Health Assessment and Parasites of Willow Grouse (Lagopus lagopus) in Sweden

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Abstract

A clinical examination of 530 willow grouse (*Lagopus lagopus*) was performed between 1989 and 2002 from four different areas in the northern part of Sweden. The result show that 90 % of the birds were infected with parasites and eight species of parasites were identified from the intestines. Only three species occurred in high numbers; the coccidian *Eimeria* sp., the nematode *Ascaridia compar*, and the cestode *Hymenolepis* sp. The average bodyweight of the birds varied significantly between the years and primarily for the juveniles. Statistical analyses indicated that that there was a significant negative effect of both *Eimeria* sp. and *A. compar* on the bodyweight, but only in juvenile birds. Neither ectoparasites nor bloodparasites were found, and *Campylobacter* spp. were not isolated in this study.

Sammanfattning

En hälsoundersökning mellan 1989 och 2002 utfördes på 530 dalripor (*Lagopus lagopus*) från fyra lokaler norra Sverige. Resultaten från studien visar att 90 % av fåglarna var infekterade med parasiter och sammanlagt åtta parasitarter från dalripornas tarmkanal identifierades. Endast tre arter var vanligt förekommande; koccidien *Eimaria* sp., rundmasken *Ascaridia compar*, och bandmasken *Hymenolepis* sp. Medelkroppsvikten hos fåglarna varierade signifikant mellan de olika åren, men i första hand hos de unga fåglarna. Statistiska analyser visade att det fanns ett signifikant negativ samband mellan antalet *Eimeria* sp. och *Ascaridia compar* samt kroppsvikten hos ungfåglarna. Varken ektoparasiter eller blodparasiter påträffades, och *Campylobacter* spp. isolerades inte vid något tillfälle.

Introduction

The willow grouse (*Lagopus lagopus*) has a circumpolar distribution on the northern hemisphere. It inhabits treeless low alpine areas and boreal forests during summer, and birch forest (*Betula betula*) and willow shrub (*Salix* spp.) are preferred habitats in winter (Brittas, 1992). From spring to autumn, willow grouse feed mainly on dwarf shrubs (*Vaccinium vitis idea*), while birch (*B. betula)* or willow twigs (*Salix* spp.) serve as the main winter food (Brittas, 1992). The willow grouse form pairs in spring and breeds at highest densities above the forest edge in the mountains. The females lay up to 10 eggs (Brittas, 1992).

The Swedish willow grouse population is estimated to fluctuate around 200000 adult pairs, which correspond to an autumn population widely varying around 900000 birds (Brittas, 1992). Around 70000 birds are shot annually in Sweden (Swedish hunting and management association, 2002).

Willow grouse hunting is an esteemed activity and has large recreational value to many hunters. Hunting with pointing dogs is a common hunting form in Sweden that occurs mainly in autumn. To some extent, birds are flushed and shot without the help of dogs. During winter, willow grouse are also stalked by skiing hunters or caught by snares (Brittas, 1992).

Despite this great interest for hunting and consumption of willow grouse in Sweden, few sick or dead birds have been investigated for parasites and diseases, probably because the species lives in remote areas and people does not commonly find debilitated or dead birds. There are also a great number of predators in these areas probably leading to that a majority of sick birds are preyed. Accordingly, knowledge about diseases among willow grouse in Sweden is therefore sparse.

Wild animal populations are regulated by a combination of biotic and abiotic factors. Although parasite infection is one of these factors, parasites can be present without affecting the individual host. On the other hand, if many animals of a population become infected with parasites, which have a density dependent harmful effect, then the dynamics of the host population may be partly or greatly regulated by the parasites (Borgsteede, 1996).

The caecal nematodeparasite, *Trichostrongylus tenuis*, is one of the most serious pathogens in the closely related red grouse (*Lagopus lagopus scoticus*) (Hudson, 1992). In Scotland it has been demonstrated that this parasite affect both the mortality and breeding success of grouse (Hudson, 1986; Hudson, 1992). Accordingly, this parasite have been demonstrated to be a key factor in generating the cyclic changes of the grouse population that has been observed both in Scotland and Northern England (Hudson & Dobson, 1990). In addition *T. tenuis* is believed to have negative effects on winter survival and chick mortality, probably due to an increased predation pressure because massive infections interferes with the birds ability to control scent emission (Hudson, 1992). Before this study was carried out, it

was not known whether this nematode was present in Swedish willow grouse.

