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AN EXPERIMENTAL STUDY ON THE EFFECTS OF POLYMORPHIASIS IN COMMON EIDER DUCKLINGS

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ABSTRACT: Eight common eider (*Somateria mollissima*) ducklings were experimentally infected from 1 June through 13 June, 1995 with acanthocephalans (*Polymorphus minutus*) by allowing the birds to feed on *Gammarus* spp. (*Gammarus oceanicus, G. salinus, G. zaddachi,* and *G. lacustris*) containing acanthocephalan cystacanths. Uninfected *Gammarus* spp. were fed to a control group of seven ducklings. No mortality of ducklings occurred during the experiment. However, the infected ducklings gained weight more slowly than the control birds. After the 2 wk study period, the mean serum concentrations of total protein, albumin, β-globulin, γ-globulin, fructosamine and creatine kinase were lower in the infected group than in the controls. The mean (±SE) number of acanthocephalans in the intestine of the infected ducklings was 21 (±4). The parasites were attached to the mucosa of the posterior small intestine of the infected ducklings with a mixed inflammatory reaction consisting of heterophils and mononuclear lymphocytes surrounding the attachment sites.

Key words: Acanthocephala, common eider, experimental infection, pathology, *Polymorphus minutus,* serum chemistry, *Somateria mollissima.*

INTRODUCTION

The common eider (*Somateria mollissima*) is the most abundant waterfowl species in the Baltic Sea of northern Europe. The number of common eiders breeding in the Finnish archipelago increased from the 1940's to the mid-1980's, reaching an estimated maximum of about 200,000 pairs (Hario, 1998). Sporadic mortalities have occurred in the population for many years, but in the late 1980's duckling survival dropped to as low as 1 to 5% in some breeding areas in the Gulf of Finland. As a result, the recruitment rate decreased and numbers of breeding pairs began to decline. Recently, mortalities of both ducklings and adults have been reported from various breeding areas in coastal Finland (Hollmén et al., 1996; Hario, 1998).

Common eiders are infected with a variety of intestinal parasites. Of these, acanthocephalans in particular have long been implicated in the mortality events and have been considered a potential factor in the population decline (Grenqvist, 1951; Persson, 1974; Hario et al., 1992). *Polymorphus minutus* is the only acanthocephalan species reported in eider ducklings from Finland. Suitable intermediate hosts for *Polymorphus botulus* are not found in the eider breeding areas, and these parasites have not been associated with eider duckling mortality in the area. Elsewhere, acanthocephalans also have been commonly found in parasitologic surveys of wild waterfowl, including eiders (Garden, 1964; Bishop and Threlfall, 1974; Thompson, 1985), but experimental studies of the possible health effects of these parasites on their avian host are rare.

Breeding populations of eiders have been closely monitored at the Söderskär Game Research Station in the central Gulf of Finland since the late 1940's. At Söderskär, duckling survival also dropped to 1 to 5% in the late 1980's. Recent studies at Söderskär suggest that the acanthocephalan *Polymorphus minutus* may be a contributing factor to the mortality but are probably not the primary cause of it (Hario et al., 1995; Hollmén et al., 1996). We

have previously found that the infection rate (number of parasites infecting the host per day) of *Polymorphus minutus* was similar in apparently healthy eider ducklings and those found dead (Hario et al., 1995).

We studied the effects of acantocephalan infection in eider ducklings during their first 2 wk of life to further evaluate the role of these parasites in the mortalities. Our aim was to determine if parasite intensities reported from free-living ducklings (Hario et al., 1995) were capable of causing death, illness, or subclinical health effects in ducklings reared in captivity at Söderskär.

