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Long-term Pathological Survey of Icelandic Rock Ptarmigan (*Lagopus muta*); Renal, Dermatological, and Traumatic Lesions

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ABSTRACT
Pathological findings in wild birds are rarely reported. This paper reviews gross and histological findings in samples from Icelandic rock ptarmigan (*Lagopus muta*), obtained through both systematic and lesion-based organ sampling. The study was part of larger project in which ptarmigan were collected and dissected over a 13- year period to study health and population changes. The primary pathological conditions identified were mange and renal tubular oxalosis, with prevalences of 18.3% and 25.3%, respectively. Mange resulted from skin mite infestations, which were more common in juveniles than in adults and more prevalent in females than males. The sex difference was attributed to a higher prevalence in adult females compared to adult males. The prevalence of renal tubular oxalosis did not vary by age or sex, but juveniles exhibited higher average severity scores for the condition than adults. Despite coccidia being found in faecal samples and in histological sections of the intestine, negligible histopathological lesions were seen in the intestinal mucosa. Lesions due to trauma showed a notably high prevalence of 2.6%. The study highlights the importance of histological examination of organs from hunter-collected specimens.

Keywords: disorders, histology, mange, oxalate, parasites, wild birds

YFIRLIT
*Langtíma meinafræðileg rannsókn á rjúpu (*Lagopus muta*); Breytingar í nýrum og húð ásamt áverkum*
Fáar birtingar eru til um meinafræði villtra fugla. Í greininni er lýst stórsæjum og vefjafræðilegum breytingum í efnivið sem fékkst bæði við kerfisbundna sýnatöku og sýnatöku af tilfallandi skemmdum í líffærum íslensku rjúpunnar (*Lagopus muta*). Meinafræðilega rannsóknin var hluti af stærra verkefni þar sem rjúpur voru krufðar á 13 ára tímabili til að rannsaka heilsufar og breytingar á stofnstærð. Helstu meinafræðilegu niðurstöðurnar voru kláðabreytingar, með tíðni upp á 18,3%, og útfellingar af oxalat kristöllum í nýrapíplum, með tíðni upp á 25,3%. Kláðinn stafaði af húðmaurasýkingu og var kvillinn algengari hjá ungum fuglum en þeim fullorðnu, og algengari hjá kvenfuglum en körrum. Kynjamunurinn var rakinn til hærri tíðni kláðans hjá fullorðnum kvenfuglum samanborið við fullorðna karra. Tíðni útfellinga oxalat kristalla í nýrapíplum var ekki mismunandi eftir aldri eða kyni, en meðalgildi alvarleikastigs kvillans var hærra í ungfuglum en í þeim fullorðnu. Þrátt fyrir að hníslar hafi fundist í saursýnum og í vefjasneiðum af þörmum, sáust hverfandi litlar vefjameinafræðilegar skemmdir í slímhúð þarma. Óvænt var að sjá skaða vegna áverka í 2,6% fuglanna. Rannsóknin sýnir mikilvægi vefjafræðilegrar rannsóknar á líffærasýnum úr fuglum sem veiðimenn hafa safnað.

INTRODUCTION

Pathological examination, including autopsy and histopathology, along with ancillary laboratory tests such as bacteriology, parasitology and virology, are important tools in diagnosing clinical and subclinical diseases (Cooper 2002). However, pathological findings are rarely reported in studies of wild birds, and similar studies to those presented in this paper have not been found in the literature, bar one (Hensel et al. 2018). A few papers describe pathological findings in sick or deceased wild birds brought to wildlife hospitals, rehabilitation centres or research centres (Cummings et al. 2022, Garcês et al. 2018, Gottdenker et al. 2008, Magagna et al. 2019, Montesdeoca et al. 2017). A Norwegian study on mortality causes in captive-reared willow (*Lagopus lagopus*) and rock ptarmigan (*Lagopus muta*) chicks describes pathological findings from hatching through their first three weeks (Hanssen & Ness 1982). Hanssen's 1982 study identified the main causes of death as congenital conditions, infectious and non-infectious gastrointestinal diseases, and fungal pneumonia.

The rock ptarmigan, hereafter called ptarmigan, is the only grouse (family Phasianidae) found in Iceland. The ptarmigan is a common and widespread breeding bird that plays a key role in the terrestrial ecosystem, serving as the main wild vertebrate herbivore in dry habitats and the primary prey of the gyrfalcon (*Falco rusticolus*) (Petersen 1998). Ptarmigan numbers in Iceland have traditionally shown an approximately 12-year population cycle (Guðmundsson 1960, Nielsen & Pétursson 1995). The density difference between peak and through years can be up to 28-fold (Ferrarini & Nielsen 2025). Recently, the dynamics have changed, and the cycle no longer exists, except in Northeast Iceland, where it has a 5-year period (Johnson & Nielsen 2024). The 12-year cycle was thought to be driven by the predator-prey relationship between the gyrfalcon and the ptarmigan (Nielsen 1999). Currently, other factors influence the cycle dynamics in Northeast Iceland, and the system appears to be regulated from the bottom up rather than from the top down, as it was previously (unpublished data).

In 2006, a study on ptarmigan health and population changes was initiated. The study lasted 13 years and concluded in 2018. Each fall, birds were collected for the research (Nielsen et al. 2013). One part of this project examined potential recurring pathologies and their relationship to the birds' age and sex. In 2006, a preliminary pathological examination was conducted on organs from some of the sampled ptarmigan using histopathology. Based on the 2006 results, a sampling protocol was implemented for the remaining years, focusing on the kidneys, skin, and intestinal tract. This paper describes gross and histopathological lesions observed in organ samples collected systematically or from incidental macroscopical lesions detected during post-mortem examinations of ptarmigan sampled for the health study. The paper also includes a statistical analysis of the two main pathological lesions, specifically mange and renal tubular lesions oxalosis.

