult wolves were tested seropositive.

The most parsimonious model was the combined model, and two submodels of the intrinsic model were within the AICc cut off value 4. According to the relative AICc weight ratio, the combined model had 4.4 times higher evidence than the next model containing only intrinsic

factors (Table 1). The submodels based on the intrinsic variables increased in evidence by removing f, Age and Body Condition, and decreased in evidence by removing Sex or Pack Size. The submodels based on the density factors increased in evidence by removing the factors distance to the next three neighbouring territories and Pack Size but decreased in evidence by removing the factor Latitude. I entered therefore the interaction of Sex and Pack Size together with Latitude in the combined model. No other combination of intrinsic and density variables was further included in the analysis.

Table 1: Model comparison based on generalized linear models on effects of latitude (Lat), sex, number of wolves in the territory (Pack Size), Body Condition, inbreeding coefficient (f), Age, Euclidiean distance to the next three neighbouring territories (Ndist) and number of wolves in the territory (Pack Size) on the probability of detecting antibodies against sarcoptic mange on captured Scandinavian wolves. N=92. Presented are the two main models (Intrinsic, Density), the submodels within delta AICc < 4, the model combining the top submodels, which at the same time is the most parsimonious model and the null model.

Model	Parameters	K	AIC_c	ΔAIC_c	ω_i
Combined	Lat + Sex * Pack Size	5	75.05	0	0.66
Submodel intrinsic	Sex * Pack Size	4	78.06	3.01	0.15
Submodel intrinsic	Sex * Pack Size + Body Condition	5	78.25	3.2	0.13
Submodel density	Lat	2	81.25	6.2	0.03
Intrinsic	Sex * Pack Size + Body Condition + f +Age	6	83.02	7.98	0.01
Density	Lat + Pack Size + Ndist	4	83.63	8.58	0.01
Null model	1	1	87.06	12.01	0

Due to the exclusion of the variables f, Age and Body Condition which contained numerous missing values, I could add 35 samples to the top model, which increased the sample size from N=92 to N=127. I tested the same models containing only the factors Sex, Pack Size and Latitude again for parsimony and compared beta estimates without finding substantial differences except of smaller standard errors (data not shown). In the combined model, the

probability to find seropositive samples was in general higher in the southern range of the wolf area and the probability of seropositive females was increasing with the number of wolves in the territory (Table 2)

Table 2: Cobined model describing effects of latitude (Lat) and the interaction of sex and number of wolves per territory on the probability to detect antibodies against sarcoptic mange on captured Scandinavian wolves. Estimates are based on a logarithmic link function. N=127

Factors	Beta	SE
Intercept	43.75	19.73
Sex *female*	-2.78	1.64
Pack Size	0.11	0.13
Lat	$-6.81\ e^{-6}$	$2.95\ e^{-6}$
s	0.27	0.26

Given the data, the north-south gradient of increased chance to find seropositive wolves was valid for female and male wolves in all group sizes (Fig. 3a). The chance to find seropositive wolves was 3.8 to 4.8 times higher in the southern part (UTM 6 600 000 ,seroprevalence females 15%, males 37%) in packs with 6 wolves (mean size of reproducing packs), as compared to the northern distribution limit of the wolf population. The increasing probability of finding seropositive wolves with increasing pack size had a linear relationship for males and increased in the south from 27% seroprevalence in pairs to 37% seroprevalence in packs of six wolves. The probability to find seropositive females in the south depending on pack size increased constant proportionally and is 4 times higher in packs of six wolves than in pairs, with a seroprevalence of 4% and 15% respectively. Females in pairs have the lowest chance to be seropositive and the north south gradient had a smaller effect.