MATERIALS AND METHODS

The study was conducted at Söderskär Game Research Station, in the Gulf of Finland (60°06′N, 25°25′E). Fifteen common eider hatchlings were collected on 1 June 1995 from four separate nests of three to five hatchlings each at the Söderskär Sanctuary. The ducklings from each nest were allocated in turns into the experimental and study groups of eight and seven birds, respectively. All ducklings were housed in a wooden shelter in a 3 × 3 m fenced area, and fed *ad libitum* with a commercial pelleted formula for ducklings (crude protein content 17.5%, Ankka FC, Oy Feedex Ab, Kolppi, Finland). They spent the night indoors, roosting in creches in semi-open wooden boxes layered with cotton. The temperature of the room ranged from 15 to 20 C and a supplemental heat source (insulated gasoline heater) was available for the ducklings next to the roosting box. During the day birds were moved to outdoor cages where they could swim and dive in natural pools that formed on rocky island terrain.

Gammarus spp., which serve as the intermediate host for *Polymorphus minutus,* were used to infect the experimental birds. *Gammarus* spp. were caught with fine-meshed nets from Söderskär and from lake Ormajärvi near Lammi Biological Station (61°03′N, 25°02′E). *Gammarus oceanicus, G. salinus,* and *G. zaddachi* were collected from Söderskär and *G. lacustris* from lake Ormajärvi. Each gammarid was examined visually for cystacanths, and separated into infected (containing one or more cystacanths) and uninfected groups. Ducklings in the study group were allowed to feed on infected gammarids released into a small pool, and the number of gammarids consumed was recorded. Uninfected gammarids were fed to the control group in a similar manner. Feeding of gammarids was started on 1 June and continued until 13 June. With this protocol, we tried to imitate conditions in the wild, where ducklings are likely to accumulate their parasite loads over a period of several days or weeks. The amount of gammarids fed daily was also based on the energy requirement formula for eider ducklings provided by Swennen (1989).

Birds were examined daily for clinical signs of infection. Growth of the ducklings was monitored by weighing the birds twice daily (0600 and 2200 hours) with a Pesola® scale, but only the evening (2200 hours) weight was used in statistical analysis. Tarsal and combined skull and bill lengths of the ducklings were measured every 3 days. After 14 days, the ducklings were anesthetized with 0.2 ml of medetomidine-ketamine 1:1 solution (Domitor® 1 mg/kg, Orion-Farmos, Helsinki, Finland; Ketalar® 50 mg/kg, Parke-Davis, Barcelona, Spain) intramuscularly and 2 ml of blood was drawn from the heart of each duckling. After the sample was taken the ducklings were killed with an intracardiac injection of 2 ml of pentobarbital (Mebunat®, Orion-Farmos, Helsinki, Finland).

Blood smears were prepared, and fixed with methanol for 10 min. Seventy μl of blood was drawn into a heparinized microcapillary tube and centrifuged in a microhematocrit centrifuge for 5 min for packed cell volume (PCV) measurement. The blood remaining in the syringe was transferred into a glass tube without anticoagulant. Blood samples were allowed to clot at room temperature for 60 min, centrifuged at 1,500 × g for 10 min, and serum was separated and stored at −70 C before the laboratory analyses.

Complete necropsies were performed on all carcasses. Sex was determined by the presence or absence of bulla tympaniformis of the syrinx (Broman, 1942), and carcasses were examined for gross abnormalities. The intestinal tract was divided into 10 cm segments. The segments were cut open lengthwise, and the contents of each were washed into a petri dish. Contents and mucosa of each segment were examined with a dissecting microscope. Species, number and location of macroparasites in intestinal segments were recorded. The identification of the acanthocephalans was based on the size of the parasites and the appearance of the proboscis and hooks (McDonald, 1988). A voucher specimen of *Polymorphus minutus* was deposited in the Finnish Museum of Natural History (Helsinki, Finland; accession number 379/1999).