MATERIAL AND METHODS

Ptarmigan sample

A total of 2,730 ptarmigan were collected for the health study by shooting in autumn from 2006 to 2018, in upland areas west, east, and north of Lake Mývatn, northeast Iceland (65° 37′ N, 17° 00′ W) (Table 1, Figure 1). All but one of the juvenile birds (n = 2,162) and 461 of 567 adult birds were collected between September 28 and October 10 under a license issued by the Natural Science Institute of Iceland, authorized under law 64/1994, chapter 4, article 7 (http://www.althingi.is/lagas/140a/1994064.html). The remaining 106 adults and one juvenile were acquired from regular hunters and were shot during the hunting season from October 20 to November 8 in 2006–2009. Juvenile birds were approximately three months old, while adults were at least one year old. The yearly goal for dissecting and sampling birds was 100 individuals, including 40 adults and 60 juveniles, with a balanced sex ratio in each

Table 1. Age and sex composition of rock ptarmigan collected for studies on health and condition, Northeast Iceland 2006−2018. Adult birds are one year old or older, and juvenile birds are approximately three months old.

Groups	Adult males	Juvenile males	Adult females	Juvenile females	Total
Dissected birds	313	405	199	377	1294
Extra birds	36	816	19	565	1436
All Groups	349	1221	218	942	2730

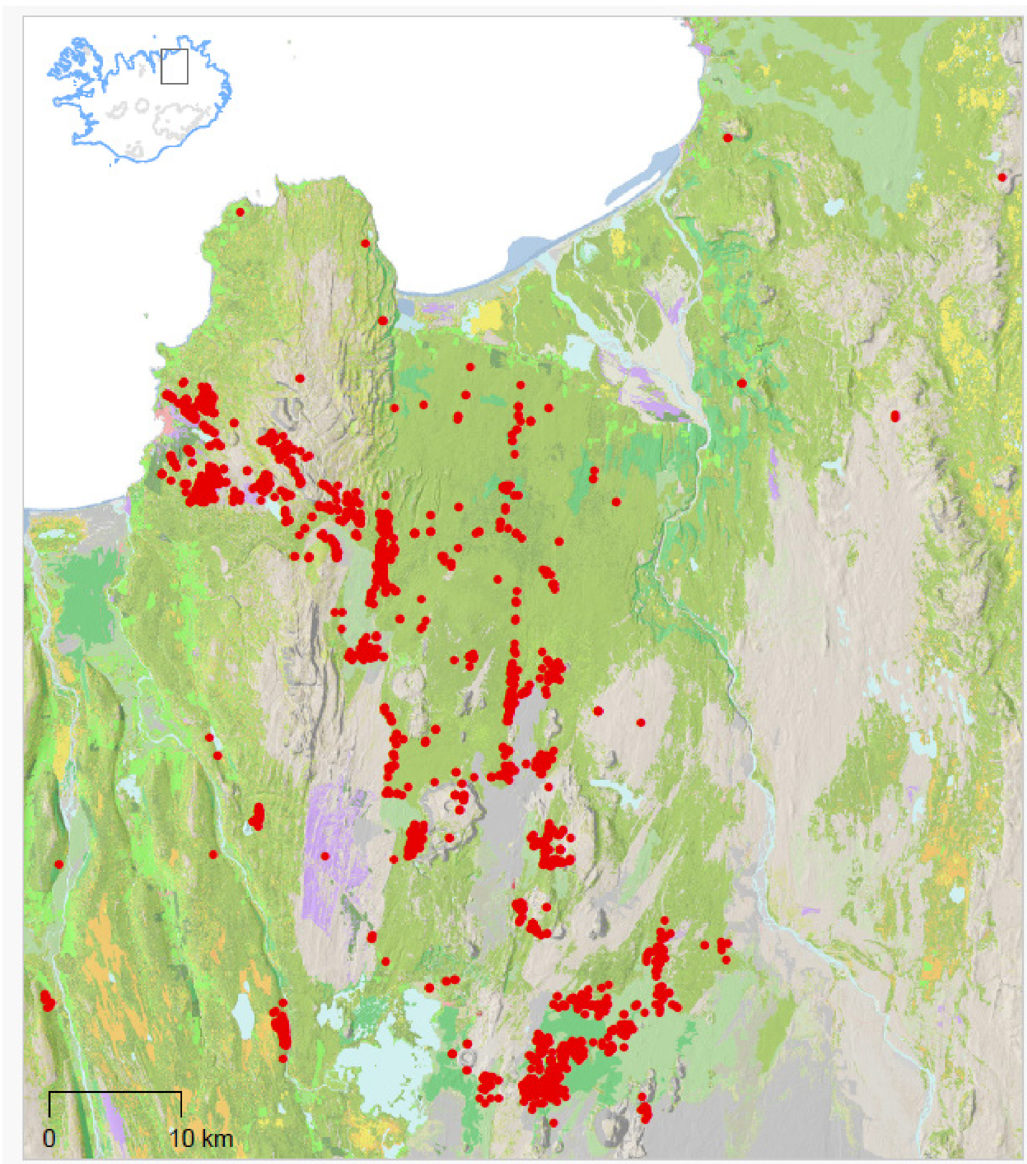

Figure 1. The ptarmigan health study area in Northeast Iceland, along with the sampling locations for the rock ptarmigan analyzed from 2006 to 2018 (n = 2,730). A red dot may represent one or more ptarmigan.

group. To meet this goal, birds were collected in excess each year, except in 2018, because juveniles dominated the collections and the sex ratio was male-biased, as females migrate out of the study area more than males in autumn. The surplus birds, called “extra birds,” were all aged, sexed, and sampled for external morphologies. Each year, either 20 or 30 birds were kept frozen whole for later analysis. The annual quota for adult females was only met in 2006–2009 by sampling birds from regular hunters during the open season.

Study design

In a pilot study in fall 2006, tissues were collected from various organs for histological examination of 18 dissected birds, 11 juveniles, and seven adults. Additionally, some incidental lesions found during the dissection from other birds were sampled and are included in the description of the relevant organ systems below. The organs examined from these 18 birds included the heart, lungs, liver, kidney, spleen, alimentary tract, and brain. The main histological findings were in the kidneys where oxalate crystals were observed in nine birds. Based on the prevalence of the crystals, renal tissues were sampled annually from all dissected birds, starting in 2007. Furthermore, since some extra birds were kept frozen in stock, it was possible to revisit these samples at the end of the study period to increase the sample size for both 2006 and adult birds. In total, renal tissues from 1,263 ptarmigan were sampled and examined histologically, comprising 1,217 samples from dissected birds and 46 samples from extra birds.