Norwegian studies have shown that the willow grouse carry a community of at least eight species of parasites (Holmstad & Skorping, 2000), but *T. tenuis* has so far only been found in island and coastal areas (Holstad, Karbøl & Skorping, 1994). In contrast, the composition of the parasite community in Swedish willow grouse was largely unknown.

To get a better knowledge about the health status of willow grouse in Sweden, a monitoring program was initiated 1989. During 14 years, basic data from willow grouse were collected from four different localities to obtain information about local and annual variations. The birds were collected in late August and early September every year and examined for diseases and parasites. Effects of the parasite community on host body weight and body condition at that time were assessed. In addition the birds were screened for *Campylobacter* sp., as campylobacteriosis is a zoonotic disease and wild birds have been suggested as reservoirs (Broman et al, 2002). The aim of this study was to establish baseline data for certain micro- and macroparasites of willow grouse in northern Sweden.

Materials and methods

Collection of hosts

A total number of 530 birds were collected between 1989 and 2002 and each year between late August and early September from four different areas in the northern of Sweden (Arjeplog (66°15'N, 16°30'E), Norrbotten County, Funäsdalen (62°35'N, 12°45'E), Härjedalen County, Idre (61°55'N, 13°10'E), Dalarna County and Kittelfjäll (65°15'N, 15°30'E), Västerbotten County. Funäsdalen and Idre belong to almost the same geographical area, and the birds from both of these areas were therefore regarded as one and the same population. The birds from this area were therefore referred to as the southern group. The birds from Arjeplog and Kittelfjäll were also combined in the analysis and were regarded as the northern group. Totally 278 birds from Funäsdalen and Idre, and 252 from Arjeplog and Kittelfjäll were collected. Between 5 and 20 birds from each area were examined each year, but each locality was not represented every year. Hunters using pointing dogs shot all birds.

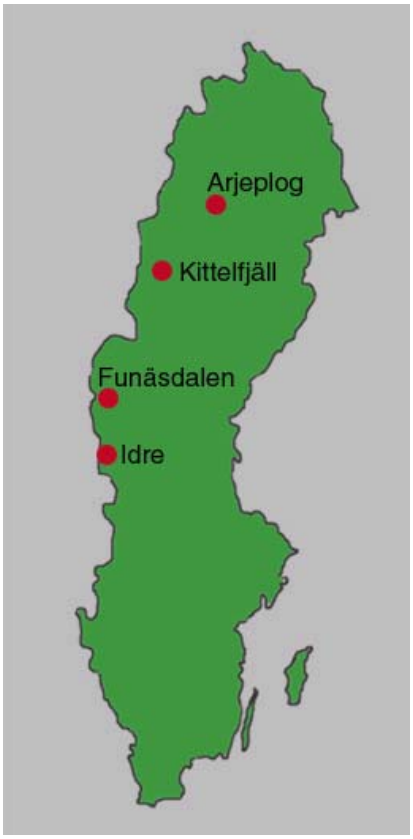


Fig. 1. Location of examined areas

Examination of hosts

The birds were inspected for ectoparasites and weighed immediately in the field. The wings and chestbone were measured to the nearest cm. The birds were classified as juveniles (2-3 months old) or adults (older than 14 months) based on the moulting sequence and pigmentation of the primaries (Brittas, 1988). Of the birds examined, 343 were classified as juveniles and 187 were adults. The remaining birds could unfortunately not be classified according to age. The birds were sexed the gonads were inspected at necropsy. Of the birds that were sexed, 262 were females and 261 were males.

A sample from the cloacal content was collected from 286 birds with a cotton wool swab supplied with Amies transport medium for culturing of *Campylobacter* sp. The samples were cultured in Preston broth (*Campylobacter* selective medium) for 24 hr at 42°C and plated on Preston agar, incubated for 48 hr at 42°C under microaerobic conditions. (NMKL no 119 2^{ed}). Screening for *Campylobacter* sp. was carried out between 1996 and 2002.