Samples of skeletal muscle, heart, lung, liver, kidney, adrenals, proventriculus, gizzard, duodenum, pancreas, jejunum, ileum, caecum, and cloaca were fixed in 10% neutral buffered for-

malin. Samples of duodenum, jejunum, and ileum were cut transversely approximately midway along the length of the intestinal segment. Fixed tissue samples were embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (Stevens, 1990), and examined by light microscopy. Special staining methods were employed for detection of fibrous tissue (Herovici, 1963) and mast cells (toluidine blue by Francis, 1990). Blood smears were stained with May-Grünwald Giemsa (Slider and Downey, 1950) for 60 min, and examined for 10 min under a 40× objective for hematozoa. The heterophil/lymphocyte ratio was determined by differential count of 100 leucocytes from the smears and dividing the number of heterophils by the number of lymphocytes.

Serum chemistry analyses were performed at the Central Laboratory of the Faculty of Veterinary Medicine (University of Helsinki, Helsinki, Finland). Activities of serum aspartate aminotransferase (AST, EC 2.6.1.1), alkaline phosphatase (AP, EC 3.1.3.1), creatine kinase (CK, EC 2.7.3.2), and lactate dehydrogenase (LDH, EC 1.1.1.27) were determined according to the recommendations of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974, 1979). Sorbitol dehydrogenase activity (SDH, EC 1.1.1.14) was analyzed using D-fructose as the substrate (Gerlach and Hiby, 1974). Total protein was analyzed by the biuret method (Weichselbaum, 1946). Triglycerides were determined using an enzymatic, colorimetric method (Wahlefeld, 1974). Enzymatic methods were used to determine free fatty acids (FFA, Shimizu et al., 1980), glycerol (Eggstein and Kreutz, 1966), and urates (Fossati et al., 1980). Fructosamine (glycosylated protein) was determined with a colorimetric reduction test with nitroblue tetrazolium (Johnson et al., 1982). Serum protein electrophoresis was performed on agarose film according to manufacturers instructions (Beckman Instruments, Inc., Application Manual, Brea, California, USA). Films were dried and scanned at 600 nm in a Beckman Appraise™ densitometer. Serum protein fractions were separated into pre-albumin, albumin, α_1-, α_2-, β-, and γ-globulins.

Student's *t*-test (Sokal and Rohlf, 1995) was used to test for differences in the mean starting weights and tarsal lengths between the experimental and control groups. Analysis of covariance (Sokal and Rohlf, 1995) using initial weight or length as a covariate was used to test for differences in growth. A nonparametric Mann-Whitney *U*-test (Sokal and Rohlf, 1995) was used to test for differences in blood parameters, as many of the variables showed a non-

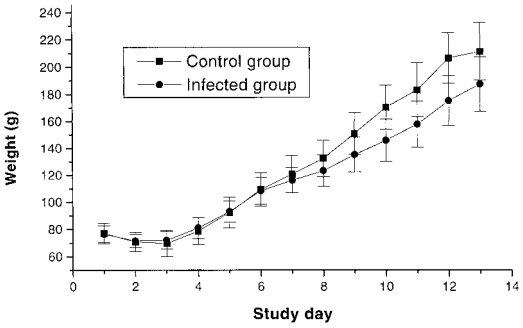

FIGURE 1. Evening (2200 hours) weight (mean ± SD) of ducklings infected with *Polymorphus minutus* and those of controls during the 2 wk study period.

normal frequency distribution. Significance was determined at $P \leq 0.05$.

RESULTS

Each duckling consumed 160 to 170 gammarids and each duckling in the experimental group consumed 160 to 170 cystacanths during the 14 day study period. No apparent signs of disease were observed in the ducklings. There was no difference in the mean starting weights between the infected and control ducklings ($P = 0.907$), but the ducklings in the control group gained weight faster ($P = 0.022$) than the infected ducklings (Fig. 1). Mean (±SD) weight after the 2 wk study period was 211.3 (±21.1) g in the control group and 187.4 (±20.0) g in the experimental group. A significant difference in weight ($P = 0.029$) between the groups was first observed after nine days of the study. After the 2 wk study period, the tarsi were longer ($P = 0.011$) in the control group (35.7 ± 1.6 mm) than in the experimental group (34.8 ± 1.3 mm), although lengths at the beginning of the study were not different ($P = 0.283$). No significant difference was detected in the combined skull and bill length measurement between the groups after two weeks ($\bar{x}$ ± SD were 64.4 ± 2.2 mm and 63.8 ± 1.4 mm for the experimental and control groups, respectively).