In 2006, skin lesions characterized by dry, scaly crusting of the skin surface were observed in several ptarmigan (Figure 2), and skin samples were examined histologically. As these lesions were found to be associated with skin-burrowing mites, skin crusting is referred to as mange. Mange was quite common among the ptarmigan collected and was documented every year of the study. During dissection after 2006, all birds were scored for the presence or absence of mange. Additionally, the severity of the lesions was objectively classified as mild (1), moderate (2), or severe (3). To score the birds, feathers were first plucked from both the breast and the back, and the exposed areas of scaly skin were estimated: mild indicated 25% or less cover, moderate represented 25–75% cover, and severe indicated 75–100% cover.

Histopathology was performed on skin samples from 33 birds collected from 2006 to 2017, including 28 birds for which data on the presence or absence of mange and the number of skin mites collected were available (see below for collection method).

From 2007 to 2011, tissue samples were collected from the intestinal tract of 52 birds, including 32 dissected birds and 20 extra birds, in total three adults and 49 juveniles. The samples were from 10 different areas along the whole length of the intestinal tract, including

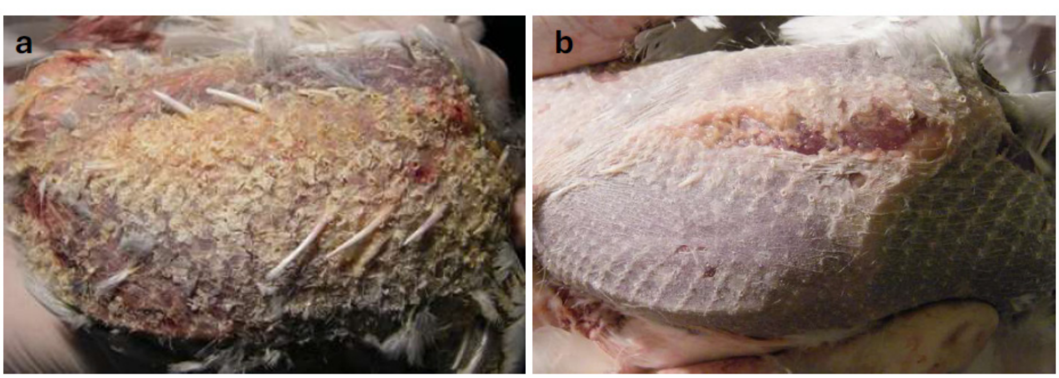

Figure 2. Lateral view of a plucked rock ptarmigan breast and belly. (a) A bird with severe mange; note the scaly, crusted skin, a characteristic of skin mite infestation. (b) The skin of a normal bird for comparison.

Table 2. Overview of organs sampled from rock ptarmigans in Northeast Iceland from 2006 to 2018, and the histopathological findings.

Anatomical location	*n*	Diagnosis / no. of cases	Sampling protocol *
Kidney	1,263	Renal tubular oxalosis / 319	SO
Intestine	52	*Eimeria* infection / 44 *Capillaria caudinflata* infection / 6	SO
Crop	13	Granulomatous ingluvitis / 5 Granulomatous periingluvitis / 8	IL
Skin	33	Hyperplastic dermatitis / 17 With intralesional mites / 15	SO/IL
Musculo-skeletal system	13	Fracture / 10 Myositis & muscle necrosis / 3	IL
Coelomic cavity	8	*Mesocestoides canislagopodis infection* / 4 Inflammation, bursa fabricii / 1 Diverse lesions, reproductive organs / 3	IL

* SO = Systematic organ sampling. IL = Incidental lesions found at autopsy and/or histology

one from the duodenum, three from the jejunum, two from the ileum, three from the caecum, and two from the rectum. Endoparasite data exist for the 32 dissected birds.

Throughout the study period from 2006 to 2018, samples from some of the incidental macroscopic lesions observed at autopsy were collected for histopathology from a total of 34 ptarmigan (Table 2).

Histopathological examination

The sampled tissues were fixed in 10% neutral buffered formalin, processed by routine paraffin embedding, and 4 µm thick sections were cut, mounted, and stained with haematoxylin-eosin (HE). Samples from bone tissue were decalcified in 10% formic acid before processing. Additional histochemical stains included Von Kossa for calcium, Luna stain for eosinophilic granulocytes, Masson-trichrome for connective tissue, Gram stain for bacteria, and PAS and Grocott's methenamine silver stain for fungi, when indicated based on initial histological findings. A selection of renal samples was stained with Alizarin Red S at pH 4.2 and pH 7.0, and some renal samples were also treated with 2M acetic acid and 0.1N hydrochloric acid to ascertain the presence or absence of oxalate crystals. Oxalate crystals dissolve in hydrochloric acid but not in acetic acid, and they stain positive with Alizarin Red S at pH 7.0 but not at pH 4.2 (Proia & Brinn 1985).

Formalin-fixed renal samples from 38 birds examined in 2013 were embedded in OCT in plastic moulds and immediately snap-frozen, sectioned at 7 µm, and stained with Oil-Red O and Sudan-III to ascertain the presence or absence of lipids in the glomeruli.

One histological slide with one to two sections of formalin fixed renal tissue was examined for each of the 1,263 birds. As the area of renal sections varied in size and the distribution of the unevenly sized crystals was irregular, the amount of oxalate crystals in samples from each bird was objectively evaluated. Their abundance was categorized as mild (1), moderate (2), or severe (3), with the approximate number of crystals per histological slide being < 50 for category 1, 50-150 for category 2, and >150 for category 3.

Parasitological examination

The collection and quantification of endo- and ectoparasites were described by Skirnisson et al. (2012). Coccidian oocysts and helminth eggs were quantified from the faecal material of 32 birds out of 52 sampled for histopathology of the intestinal tract, following a modified McMaster procedure, with the identification of coccidians based on Skirnisson and Thorarinsdottir (2007) and Matsubayashi et al. (2024). Skin mites were collected using a handheld vacuum cleaner

(Princess, Turbo Tiger, Type 2755) connected to an external collection chamber fitted with a circular sack-like filter. Each bird was vacuumed systematically, covering the whole body in approximately 2 minutes. Skin mites were mounted in Hoyer's medium (Gaud and Atyeo, 1996), with mite identification based on Mironov et al. (2010). No data on skin mite infestations were available for 2018.