Tissue samples (0,5 cm^3) from lung, liver, spleen and kidney, in addition to gonads and pieces from different parts of the gastrointestinal tract were fixed in 3 % formaldehyde for later histopathological examination. Samples from the lungs (2 cm^3) were stored in 2 ml phosphate buffered saline solution (PBS) for screening of infectious agents/microbes as described by Mörner et al. (1988). The liver and kidneys were frozen and stored for chemical analyses. These results will be presented elsewhere.

Between 1989 and 1995, blood was collected by venipuncture of the heart and bloodsmears were prepared and examined for parasites. Prior to the microscopic examination the bloodsmears were stained in phosphate-buffered Giemsa as described by Godfrey et al. (1987).

The whole gastrointestinal tract was removed, and the length of the small intestine, caecum and the large intestine was measured to the nearest cm. Faeces from the large intestine was examined for coccidian oocysts. The oocysts were isolated by flotation in saturated NaCl solution and counted in a counting chamber using the microscope. The amount salt was correlated to the amount of faeces, in order to control for the mass of faeces examined. The oocysts observed in 2002 were identified according to Pellérdy, (1974).

The intestines including the caeca were sectioned longitudinally and carefully washed with water. The washings were screened through a sieve with a mesh width of 150 μm, and the material remaining in the sieve was fixed in 70% ethanol and then examined in a stereo-microscope. The nematodes were counted and the number of cestodes was graded as low, medium or high. All intestinal parasites that were found were stored in 70% ethanol, and representative specimens were mounted in polyvinyl lactophenol for later identification. The nematodes were identified according to Sprehn, (1932).

Some nematodes, stored in 70% ethanol, were prepared for scanning electronmicroscopy (SEM). The ethanol was gradually replaced with filtered freon TF and the specimens were critical-point dried, using CO_2 as the transitional fluid. The dried specimen were mounted on stubs with double-stick tape, coated with gold-palladium and examined at 10 kV in a Jeol LSM-820 electron SEM microscope.

Statistical analyses

Statistical analyses were performed using Excel 2000 version (Microsoft Corporation) in data summary and to perform descriptive analyses. One-way and multi-way of analysis of variance (ANOVA) were performed in order to analyse fluctuations in parasite burdens between years, geographical areas as well as in relation total body weight (TBW) and body condition (BC). An index for body condition was developed based on the residuals of the linear regression between the length of the wings and the total body weight. These analyses were all performed with StatView^{TM} 5,0 (SAS Institute Inc.) and were considered significant at $p \leq 0.05$ level.

Results

Body measures

Bodyweight

The bodyweight of both adult and juvenile birds varied significantly between the years ($p=0.0423$) and ($p<0.0001$), respectively. The adults had a significantly ($p<0.0001$) higher bodyweight than the juveniles, ranging from 390 g to 685 g, and the average weight was 558 g. The bodyweight of the juvenile birds ranged from 210 g to 605 g, and the average weight was 445 g. As can be seen in Fig. 3., it is evident that the bodyweight varied more between the different years among the juveniles than the adults.

Chestbone

The adult birds had a longer chestbone than the juvenile birds, and this difference was highly significant ($p<0.0001$).
The length of the chestbone measured on the adults ranged from 6.1 cm to 9.0 cm, and the average length was 7.5 cm. The length ranged from 4.3 to 8.3 cm on the juveniles, and the average length was 6.6 cm.

Wings

The adults had longer wings than the juveniles, and this difference was also significant ($p<0.0001$). The length of the wings ranged from 17 to 22 cm on the adults, and the average length was 20 cm. On the juveniles the length of the wings ranged from 15 to 21 cm, and the average length was 19 cm.

There was a significant ($p<0.0001$) positive relationship between the length of the wings and the length of the chestbone ($n=398$, $r^2=0.43$). The length of the chestbone increased with an increasing body weight.

There was a significant ($p<0.0001$) positive relationship between the length of the wings and the body weight ($n=442$, $r^2=0.57$).

Parasites

Of the willow grouse examined 90 % (n=477) were infected with at least one parasite species. Eight genera of parasites were identified, *Eimeria* sp., *Ascaridia* sp., *Hymenolepis* sp., *Capillaria* sp., *Ancylostoma* sp., *Heterakis* sp., *Syngamus* sp. *and Trichostrongylus* sp.