a *b*

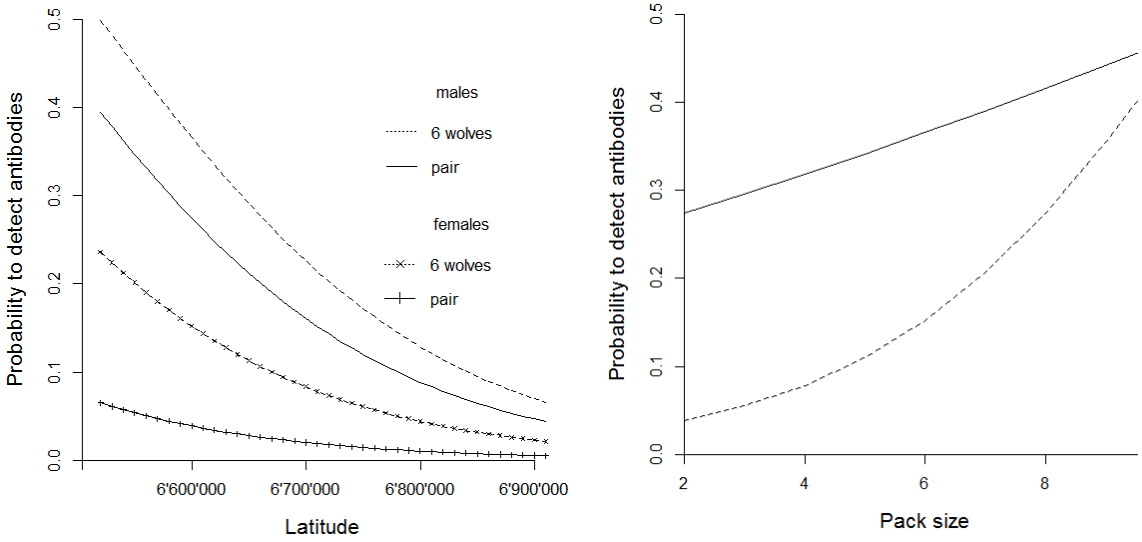


Figure 3*: The probability to detect sarcoptic mange specific antibodies in serum samples of captures wolves in Scandinavia in relation to latitude in combination with pack size and sex (a), and wolf pack size in combination with sex (b). The predicted lines in Figure 3b) are estimated for wolves captured at latitude 6'600'000 (UTM 33n, Figure 1), with dashed line for females and solid line for males Both figures show the backtransformed predictions of the combined model with* $e^{(Combined/(1+Combined)}$.

3. Discussion

Sarcoptic mange is a common disease in the Scandinavian wolf population and seropositive animals were found every year except for 2010. The probability of detecting seropositive individuals among all sampled wolves increased along a north – south gradient with the size of the wolf packs and was higher for males than females. Models that predicted the probability of detecting seropositive wolves had less support if they were based on wolf density factors than the models based on intrinsic factors, especially sex and pack size. Mange - specific clinical signs or antibodies were not found among the 56 captured pups, but at necropsy, 38% of the mange positive wolves were pups. Four out of six recaptured individuals turned from seropositive to seronegative, suggesting regular recovery from sarcoptic mange. Whenever a seropositive sample was collected at the same time as a sample from other pack members, I found a seronegative sample as well, indicating a low probability of wolf-to-wolf transmission within the territory.

For the time period of 1999 to 2013, the estimated seroprevalence of sarcoptic mange was 10% in this study is lower than earlier reports for the Scandinavian wolf population with 21% and 25% respectively (Olsen 2003; Ledin *et al.* 2008). It is also lower than the seroprevalence of 20% reported in the Iberian population (Oleaga *et al.* 2011), the only other population where ELISA was used to estimate the prevalence of sarcoptic mange on numerous wolves (N=17/88). Prevalence of mange on wolves in the Bialowieza primeval forest was 6% (N=2/27) (Kołodziej-Sobocińska, Zalewski & Kowalczyk 2014). The declining seroprevalence reported in my study is consistent with a declining seroprevalence observed in red foxes in Norway between 1993 - 1995 and 2003 - 2005, despite an increasing red fox density (Davidson, Bornstein & Handeland 2008). Even though not significant, an underlying two year cycling pattern of sarcoptic mange seems to occur in the Scandinavian wolf population. Such temporal fluctuations are not uncommon for mange and are reported for wolves in Spain and Canada, as a result of the one year delayed morbidity peak in red foxes and coyotes respectively (Todd, Gunson & Samuel 1980; Oleaga *et al.* 2011). Detailed long - term time series of mange prevalence in red foxes in Scandinavia are to my knowledge not available.