Statistical analysis

Statistical analysis was conducted using the software package Statistica (Dell Inc. 2015) and the website QPweb (Reiczigel et al. 2019). The mean intensity of skin mites refers to the average number of mites per infected host. The prevalence of mange, skin mite infestation, oxalate deposits in the kidneys, and injuries was defined as the proportion of birds showing each condition. The Sterne method was used to calculate the 95% confidence limits for prevalence values (Reiczigel et al. 2019). Fisher's exact test was applied to compare the prevalence between two groups. To analyse the relationship between skin mite prevalence and mean intensity, two types of GLMs were employed. For prevalence, a GLM with a binomial distribution and a logit link was used, with explanatory variables including age, sex, and mange severity stage (four stages: no mange, mild, moderate, and severe mange), along with their two-way interaction terms. For mean intensity, a GLM with a negative binomial distribution and a log link was utilized, with the same explanatory variables. To examine the relationship between mange and oxalate deposit prevalence with ptarmigan age and sex, as well as their interaction, a generalized linear model (GLM) with a binomial distribution and a logit link function was used. The Mann-Whitney U test was used to compare the severity index across different age groups for oxalate deposits. Spearman's rank correlation was used to assess the relationship between the number of mites per host for the two skin mite species. The alpha level, two-tailed, was set at $p = 0.05$ for all tests.

RESULTS

Integument

Data on the presence or absence of mange and skin mite infestations were collected for a total of 1,108 birds, including 272 adult males, 330 juvenile males, 175 adult females, and 331 juvenile females. There was a highly significant positive correlation between the number of skin mites per host for the two skin mite species, *Metamicrolichus islandicus* (*M. islandicus*) and *Myialges borealis* (*M. borealis*) (Spearman's rank coefficient = 0.672, n = 1108, $p < 0.001$). As a result, these mites were grouped in the analysis below. Mange was observed in 203 birds (prevalence, 18.3%; 95% confidence interval, 16.1–20.7), and skin mites—when combined for both species—were found on 275 birds (prevalence, 24.8%; 95% confidence interval, 22.3–27.5). A significant difference in prevalence was noted, with mite infestations being slightly more common than mange (Fisher's exact test, two-sided p-value < 0.001). The same trend was observed across different ptarmigan age groups; mite prevalence was higher than mange prevalence (Figure 3).

The prevalence of mange was associated with the age and sex of the ptarmigan, as well as their interactions. Mange was more common in juvenile than in adult ptarmigan (Wald Stat. = 12.493, df = 1, $p < 0.001$), and more prevalent in females compared to males (Wald Stat. = 3.246, df = 1, $p = 0.071$). The age-by-sex interaction was significant, with adult females showing slightly higher prevalence than adult males, which differed from the pattern in juveniles where both sexes had similar prevalence (Wald Stat. = 4.507, df = 1, $p = 0.034$; Figure 3).

Both the prevalence and mean intensities of skin mite infestations clearly correlated with mange, with this relationship explained by the age of the birds, the stage of mange, and their interaction (Figure 4). The prevalence and mean intensity varied according to the severity score for mange (prevalence: Wald Stat. = 159.416, df = 3, $p < 0.001$; mean intensity: Wald Stat. = 57.076, df = 3, $p < 0.001$). Values were lowest in birds without mange and increased with higher severity scores (mild, moderate, severe

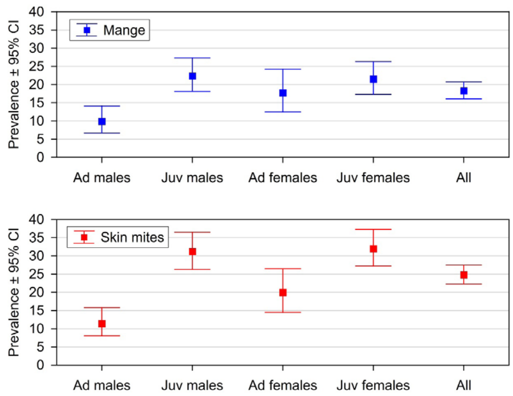

Figure 3. Prevalence and 95% confidence intervals of mange and skin mite infestations in rock ptarmigan in Northeast Iceland from 2006 to 2017 (n = 1,108 birds). "Ad" refers to adult birds, one year old or older; "Juv" refers to juvenile birds, approximately three months old; and "All" represents the total sample.

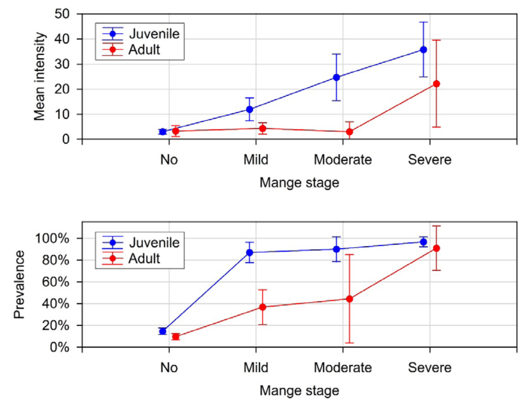

Figure 4. Prevalence and mean intensities of mite infestations, with 95% confidence intervals, in rock ptarmigan in Northeast Iceland from 2006 to 2017, by mange stage. Values displayed are weighted marginal means derived from a generalized linear model. Mange was categorized into four stages: no sign of mange (No); mild mange; moderate mange; and severe mange.

mange). Both parasite measures were higher in juveniles than in adults (prevalence: Wald Stat. = 14.062, df = 1, $p < 0.001$; mean intensity: Wald Stat. = 18.237, df = 1, $p < 0.001$). The interaction between age and severity score was significant for both measures (prevalence: Wald Stat. = 16.432, df = 3, $p = 0.001$; mean intensity: Wald Stat. = 16.209, df = 3, $p < 0.001$). This interaction illustrated how parasite levels change across mange severity stages based on the birds' age (Figure 4). The prevalence of skin mites was low in juvenile hosts without signs of mange (15%) but increased across severities (87-97%). In adults, prevalence was low without mange (10%) and rose gradually with severity, reaching 91% in the most severe cases. The mean intensity in juveniles followed the expected pattern: low in birds without mange (average 3.0 mites) and increasing with severity, peaking in those with severe mange (average 35.8 mites). In adults, the pattern was different, with low and similar intensities in birds without mange and with mild to moderate mange (average 3.0–4.5 mites), but it increased sharply in severe cases (average 22.2 mites).