Eimeria sp.

Oocysts of *Eimeria* sp were exclusively found in the large intestine. The unsporulated oocysts were ovoid-cylindrical in shape and measured approximately 24 x 15 µm and was identified in 2002 as *Eimeria lagopodi* (Pellérdy, 1974). Of the examined birds were 76 % (n=404) excreting oocysts.

There were a significantly ($p<0.0001$) higher number of oocysts in juvenile birds as compared to adults. As can be seen in Fig. 2, this relationship was observed in 12 out of the 14 years. In 1994 the number of oocysts were higher in the adults, but in this year one of the adult birds had of 876000 opg, which may contribute to this result. One of the juvenile birds from 2001 had 600000 opg, which may contribute to the high average opg for the juveniles this year.

There were also a significant ($p<0.0001$) higher number of oocysts in the birds from the northern area. The annual mean intensity of oocysts in the juveniles was 35000 opg in the northern area and 16000 opg in the southern area. The annual mean intensity of oocysts in adults was 21000 opg in the northern area and 5100 opg in the southern area.

There was no liniear relationship between number of oocysts and the condition ($p=0,5056$) for the juvenile and the adult birds. However there was a significant negative regression ($p=0,0008$) between the number of oocysts and the body weight for the juvenile birds ($n=330, r^2=0,034$). In contrast there was no association between the number of oocysts and the body weight for the adult birds ($p=0,2556$).

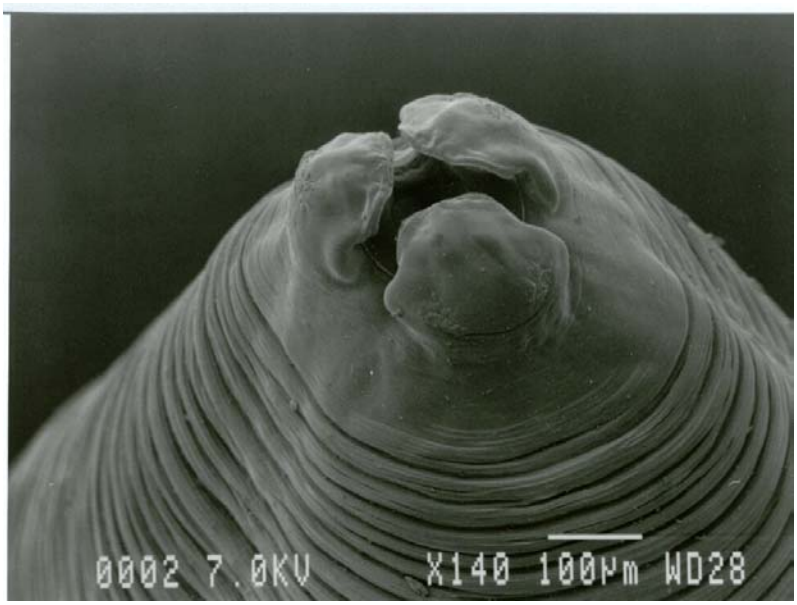
Ascaridiae sp.

Of the birds examined 40 % ($n=212$) were infected with *Ascaridiae* sp. The *Ascaridiae* sp. was identified as *A. compar* according to Sprehn, (1932). It seemed to be a yearly cyclic pattern in the number of *A. compar* in the willow grouse. The number was high during the first two years of the study. Then the number was low for four years, from 1991 until 1995, before numbers rose again and remained higher for four years until 1999. Then the number seemed to drop again.

Over the study period there was no significant ($p=0.8794$) difference in number of *A. compar* between the juvenile and the adult birds. The highest number of *A. compar* ($n=63$) was found in a juvenile bird that was shot in Arjeplog 1995.

There was a significantly ($p<0.0001$) higher number of *A. compar* in the northern as compared to in the southern area. The average number of *A. compar* in juveniles was 3.4 in the northern area and 0.9 in the southern area. The average number in the adult birds was 2.4 in the northern area and 0.7 in the southern area.

There was no significant ($p=0.48$) relationship between the number of *A. compar* and the condition factor. However, there was a significant ($p<0.001$) negative relationship between the number of *A. compar* and bodyweight in the juvenile birds.