At the end of the 2 wk study period, the mean serum concentrations of total protein, fructosamine (glycosylated protein), and creatine kinase were lower ($P = 0.004$,

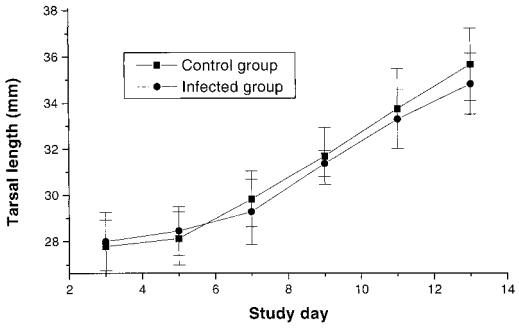

FIGURE 2. Tarsal length (mean ± SD) of ducklings infected with *Polymorphus minutus* and those of controls during the 2 wk study period.

$P = 0.023$, and $P = 0.010$, respectively) in the infected ducklings than in the controls (Table 1). The infected ducklings also had lower serum concentrations of albumin ($P = 0.004$), β-globulin ($P = 0.007$), and γ-globulin ($P = 0.048$) (Table 2). No significant differences were detected in the other serum biochemistries (Table 1). The mean (±SD) PCV and heterophil/lymphocyte ratio of the infected ducklings (42 ± 3% and 1.39 ± 0.80, respectively) were not significantly different from those of the controls (33 ± 9% and 0.73 ± 0.32).

Polymorphus minutus was the only intestinal macroparasite found in the infected ducklings. One hundred and sixty five of 1342 (12%) cystacanths fed to the ducklings established in the intestines over the 2 wk study period. The mean (±SE) number of parasites in the infected ducklings was 21 (±4) and the mean infection rate was 1.5 parasites per day (number of parasites found at the end of the study divided by number of days of the study). The mean (±SD) total length of the intestine of the infected ducklings was 96.3 (±8.0) cm and of the controls 101.9 (±10.2) cm. The acanthocephalans were found between 30 to 90 cm posterior to the gizzard in the small intestine (jejunum and ileum). No macroparasites were found in the intestines of the control birds. No hemoparasites were detected in blood smears.

There were two males and five females in the control group and four males and four females in the experimental group. No gross abnormalities or histopathological changes were observed at necropsy of the ducklings in the control group. The small intestines of the ducklings in the experimental group were distended and fluid-filled but no gross abnormalities were observed in other tissues. Histologically, a mixed inflammatory reaction consisting of heterophils and mononuclear lymphocytes surrounded the attachment site of the parasites in the lamina propria of the small intestinal wall. Parasites did not penetrate into the muscular layer of the intestinal wall. No other pathological changes were detected in histologic sections.

TABLE 1. Comparison of the mean (±SD) and range for serum biochemical values for eider ducklings infected with *Polymorphus minutus* and those of controls.

	Infected group		Control group	
	Mean ± SD	Range	Mean ± SD	Range
Aspartate aminotransferase (AST) (IU/L)	32 ± 14	22–63	73 ± 75	13–185
Alkaline phosphatase (AP (IU/L)	846 ± 226	484–1,257	694 ± 159	506–915
Creatine kinase (CK) (IU/L)[a]	15 ± 7	7–28	95 ± 97	22–233
Lactate dehydrogenase (LDH) (IU/L)	781 ± 241	574–1,288	628 ± 460	167–1,401
Sorbitol dehydrogenase (SDH) (IU/L)	3.0 ± 0.7	2.2–3.8	13.1 ± 10.7	5.5–20.7
Triglycerides (mmol/L)	1.8 ± 0.5	1.3–2.5	1.4 ± 0.3	1.1–1.7
Free fatty acids (FFA) (mmol/L)	1.7 ± 0.6	0.8–2.7	1.6 ± 0.5	1.0–2.3
Glycerol (μmol/L)	397 ± 111	286–590	326 ± 61	232–400
Urates (μmol/L)	293 ± 82	187–417	338 ± 49	263–386
Fructosamine (μmol/L)[a]	229 ± 38	164–268	278 ± 13	266–292
Total protein (g/L)[a]	27.3 ± 2.8	24.1–31.2	34.5 ± 2.8	31.7–38.4

[a] Significant difference detected between the infected and control groups ($P \leq 0.05$).