A major result of this study is the absence of mange - specific antibodies among all of the 56 captured pups. I have three primary hypotheses to approach the interpretation of that surprising finding:

First, sarcoptic mange causes a high mortality among the wolf pups, making it unlikely to capture them in late winter. Mange - caused decreased pup survival is reported from several wolf populations in North America (Todd, Gunson & Samuel 1980; Kreeger 2003; Schultz, Wiedenhoeft & Wydeven 2003; Wydeven *et al.* 2004; Jimenez *et al.* 2010). In Yellowstone National Park, pups observed with clinical signs of mange disappeared from the pack at 4 to 5 months of age (E. Almberg 2014, Department of Biology, Pennsylvania State University, pers. com.). After strong winters, seasonal effects with low mange prevalence in early spring due to hyperthermia were found in wolves and coyotes (Todd, Gunson & Samuel 1980; Wilson 2012). In my study however, the mange infested pups found dead or shot, had died from starvation or were euthanized in an emaciated state between October and January; hyperthermia was not reported as the cause of death in any wolves suffering from mange in Sweden. Further, I found a positive linear relationship between the number of wolves in a territory and the probability of a seropositive adult. However, if mange prevalence in a wolf pack causes high pup mortality, I would expect to have higher occurrence of seropositive adults in packs with low numbers of pups. The observed mortality among pups in Sweden were from two packs, which subsequently both collapsed. I would have expected to have observed more of these mortality events if they were a regular occurrence.

Secondly, wolf pups have less exposure to sarcoptic mange. The transmission of the mite is normally related to close skin contact (Pence & Ueckermann 2002). Considering the heterogenic distribution of seropositive individuals within the packs, this hypothesis would preclude a wolf-to-wolf transmission of the mite. In fact, the coexistence of seropositive, clinically effected animals with seronegative, healthy individuals over time in the same pack, as well as the observed recoveries from seropositive wolves, support a restricted wolf-to-wolf transmission. Experimental cross - infection of mites between different host species often fail (Arlian *et al.* 1984a; Arlian 1989). For the free - ranging wolves of Scandinavia and Spain, the red fox is the most possible origin of the Sarcoptes mites (Mörner *et al.* 2005; Oleaga *et al.* 2011) and cross species host transmission is possible even from ungulates to wolves (Oleaga *et al.* 2013). In Scandinavia, there is no evidence of any successful hunting behaviour of ungulates by wolf pups within the parental territory and both parents alive have been observed so far. During predation studies in 15 Scandinavian wolf packs one red fox was killed by a

collared wolf pup shortly before dispersal (Zimmermann 2014). Moreover, during winter, red foxes are mostly killed by adult wolves but are usually not consumed (P. Wabakken, Høgskolen I Hedmark, pers. com.). Thus, wolf-killed red foxes are most likely not brought to the den when the pups are young. I assume a low probability of direct exposure to mange infested red foxes by wolf pups. However, host seeking behaviour and a potential three week off - host survival of the mite, allows for transmission from red foxes at consecutively shared bedding sites or kills (Arlian *et al.* 1984a; Arlian *et al.* 1984b). However, pups in the Spanish population are equally effected as the adults (Oleaga *et al.* 2011). Also mange infested pups are also reported from the German population (I. Reinhardt, Büro Lupus, Germany 2013 pers. com.). Eight mange infested pups collected for necropsy from two different packs in the Scandinavian population as well as one pup with clinical signs, captured in February 2014, confirm potential exposure.

Third, the ELISA may not be sensitive enough to detect a weak antibody response of wolf pups. The wolf pups delivered for necropsy tested seropositive with the same method at the same institute which is applying it yearly on > 2000 domestic dogs of all ages (E. Osterman – Lind, SVA, Uppsala, pers. com.). Age of the captured pups ranged between 7 and 10 months, old enough for seroconversion which normally takes 1 – 5 weeks post infection (Bornstein & Zakrisson 1993; Bornstein, Zakrisson & Thebo 1995). Alopecia, a major clinical sign, partly caused by intense pruritus and the related scratching, was not observed on any of the 56 captured pups. Passive transfer of antibodies through colostrum milk from recently or currently exposed females support the immune system of the pup, but the effective period might be short (Wobeser 2006).