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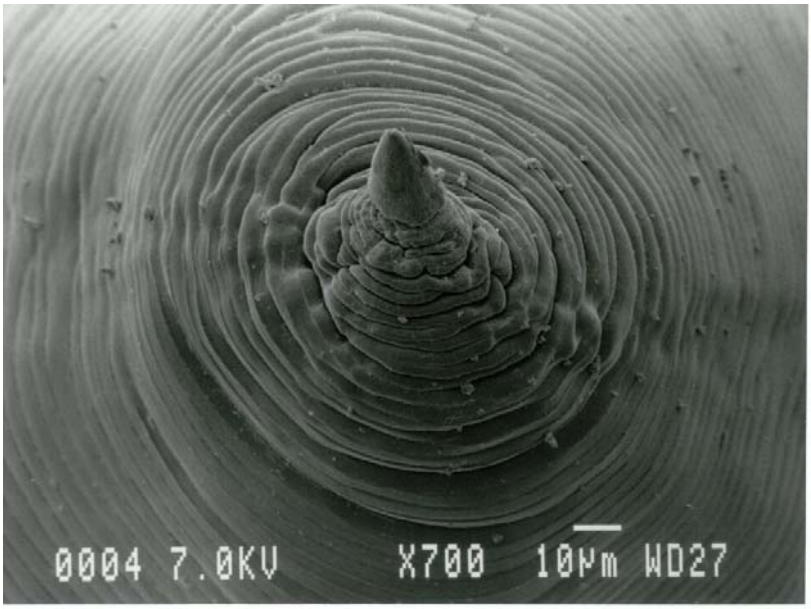

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Fig. 2. SEM micrographs of *Ascaridia compar*.
A: The anterior part. B: The posterior end.

Hymenolepis sp.

Of the willow grouse examined 41 % (n=217) birds were infected with *Hymenolepis* sp. The number varied significantly ($p<0.0001$) between years, but no obvious yearly cyclic pattern was observed. The *Hymenolepis* sp. was not identified at the species level.

There was no significant ($p=0.31$) difference in the amount of *Hymenolepis* sp.between the juvenile and the adult birds.

Birds from the southern area had a significant ($p<0.0001$) higher load of cestodes than the birds in the northern area. The mean score of *Hymenolepis* sp. for the juveniles was 0.4 in the northern area and 0.6 in the southern area. The mean intensity for the adults was 0.3 in the northern area and 1.0 in the southern area.

Other parasites and Campylobacter sp.

The nematode *Capillaria* sp. was found in 3 % (n=15) of the birds, but the intensity was low and no more than 10 worms was registered. This parasite was found occasionally in both the northern and the southern area from 1994 to 2000. Three specimens of the nematode *Ancylostoma* sp. from two birds from Kittelfjäll were identified in 1998. Only a single specimen of *Trichostrongylus* sp. was found in Arjeplog in 1995, one *Heterakis* sp. was found in Funäsdalen in 1989 and one single *Syngamus* sp were found in Kittelfjäll in 1999. Due to the limited number no further statistical analysis was performed. Furthermore parasites were not found in the blood smears. Neither were arthropod ectoparasites such as mites, ticks, lice nor fleas found on the birds.

Campylobacter sp were not isolated in any of the 286 samples.