Histopathology of skin from 11 adult birds and 17 juvenile birds further supports the

Table 3. Results from histopathology of the skin in 28 rock ptarmigan that had also been scored for the presence or absence of mange and also had mites collected from their plumes and the surface of their skin. Northeast Iceland, 2006 to 2017.

Age group	Sample size	Mange	Histopathology: birds with dermatitis	Histopathology: birds with intralesional mites	Collection of mites: birds with mites
Adult	4	No	0	0	0
Adult	7	Yes	2	1	0
Juvenile	6	No	1	1	2
Juvenile	11	Yes	10	8	10

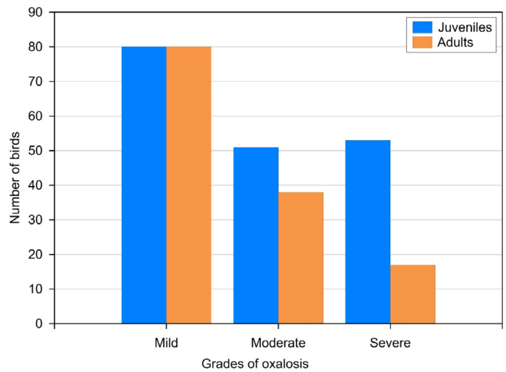

Figure 5. Renal tubular oxalosis in rock ptarmigan in Northeast Iceland from 2006 to 2018, categorized by severity. Juveniles were around three months old, and adults were at least one year old.

observed age difference in mange and skin mite infestations (Table 3). Of the four adults classified as without mange, all had normal skin, and no mites were collected from the plumage. Of seven adults with mange, only two exhibited dermatitis, including one with mites present in the skin; mites were not collected from any of those birds. Out of six juveniles scored as free of mange, one had dermatitis and also yielded skin mites during ectoparasite collection, along with one additional bird. All but one of the 11 juvenile birds scored with mange had dermatitis, and this individual was also the only bird that did not have skin mites collected. For the 10 birds with dermatitis, intralesional mites were observed in eight of them.

Histologically, mange lesions were those of hyperplastic dermatitis. As seen in one of seven adults and 14 of 15 juveniles with dry, scaly crusting skin surface, the lesions were characterized by a variable thickening of the epidermis due to orthokeratotic hyperkeratosis, epidermal hyperplasia, and hypertrophy in the stratum germinativum (Figure 6d). There were subcorneal and occasional intraepidermal pustules, and sometimes serocellular crusting. Exocytosis of granulocytes and epidermal necrosis were present in a few cases. The inflammatory reaction in the dermis varied from mild to moderate, with infiltration of eosinophilic granulocytes and small mononuclear cells. Lymphoid aggregates in the deep dermis were seen in a few skin samples, and occasionally neovascularization was present. Apart from a small focal granuloma in the deep dermis in two ptarmigan, an overt granulomatous reaction was not a feature of the mite infestation. Mites, and occasionally their eggs, were seen in the stratum corneum in all but two juvenile ptarmigan (Table 2, Figure 6d).

Urinary system

A total of 1,263 birds were examined histologically for oxalate deposits in their kidneys, including 317 adult males, 391 juvenile males, 191 adult females, and 364 juvenile females (Table 2). Oxalate crystals were present in 319 birds, with a prevalence of 25.3%, (95% confidence interval: 22.9–27.8%), including 89 adult males (28.1%, 23.3–33.3%), 100 juvenile males (25.6%, 21.5–30.2%), 46 adult females (24.1%, 18.5–30.6%) and 84 juvenile females (23.1%, 18.9–27.7%). There were no significant differences based on age (Wald Stat. = 0.459, df = 1, p = 0.498) or sex (Wald Stat. = 1.610, df = 1, p = 0.205), nor age-sex interactions (Wald Stat. = 0.070, df = 1, p = 0.791). Nonetheless, a highly significant age difference was found in the average severity score of oxalate deposits between age groups (z = 3.393, p < 0.001). Juveniles exhibited higher mean severity scores than adults, 1.85 (sd = 0.840) versus 1.53 (sd = 0.710) (Figure 5).

Histologically, oxalate crystals were present in the renal tubules, and only rarely in the glomeruli, and inflammatory reaction was minimal. Tubular epithelial cells were attenuated, and multinucleated giant cells (MNGCs) frequently surrounded the crystals (Figures 7a and c). MNGCs without crystals were also seen in kidneys, both with and without oxalate deposits, sometimes contained thread-like material (Figure 7c). A variable amount of calcium was present in the crystals, and calcium deposits were sometimes seen in the glomeruli, independent of the presence or absence of oxalate crystals in the renal samples. In some kidneys, there were the occasional swollen glomeruli