TABLE 2. Comparison of the mean (±SD) and range for serum protein values for eider ducklings infected with *Polymorphus minutus* and those of controls.

	Infected group		Control group	
	Mean ± SD	Range	Mean ± SD	Range
Pre-albumin (g/L)	3.1 ± 1.3	1.8–5.7	2.9 ± 0.9	2.0–4.2
Albumin (g/L)[a]	12.0 ± 2.2	9.2–14.6	17.5 ± 2.0	16.3–18.9
α_1-globulins (g/L)	1.9 ± 0.3	1.6–2.3	2.4 ± 0.8	1.7–3.8
α_2-globulins (g/L)	6.7 ± 0.7	5.7–7.7	7.0 ± 1.1	6.1–8.8
β-globulins (g/L)[a]	3.0 ± 0.2	2.7–3.3	3.9 ± 0.5	3.3–4.3
γ-globulins (g/L)[a]	0.6 ± 0.1	0.5–0.8	0.9 ± 0.3	0.6–1.3

[a] Significant difference detected between the infected and control groups ($P \leq 0.05$).

DISCUSSION

We found significant differences in serum protein levels between the infected ducklings and controls at the end of the 2 wk study period. The eider ducklings infected with acanthocephalans had lower serum concentrations of total protein and albumin, both of which may be a consequence of a parasitic infection of the alimentary tract (Hochleithner, 1994). Dietary proteins are enzymatically digested and the resulting amino acids are absorbed in the small intestine, primarily in the jejunum of birds (Brue, 1994). Acanthocephalans use amino acids for growth and reproduction (Starling, 1985), and the direct absorption of amino acids by the parasites may decrease the availability of these nutrients to ducklings. Furthermore, damage to the intestinal mucosa caused by the parasites may interfere with absorption of dietary nutrients. Histopathological lesions associated with acanthocephalans have been reported in the intestines of wild birds (Thompson-Cowley et al., 1979; Moore and Bell, 1983; McOrist and Scott, 1989), and the inflammatory response found in eider ducklings was similar to that earlier described in other birds. The reaction consisted of local infiltrations of mixed inflammatory cells and was restricted to the mucosal layer of the small intestine. Although localized, the inflammatory response in the intestinal mucosa may have interfered with absorption of amino acids and contributed to the lower serum concentrations of proteins in the study group. Other than parasitic infections, potential causes for low levels of protein in the avian blood include chronic intestinal diseases of other etiology, hepatic and renal diseases, and overhydration (Hochleithner, 1994). As we found no evidence for other potential causes for the difference in serum protein concentrations between the infected ducklings and the controls, we attribute this finding to the acanthocephalan infection.

Serum fructosamine also was lower in the infected ducklings than in controls. Fructosamine concentrations have been used in veterinary medicine as an indicator of serum glucose levels within the most recent days or a week (Kawamoto et al., 1992). The rate of synthesis and elimination of fructosamine depends on the serum protein concentration and composition, and on the turnover rate of various serum proteins (Jensen, 1992). Although the clinical use of serum fructosamine has not been reported in birds, our finding of lower fructosamine levels in ducklings infected with acanthocephalans may reflect lower serum protein concentrations and possibly also lower serum glucose levels in these birds during the study period.