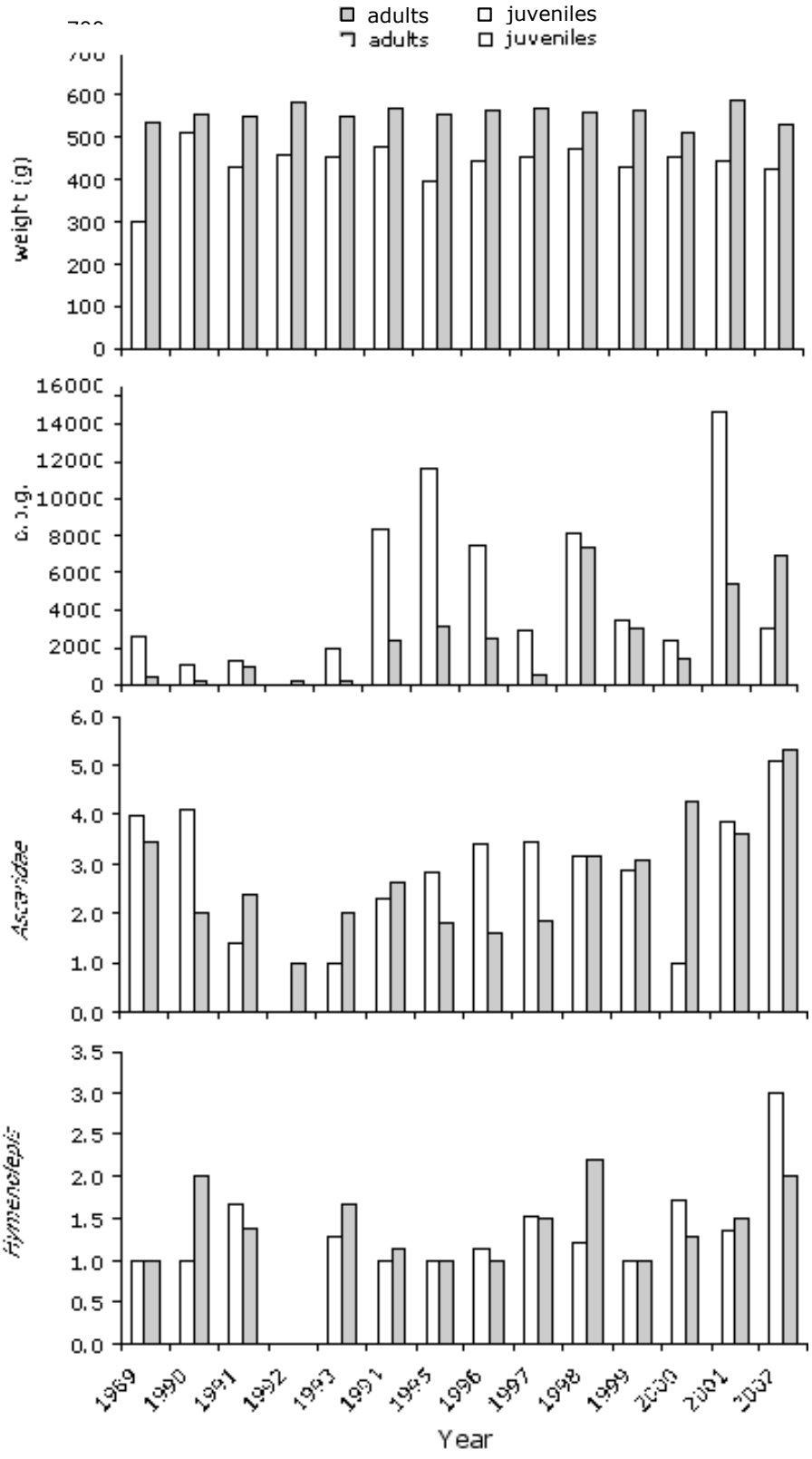

Fig.3. The annual average bodyweight and the annual average amount of *Ascaridia compar*, *Hymenolepis* sp. and oocysts of *Eimeria* sp. for the juvenile and the adult birds from 1989 to 2002.

Discussion

In this study we found that 90 % of the willow grouse were infected with parasites. Eight species of parasites were identified and of those did only the coccidian *Eimeria* sp., the nematode *Ascaridia compar* and the cestode *Hymenolepis* sp. occur at high levels. For these parasites we also found significant regional differences. The number of *Eimeria* sp. and *A. compar* were higher in the northern than in the southern area, while the number of *Hymenolepis* sp. were higher in the southern than the northern area. No reasonable explanation for this can be offered.

Throughout the study the variation in body weight was higher among the juvenile than in adult birds. The study showed that *A. compar* and *Eimeria* sp. had a negative influence on the body weight, but this was only observed for juvenile birds. An alternative explanation is that the point of time for hatching and the supply of food vary between years as cold and rainy weather also has a major influence on the weight gain of birds.

Still, microparasites like the coccidian *Eimeria* sp. have the qualifications for being involved in the regulation of the host population (Holmstad, 1998). In as short as five days unsporulated oocysts are liberated from the body together with the faeces. Outside the host they then develop to infective sporulated oocysts. Accordingly, coccidian parasites can be transmitted from the hen to the newly-hatched chickens before they are able to resist the infection (Urquhart et al, 1996). This may result in a high mortality rate and especially among the chickens if they are exposed to large numbers under conditions that are suitable for transmission (Holmstad, 2002).