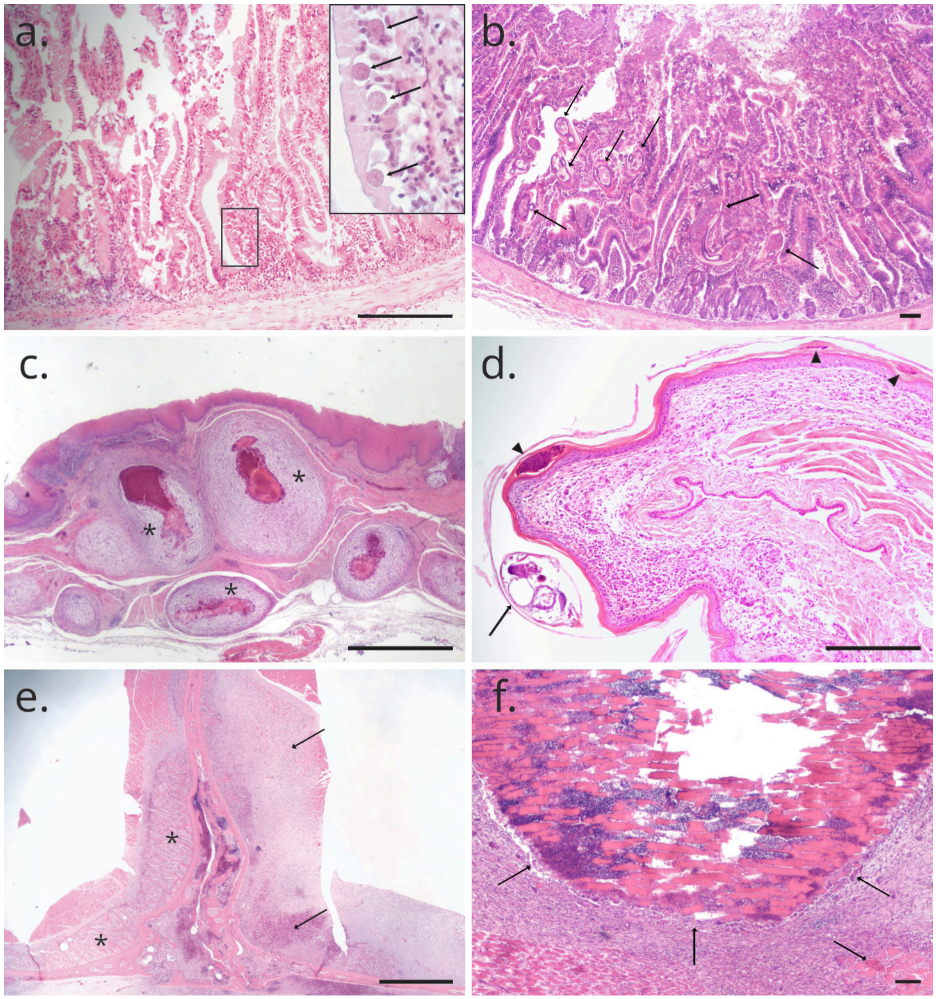

Figure 6. Examples of histological lesions observed in rock ptarmigan from Northeast Iceland between 2006 and2018.

a. Proximal small intestine of a juvenile female with mild coccidia infection. There is minimal inflammation, but autolytic desquamation of the mucosal epithelial lining is present. The inset shows a higher magnification of an area of the mucosa that has a few endogenous stages of coccidia in the epithelium and subepithelial lamina propria, as indicated by the arrows. The faecal sample was positive for *Eimeria muta*, but not for *Eimeria rjupa*. HE. Bar = 100 mm.
b. Duodenum of a juvenile male with nematode infection. The nematodes are closely associated with the mucosa (arrows). There is hyperplasia of the crypts. *Capillaria caudinflata* was identified on parasitological examination. HE. Bar = 100 mm.
c. Crop of a juvenile female with ingluvitis. Multiple granulomas are in the wall of the crop that is lined by intact, but hyperplastic, mucosal epithelium. HE. Bar = 1000 mm.
d. Skin of a juvenile male showing widespread mange affecting the whole body. Histologically, there is hyperplastic dermatitis with an intralesional mite (arrow) and subcorneal pustules (open arrows). Moderate inflammation is in the dermis. HE. Bar 100 mm.
e. Sternum of a juvenile male with traumatic lesions. There is a fracture of the carina and body of the sternum, with extensive callus formation (*). In the pectoral muscle, there is haemorrhage, fibrosis and muscle atrophy (arrows). Most of the pectoral muscle had been dissected free from the sternum prior to fixation. HE. Bar =1000 mm.
f. Pectoral muscle of a juvenile male with traumatic lesions. There is muscle necrosis (arrows), haemorrhage, inflammation, and granulation tissue formation. HE. Bar = 100 mm.

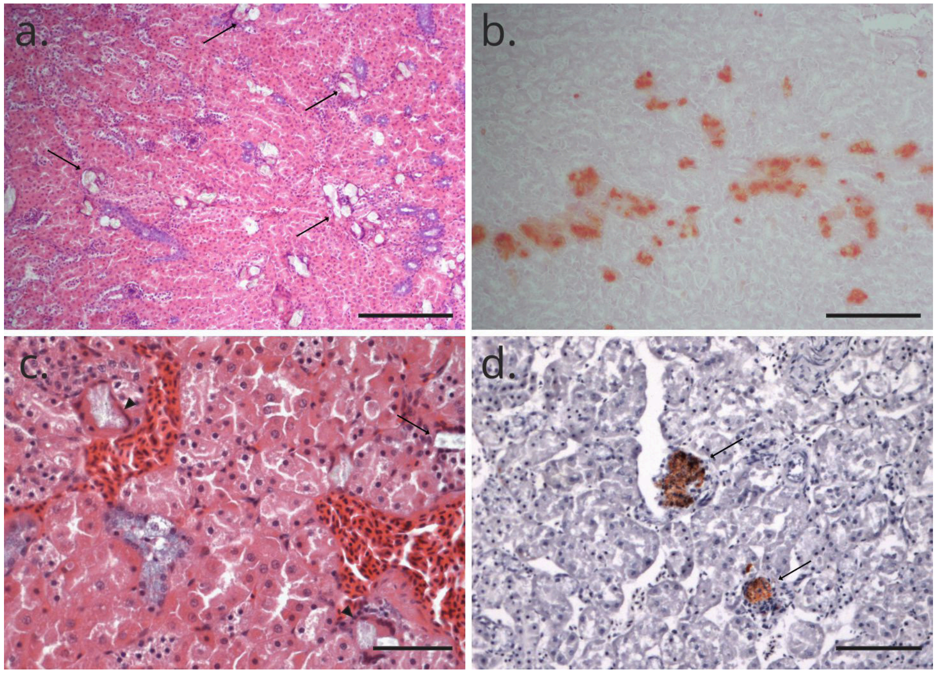

Figure 7. Renal tubular oxalosis in rock ptarmigan from Northeast Iceland between 2006 and 2018.

a. Kidney of a juvenile male with severe renal tubular oxalosis. Numerous oxalate crystals are present in renal tubules (black arrows). HE. Bar 100 mm.

b. The same ptarmigan with oxalate crystal that stain orange with Alizarin Red S pH 7.0. Bar 100 mm.

c. Kidney of an adult male with one crystal (black arrow) and multinucleated giant cells (MNGC) containing thread-like material (arrowheads) in renal tubules. The ptarmigan had very mild tubular oxalosis. HE. Bar 10 mm.

d. Kidney of an adult male without renal tubular oxalosis. Lipid in two glomeruli (black arrows). Frozen section, Sudan III. Bar 100 mm.

or glomeruli containing foamy material. In the frozen sections of the 2013 renal samples, some lipid was seen in a few glomeruli in Oil-Red O and Sudan III-stained sections (Figure 7d). Lipid was seen in the kidneys with and without oxalate crystals, including two ptarmigan that were categorized as having moderate to severe renal tubular oxalosis.