In conclusion, the wolf pups in general might be less exposed due to their behaviour and restricted mite transmission within the pack. Undetected high mortality is unlikely and probably limited to sporadic events. There was no evidence of an age - dependent bias by the ELISA.

The 21 mange infested wolves at necropsy were all collected in the southern part of the wolf range (Figure 1), consistent with the finding of the statistical model of the captured wolves. The high amount of pups among the wolves at necropsy is biased because they originated from outbreaks in only two packs. This uneven distribution of mortality rates within a wolf population is also seen in other populations (Schultz, Wiedenhoeft & Wydeven 2003; Almberg *et al.* 2012). Two - thirds of the collected wolves died due to starvation or were

euthanized in an emaciated state, consistent with Wobeser (2006) who describes mange infested coyotes and wolves as continuously scratching and biting themselves, until most of the reachable fur is removed, they stop foraging due to the intense pruritus and lose energy to thermoregulatory problems.

The statistical analysis of my study needs to be interpreted with care. First, model selection was based on 92 samples including only 15 seropositve samples and I did not account for repeated measurements of the same indivduals by treating the animal's identification as a random factor in a mixed-model design. The combination of a skewed, and in statistical terms small sample size with potential violation of independent observations, might give only a rough estimate of the real patterns. Due to the minor changes in the beta estimates of the top model, when increasing the sample size by 35 observations and having a low variance influence by the repeated measurements (data not shown), I consider the rough estimates to be credible. A further constraint is the lack of isolated sarcoptes mites from Scandinavian wolves. This might be related to the usually low total number of mites found on wolves (< 100) and the negative relation between mite numbers and the proportion of alopecia (Oleaga *et al.* 2011). Due to a non-systematic evaluation of clinical signs at capture, it was not possible to estimate sensitivity and specificity of the ELISA.

The increasing probability of seropositive wolves along the north - south gradient is consistent with other observed patterns in this wolf population. The home range size of territorial wolves is smaller in the southern part of the wolf area. Latitude is correlated to the proportion of agricultural fields and the roe deer density within the wolf area. (Mattisson *et al.* 2013). Both factors are probably important predictors of the general red fox density (Panzacchi *et al.* 2008). Red fox density might be a crucial factor for the exposure to sarcoptic mange. Data on red fox density is available on county level in Sweden based on bag statistics. However, for this study I did not include this data because I assumed a more accurate representation by latitude. This assumption is based on a contradictory development of the red fox density and the prevalence of mange in red foxes during parts of the study period (Davidson, Bornstein & Handeland 2008) as well as potential important influence of changes in the prey population to the growth rates in the red fox population. In addition, is it difficult to distinguish between fox dynamics and functional response of the hunters represented in bag statistics, especially after a regional mange outbreak. (Elmhagen *et al.* 2011). Further research could account for the red-fox density and the pack - and home range size of the wolves by creating a red fox – wolf ratio as done with other prey species estimating the probability of potential exposure

(Zimmermann 2014). Spatial differences in the occurrence of mange in Yellowstone National Park follow the wolf (host) density, which is related to the prey density (Almberg *et al.* 2012). In Scandinavia the density of the main prey within the wolf area varies between territories but is not related to the latitude (Mattisson *et al.* 2013).

Opposing other studies on mange and wolves (Todd, Gunson & Samuel 1980; Oleaga *et al.* 2011), I found a higher probability of seropositive samples from males. Sex difference in parasite infections can be based on both physiological or ecological reasons (Zuk & McKean 1996). Based on the assumption of restricted mite population development on wolves in this study as well as by Oleaga *et al.* (2013), physiological factors, such as testosterone levels, might play a role. Assuming equal chance of exposure to the mite based on ecological reasons, the time of measurable antibody response could be shorter in animals with better physiological defence mechanisms and therefore have a lower detectability. It has been shown that IgG binds sarcoptes antigen (ASA-1) inside the burrows (Ljunggren *et al.* 2006). Higher IgG levels on female Iberian Ibex *Capra pyrenaica hispanica* infested with *S. scabiei* is reported by Sarasa *et al.* (2010), but the results in that study are difficult to interpret. IgE level measured on a subset of the serum samples used for my study did not differ between males and females nor between mange seropositve or seronegative sera (Ledin *et al.* 2008). Testosterone has been shown to have an immuno - suppressing effect, a factor making males in general more susceptible to infestation of parasites. On the other hand, low sexual selection and a monogamous mating system as seen in wolves, are contradictory factors to sex-biased mange prevalence in wolves (Zuk & McKean 1996; Mech & Boitani 2003). However, the observed strong positive correlation of mange prevalence in female wolves and pack size maybe related to their increased susceptibility to parasites during parturition and lactation (Wobeser 2006).