The present survey on parasites of willow grouse indicates that there was a tendency for a cyclic pattern in the intensity of *A. compar*. The number of this nematode seemed to fluctuate according to a four-year cyclic pattern with low numbers for four years and then followed by an increase the fifth year. One reason for the generation of this pattern could be that in years with a high level of *A. compar*, it coincided with a peak in the small rodent population. It is a traditional view that when the population of lemming (*Lemmus lemmus*) and other small rhodents are high, then there is less predation on eggs and chicks from stoat (*Mustela erminea*), red fox (*Vulpes vulpes*) and corvids. Consequently, the predation pressure on willow grouse will increase dramatically when the number of alternative preys, i.e. small rodents drop. Hence the most heavily infected birds become an easier prey for the predators, which will result in a situation where the number of *A. compar* in willow grouse will drop again.

Norwegian studies have shown that cestodes can affect the health of willow grouse by causing loss of weight and by weakening the skeleton (Holmstad, 2002). It has also been reported that cestode infections reduce the condition of the birds and they are therefore more vulnerable to predators (Holmstad, 2002). An examination of golden eagles (*Aquila chrysaëtos*) in Norway showed that this birds of prey eat willow grouse and especially if heavily

infected with helminth parasites such as nematodes and cestodes (Holmstad, 2002). In the present study no such relationship were observed and the cestode parasite, *Hymenolepis* sp., seemed to present in relatively low number and it appeared to be relatively harmless.

Evidence for the negative influence of parasites on host populations comes from work on the red grouse, *Lagopus lagopus scoticus,* and particularly in relation to the caecal trichostrongylid nematode *Trichostrongylus tenuis*. In fact it has been shown that *T. tenuis* burdens can seriously affect the red grouse (Borgsteede, 1996). The direct effects of the infection are reduction of body condition, body weight, adult survival, clutch size, hatching success and chick survival (Borgsteede, 1996).

T. tenuis is present in willow grouse in Norway (Holstad, Karbøl & Skorping, 1994), but this parasite turned out to be nearly absent in populations of the Swedish willow grouse. However in Norway there was a marked pattern in the occurrence of *T. tenuis* in grouse populations from different areas. Island and coastal populations had a relatively high prevalence, while the parasite was more or less absent in inland populations (Holstad, Karbøl & Skorping, 1994). Survival of larvae on the ground is markedly affected by reduction in humidity, and a distribution limited to coastal areas, with a relatively higher precipitation than in inland alpine version, is therefore not unexpected (Holstad, Karbøl & Skorping, 1994). The samples in this study were collected solely from such inland populations, which can explain the almost complete absence of *T. tenuis.*

It is known that many mammals are carrying *Campylobacter* spp., but there seems also to be a particularly strong association with both wild and domesticated birds (Broman et al, 2002). *Campylobacter* spp. is the most common cause of food-borne bacterial enteritis in humans in Sweden. Clinical signs include diarrhoea, which may be accompanied by vomiting, fever and abdominal discomfort (Ettinger & Feldman, 2000). In a study of black-headed gulls (*Larus ridibundus*) in southern Sweden, *Campylobacter* spp. were isolated in ca 30 % of the birds, and more than 90 % among these were identified as *C. jejuni*, which is the main human pathogen (Broman et al 2002).

Campylobacter spp. Was not isolated in any of the samples from willow grouse in our study. Dietary variations between gulls and willow grouse may be a reason for why it was not isolated from willow grouse. A Norwegian study showed that pigeons, which are herbivorous, had an incidence of 4%, while crows and gulls, which are omnivorous, showed a prevalence of 90 % and 50 %, respectively (Kapperud & Rosef, 1982). Other obvious reasons for the absence of this bacteria are that it can not survive in a climate such as in the alpine areas of northern Sweden, or that the bacteria has a preference for certain hosts.

In conclusion, the present results indicate that most of the willow grouse were infected with parasites, even though *T. tenuis was* absent in Swedish populations, other parasites like *A. compar* and *Eimeria* sp. seemed to have a negative influence on the weight gain in these birds.

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