Alimentary system

The quality of the examined formalin-fixed intestinal samples varied due to often prolonged post-mortem intervals of over 24 hours. Detachment of enterocytes was frequently observed, and in some samples of the duodenum, the villi were completely devoid of mucosal epithelial lining due to autolysis.

Endogenous stages of coccidia were seen in the intestinal mucosa, primarily in the enterocytes, and/or oocysts in the intestinal lumen of all but eight of the 52 (85%) ptarmigan examined (Table 2, Figure 6a). In only two ptarmigan were meronts present in enterocytes, in the distal ileum and cecum, respectively. Five of the eight ptarmigan that were negative on histology were also negative for oocysts in faecal samples. The coccidia were seen in all 10 segments of the intestine, mainly in the mucosal epithelial cells and free in the intestinal lumen. Forty-six of the 52 ptarmigan were infected with *Eimeria muta (E. muta)*, with oocyst count per gram faeces (OPG) ranging from 25 to 51,100. Six of those ptarmigan were also infected with *E. rjupa,* with the highest OPG in faeces reaching 1,250. The oocyst count in faeces generally corresponded to the frequency of coccidia structures being observed in the histological samples. Crypt hyperplasia was not apparent, and the rare inflammatory reaction included an infrequent finding of a few granulocytes in the lamina propria in the occasional ptarmigan and small inflammatory foci in the mucosal epithelium in two ptarmigan.

Six of 49 juveniles had inflammatory lesions in the duodenum with intralesional nematodes (Table 2, Figure 6b). In three of those six ptarmigan, *Capillaria caudinflata* (*C. caudinflata*) was found on parasitological examination. In seven ptarmigan, *C. caudinflata* was detected on parasitological examination without inflammation or parasite being detected histologically. In the duodenum, adult worms and eggs were closely associated with the villi. Eggs were occasionally seen within the enterocytes or in the lamina propria. There was mild to moderate inflammation with infiltration of lymphocytes into the lamina propria. In a few instances, focal granulocyte infiltration was associated with intraepithelial eggs. The duodenal crypts showed variable degrees of hyperplasia.

Thirteen ptarmigan, including nine adults and four juveniles of both sexes, had pale to dark nodular lesions in, or closely associated with, the crop (Table 2). These lesions were observed during the dissection of 1,294 birds (Table 1). In five ptarmigan, the nodular lesions were in the wall of the crop, and three of these had multiple nodules (Figure 6c). The lesions in the remaining eight ptarmigan, found in the subcutaneous tissue near or attached to the crop, consisted of a single nodule. The nodules range from 0.5–1.5 cm in diameter and were well encapsulated. On cut surfaces, a cystic space filled with dark, soft material was present. Histologically, the nodular lesions were similar, containing amorphous to granular material mixed with cell debris centrally and a peripheral layer of multinucleated giant cells (Figure 6c). Infiltration of other inflammatory cells varied, but in some cases included numerous granulocytes and lymphocytes. The collagen-rich connective tissue stroma of the capsule varied from dense to loosely woven, with extensive fibroplasia. Nodular lesions in the crop wall were in the lamina propria, lamina muscularis, or both. The nodules elevated the mucosal surface of the crop that was lined by intact, but mildly hyperplastic epithelium. In seven ptarmigan, plant material was present in the nodules, and two of these also had fungal hyphae visible in PAS and/or Grocott's methenamine silver-stained sections. An additional five ptarmigan had fungal hyphae in the granulomatous lesion, and three ptarmigan had bacterial colonies, of which two also had plant material. Plant material was only seen in peri-ingluvial nodules, whereas fungal hyphae were seen in four of the five ptarmigan with mural granulomas and in three with peri-ingluvial granulomas.

Musculoskeletal system

Thirty-four of the dissected birds (n = 1,294; prevalence 2.6%; 95% CI 1.9–3.7%) had lesions in their bones, skeletal muscles and/or skin that were unrelated to the harvesting for the study, but caused by previous trauma that, in most cases, was consistent with impact with man-made structures. No significant difference in prevalence was observed between adults and juveniles (Fisher's exact test, p = 0.860). Among adults (n = 512) the prevalence was 2.7% (95% CI 1.6–4.6%), while in juveniles (n= 782) it was 2.6% (95% CI 1.6–3.9%).

Thirteen ptarmigan, including two adult males, six juvenile males, and five juvenile females with trauma lesions, were examined histologically (Table 2). The carina and body of the sternum were the bones most frequently affected, seen in eight birds. Macroscopically, there was nodular to irregular thickening and discoloration of the periosteum. Histologically, the thickening of the sternum was due to extensive reactive changes of variable ages, with periosteal and sometimes endosteal callus, consisting of osseous, cartilaginous, and fibrous tissue (Figure 6e). Some histological sections showed bone deviation, fractures, and necrosis. Adjacent skeletal muscle exhibited degenerative and regenerative changes, sometimes with extensive necrosis of muscle fibres, fibroplasia, and fibrosis. One juvenile female ptarmigan had multiple fractures of the carina and of the caudal part of the vertebral column with associated epidural haemorrhage. A fracture of the sternum was observed at the costal margins in one juvenile male, who also had fractured ribs. Two juveniles, a male and a female, had fractures of the body of the claviculae and of the right tibiotarsus, respectively.

Three juvenile ptarmigan had lesions primarily in skeletal muscle, of which two had macroscopic lesions in the pectoral muscle. One had green discoloration of the muscle due to multifocal necrotizing and granulomatous myositis, with sequester formation and bone fragments, indicating undetected trauma to the sternum (Figure 6f). Another ptarmigan had pale streaks in the muscle due to subacute myositis, with multifocal degeneration and necrosis of myocytes. The third ptarmigan had green discoloration of one wing. Histologically, there was ulceration of the overlying skin and serocellular crusting. In the underlying tissue, including the skeletal muscle, there was degeneration, necrosis, haemorrhage, and fibroplasia.

Miscellaneous lesions

Four ptarmigan had pathological findings in the coelomic cavity, in addition to the four previously reported ptarmigan infected with the larval stage (tetrahydride) of the cestode *Mesocestoides canislagopodis* (*M. canislagopodis*) (Table 2) (Skírnisson et al. 2016).

A juvenile female had a 1.5 cm in diameter large nodular lesion in the caudal part of the coelomic cavity, which histology revealed to be a severely atrophic and inflamed bursa fabricii.