Levels of β- and γ-globulins were also lower in the serum of the infected ducklings than in the uninfected ducklings. This finding also may have been associated with the parasitic infection. β-globulins consist of complement, hemopexin, transferrin, ferritin, plasminogen, fibrinogen, lipoproteins, and some immunoglobulins

(Kaneko, 1989; Hochleithner, 1994), and γ-globulins consist mainly of immunoglobulins. Serum levels of transferrin may decrease in acute inflammatory diseases (Kaneko, 1989), and this is one possible explanation for the lower level of β-globulins in the infected ducklings. Furthermore, as the immune defense mechanisms of a young bird rely heavily on maternally-derived immunoglobulins during the first weeks of life (Gerlach, 1994), the infected ducklings may have been using immunoglobulins acquired from the hen faster than the uninfected ducklings and thus exhibited lower levels of β- and γ-globulins in their serum after the 2 wk study period. In addition, the infected ducklings may not have been able to produce immunoglobulins of their own because of nutritional amino acid deficiency.

Mean serum creatine kinase (CK) level was higher in the control group than in the infected ducklings. Creatine kinase enzyme reacts rapidly in tissue changes and has been used in birds to evaluate muscle cell damage (Hochleithner, 1994). We attribute the difference in CK activity to high levels (164 and 233 IU/1) detected in two ducklings in the control group. These levels may have been caused by a recent, vigorous muscle activity in these birds.

Lochmiller et al. (1993) fed diets containing 8, 15, or 33% protein to northern bobwhite chicks and showed that lower dietary protein contents (8 and 15%) reduced growth rates of the chicks significantly. In our study, ducklings infected with acanthocephalans gained on average 23.5 g less weight than the uninfected ducklings. This difference in weight gain may also be explained by the decreased intestinal absorption of nutrients and increased loss of nutrients to the acanthocephalan metabolism as indicated by the lower serum protein levels in the experimental group. Furthermore, reduced appetite and food intake associated with intestinal parasitism has been documented in animals (Holmes and Zohar, 1990), and may also have affected the growth and nutritional status of the infected ducklings. The growth of the infected and control ducklings diverged after day nine of the experiment. Possibly at this point, a critical number of parasites had matured in the intestines to produce the observed effects. Also, ducklings acquire nutrients from their yolk during the first week of their life, and effects of intestinal parasitism may have been enhanced after the reabsorption of yolk is complete.

The possible role of parasites as regulators of wildlife populations has gained wide attention during recent decades (Gulland, 1995). Potentially, parasites can affect the population size of their host through various direct and indirect mechanisms. Parasites may cause mortality, disease that renders their hosts more susceptible to predation and infectious agents, nutritional stresses, and reduced reproductive success (Munger and Karasov, 1989; Toft, 1991; Gulland, 1995). Parasites with low or moderate virulence and high transmission rates have been considered potentially most effective as long-term regulators of host population size (Anderson, 1979).

Acanthocephalans have been frequently reported in common eiders in the Baltic Sea (Persson, 1974; Itämies et al., 1980; Hario et al., 1992), but their impact on the eider populations is not clear. We found that *Polymorphus minutus* caused significant changes in serum protein concentrations and reduced weight gain that may lead to clinical health effects and reduction of fitness in common eider ducklings. The parasite intensity in the experimentally infected ducklings was even lower than that reported in the wild: in an earlier study, Hario et al. (1995) reported infection rates of 12.3 parasites per day for apparently healthy eider ducklings and 9.3 parasites per day for those found dead. Therefore, the acanthocephalans may alone or together with other factors contribute to the low duckling survival rate observed during the past decade in the Gulf of Finland. In other experimental

studies, low levels of dietary protein have been shown to reduce immunocompetence of mammals and birds (Slater and Keymer, 1988; Lochmiller et al., 1993) and lowered levels of serum proteins in eider ducklings infected with acanthocephalans may render them more susceptible to other diseases. In addition, infected ducklings may be more susceptible to predation by gulls, and the parasites also may enhance the degree of malnutrition in eiders in years and areas of limited food resources. These effects may in fact be more pronounced in wild birds than in experimental birds fed *ad libitum* diets in a sheltered environment.

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