Assuming equal physiological ability to defend against the disease, extra energy consuming efforts can lead to higher susceptibility or different moving patterns to higher exposure to parasites (Zuk & McKean 1996). Due to the high sociality and the monogamous mating system, sex difference in behaviour of breeding wolves might be small, except during the early stage of nursing the pups (Packard 2003). A potential effect on different behaviour has the sexual dimorphism as male wolves are 20% to 30% heavier than females (Wabakken *et al.* 2001; Mech & Peterson 2003; Zimmermann 2014). Due to the higher body mass males have a higher food intake (Peterson & Ciucci 2003) and might spend more time on carcasses or are more likely to prey upon red foxes than females. On the other hand, females have been

observed switching to smaller prey (e.g. moose to roe deer) when losing their partner (Sand *et al.* 2006) and thus may be more likely to kill red foxes. The breeding wolf pairs in Scandinavia use their territory equally during most of the year, but in summer males have been shown to travel more frequently on roads and probably spend more time scent-marking than females (Zimmermann 2014). Relating the assumption of restricted mite development on wolves with the relatively low degree of sex - related behavioural differences and the physiological arguments I would expect physiological reasons responsible for higher prevalence in males.

The general increasing probability to find seropositive adults with increasing pack size is consistent with the density dependence of sarcoptic mange (Pence & Ueckermann 2002). The lack of seropositive pups however does not contribute to the density of infested animals. Pack size represents the number of wolves in the territory and contains pairs, pairs with first litter and pairs with litter and subadults from the year before (due to mortality only one adult is present in a few cases). Thus, the increasing chance to be seropositive related to pack size, particularly seen on females, might be related to this often consecutive reproducing status. In this case the observed pattern would be rather an intrinsic than a density dependent factor. The lacking evidence of wolf density measured as the Euclidian distance to the next three neighbouring packs gives no support to the hypothesis of wolf density dependence and is opposes the patterns found in Yellowstone National Park (Almberg *et al.* 2012), but it supports the assumption of a restricted wolf-to-wolf transmission in Scandinavia.

The intrinsic factors, age and inbreeding coefficient, did not influence the probability of detecting seropositive wolves. Little is published on the possible effects of inbreeding on resistance to parasite infestations, but inbreeding can influence the immune response to parasites (Reid *et al.* 2007), the parasite load (Cassinello, Gomendio & Roldan 2001) and parasite avoidance behaviour (Luong, Heath & Polak 2007). The ELISA used in this study is not linked to parasite load or immune abilities, at least as long as the time since infection is unknown. If the recovery time of less inbred individuals would be shorter, the probability to detect inbred seropositive wolves would be higher. On the other hand, if mortality among inbred wolves with mange is higher, the detectability could be lower. Behavioural differences for wolves with different inbreeding coefficients has not been described yet. The applied ELISA might not be the right method to find potential influences of the inbreeding factor on susceptibility or exposure to sarcoptic mange. Correlating response ability like IGg levels or parasite loads to the inbreeding coefficient could be of future research interest.

Conclusions

Even though continually present, sarcoptic mange had a small potential effect on the sustainability and recovery of the Scandinavian wolf population. Wolf pups are probably less exposed to the parasite and mange dynamics are not related to the wolf density. Heterogenic seroprevalence distribution within the packs and repeated observed recovery suggest an effective host - parasite response and a restricted wolf-to-wolf transmission. However, devastating mortality on the pack scale is possible but its trigger is not understood. The higher probability of sarcoptic mange in the southern part of the wolf distribution is probably related to landscape factors and red fox population dynamics. Observed sexual differences are more likely related to physiological rather than ecological factors. Future research should address the humoral response of wolves and other canids to different sarcoptes strains. In Scandinavia the research scale should widen up to the entire carnivore guild for a more complete view on the dynamics and effects of this itchy disease.

Acknowledgments

I am very grateful to my three eminent supervisors from the Hedmark University College, Jon M. Arnemo for access to the unique data, the extraordinary fieldwork opportunities and the spread of the positive mood; Barbara Zimmermann for statistical support, motivation and lots of very useful comments; and Petter Wabakken for many creative inputs and enthusiasm. I also want to thank Katarina Näslund and Eva Osterman-Lind at SVA for involving me in the Lab work; Alina Evans for leading me into this topic, as well as for checking the English language and medical terminology; Martin Mayer for critical and inspiring discussions and companionship during fieldwork; and Cyril Milleret, for technical support in R.

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Appendix

ELISA

Serum samples were analysed by ELISA using a crude (whole mite extract) antigen according to Bornstein and Zakrisson (1993) and a recombinant major sarcoptes mite antigen (MSA-1) (Ljunggren *et al.* 2006). In each series, samples were tested in duplicates and positive and negative control samples from dogs were included. Wells of a micro titer plates are coated with the diluted antigen (Crude: 5-10 µg/ml, MSA-1: 0.5-1 µg/ml in 0.1 M carbonate buffer pH9.6, 100 µl / well, incubated at +4°C for 48h) and blocked with PBS-T (PBS with 0.5% Tween -20) for 1 hour at room temperature. Serum samples are diluted 1:100 in PBS-T, 100 µl add to each well and incubate at 37°C for 1 h. Anti-dog lgG (clone 1-17, SVA) 1:1000 in PBS-T and rabbit anti-mouse IgG-HRP (P 0260 DakoCytomation, Denmark) 1:1500 in PBS-T are added as secondary antibody and conjugate respectively with 100 µl / well and incubated for 1 hour at 37° C. TMB (20 mM TMB 1:20 in 0.1 M citrate buffer, pH 4.2 with 230 µl H_2O_2/l).100 µl/well, 10 minutes at room temperature. The reaction is stopped by adding 50 µl 10 % H_2SO_4. Optical density (OD) measured at 450nm. In order to get a OD value relative to the positive control, mean OD values for each duplicate are calculated and the mean OD of a blank (PBS-T instead of a sample) subtracted. The values are divided by the value of the positive control. OD of the positive control for the crude antigen must be between 0.8 and 1.6 and for the MSA-1 antigen between 0.7 and 2.0. Positive corrected OD values at 450 nm from samples tested by the crude antigen should reach >0.3 and >0.5 tested by MSA-1, relative to the positive control. Doubtful results reach 0.2-0.299 by the crude antigen and 0.35-0.499 by the MSA-1 antigen, OD values below are considered to be negative.

Western Blot

All doubtful and seropositive samples are tested by Western blot. The sarcoptes protein in the crude antigen extract is separated by polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane according to the method described by Bornstein, Zakrisson and Thebo (1995). The membrane is cut into two mm stripes. The sample diluted by 1:50 in 0.8 ml of Tris Buffered Saline with 0.05% Tween-20 (TTBS) and 2 % (w/v) of non-fat milk powder and incubated for one hour at room temperature. Monoclonal anti-dog Ig G (clone 1-17, SVA) 1:600 in PBS-T and rabbit anti-mouse IgG-HRP (P 0260 DakoCytomation,

Denmark) 1:2000 in TTBS plus 2 % milk powder are add as secondary antibody and conjugate respectively with 0.8 ml per stripe and incubate at room temperature for one hour. As substrate Amersham ECL western blotting detection reagents (GE Healthcare) are used according to the product manual. Hereafter the membranes are exposed to a film (Hyperfilm ECL, GE Healthcare) for 60 seconds and developed and fixed (AGFA G150, AGFA, 354). In order to test a sample positive, the proteins of 164 kDa or 147 kDa and 105 kDa have to be visible as bars.