Three females, including one juvenile and two adults, had lesions in or associated with the reproductive organs. The juvenile had a dilated oviduct, measuring 0.5 cm in diameter, filled with tenacious, semi-transparent mucoid content. No inflammation was seen on histology; the mucosa was devoid of glands, and the mucosal lining was attenuated. An adult female had a yellow cyst, 0.5 cm in diameter, attached to the ovary by a short stalk. On histology, there was heterophilic granulomatous inflammation effacing the cellular composition of the cyst wall, and the cyst lumen appeared empty. The lesion was likely an inflamed ovarian cyst. The other adult female had an irregular, yellow-green, semisolid mass within the coelomic cavity, contained by a thick membrane. Histology showed yolk-like material, partially surrounded by a thickened, folded perivitelline membrane and an outer rim of connective tissue with granulomatous inflammation. The lesion was compatible with an inflamed ovarian follicle.

DISCUSSION

The primary macroscopic lesion in the hunter-harvested ptarmigan was scaly crusted skin, i.e., mange caused by skin mites, which was found in 18.3% of the 1,108 birds examined. Other macroscopic lesions included nodules in or near the crop and traumatic lesions of the musculo-skeletal system. Histological findings included renal tubular oxalosis, which was present in 25.3% of the 1,263 birds examined, along with parasitic enteropathy, primarily protozoa infection but also duodenitis associated with *C. caudinflata* infection.

Integuments

Epidermoptidae mites live in or on the skin of birds, and many species can cause avian mange, including *Knemidokoptes*, *Microlichus*, and *Myialges* (Gilardi et al. 2001, Low et al. 2007, Rettenmund et al. 2015). In ptarmigan, mange was linked to two species of skin mites, *M. islandicus* and *M. borealis*. Skin lesions caused by *Knemidokoptes* are usually found in unfeathered areas of the face and feet (Low et al. 2007), but infestation caused by other epidermoptid mites can become more generalized, causing dermatitis and feather loss (Gilardi et al. 2001, Rettenmund et al 2015). Dermatitis, but not feather loss, characterized ptarmigan mange. Mange is a new observation for ptarmigan and grouse in general. This is concerning because mange is known to affect birds' body condition (e.g., Gaudioso-Levita et al. 2016). In ptarmigan, mange appears to cause physiological costs, at least in terms of fat reserves, which are crucial for winter survival. Birds with mange had fewer fat reserves than those without mange (unpublished data). Not all ptarmigan infected with skin mites develop mange, as shown by a higher prevalence of skin mite infestations compared to mange. These findings are consistent with other studies (Gilardi et al. 2001, Rettenmund et al. 2015), confirming that infestation with epidermoptid mites can be subclinical.

There was an age and sex difference in mange prevalence among ptarmigan. Adults had lower prevalence than juveniles, and males had lower prevalence than females. The sex difference was due to adult females having higher prevalence than adult males and being more similar to juveniles. Within the study population, there was a juvenile age and female bias in the abundance of the more common skin mite species, *M. islandicus*. The other species, *M. borealis*, showed a juvenile age bias (Nielsen et al. 2020). This aligns with our findings on mange and its connection to the bird's age and sex, which should not be surprising since skin mites cause mange. The age bias as suggested by Nielsen et al. (2020) for skin mites, and subsequently mange, may be due to either acquired physiological immunity or more effective anti-parasite behaviors, such as preening, or because birds with skin mites and mange are more likely to die, leading to the observed bias. The adult female bias likely reflects her role in reproduction, such as egg-laying, incubation, and caring for the chicks. The associated energetic costs and close bodily contact with the offspring may explain this phenomenon. Thus, allocating limited resources to breeding might reduce the female's ability to fight skin mite infestations. Additionally, the chicks could potentially serve as a hotspot for skin mites, further challenging the brooding female. The skin mite *M. islandicus* is transmitted from mother to offspring (vertical transmission), but *M. borealis* is a hyperparasite on hippoboscid flies and transmitted by the flies to avian hosts like ptarmigan (vector transmission).

Few avian studies have examined differences in skin lesions based on the age or sex of the hosts. For stitchbirds (*Notiomystis cincta*), both the prevalence and severity of skin lesions were higher in males than in females during the breeding season (Low et al. 2007). Male bias is a common pattern in parasitism and is thought to result partly from hormone-linked suppression of the immune system among breeding males (Poulin 1996, Zuk & Stoehr 2010). This pattern contrasts with our findings for mange, where a bias toward adult females was observed, as discussed above.

Additionally, the relationships between mange scores and the skin mite measures of prevalence and of mean intensity were stronger for juveniles than for adults. Juveniles with mange had a high prevalence of skin mites regardless of the mange severity score (range 87–97%), but for adults, prevalence increased from 37% for birds with mild mange to 91% for birds with severe mange. The pattern of mean intensity was also notable: for juveniles, there was a gradual increase in mean intensity with severity score, but for adults, birds with mild or moderate mange had very low intensity values, similar to those of adult birds without mange; only adults with severe mange had high intensity values. Why does this age-related pattern occur? This could partly be due to the sensitivity of the vacuum method used to collect skin mites, how mange was identified and scored, or because of different behaviours of skin mites depending on the host's age. The vacuum method does not seem to detect mites in all infected birds; in juveniles, most with mange yielded skin mites, but this was not true for adults. The effectiveness of the vacuum method likely depends on the duration of vacuuming (2 minutes in this case) and the number of mites on the skin's surface, rather than those embedded within the skin. As a result, there are probably more false negatives for hosts with a low number of skin mites. There might also be a host age-related difference in the exposure or vulnerability of skin mites to the vacuum method, with skin mites parasitizing juvenile hosts being more on the surface than those parasitizing adult hosts. Additionally, there appears to be an issue with how mange in adults was identified and scored; only adults with severe mange showed high prevalence and intensity values for skin mites. The scoring of mange is subjective and depends on how the skin looks. Severe mange is easy to identify, but the two milder stages seem more challenging to classify accurately, especially for adult hosts. Based on the average intensity and prevalence of skin mites, there were no clear quantitative differences between mild and moderate mange among adult hosts.

An age effect for mange was also observed in histopathology. Out of the seven adults with mange, only two had dermatitis, compared to 10 of 11 juveniles with mange. One of the two adults also had intralesional mites, and eight of the 10 juveniles. While not detecting mites in histological samples of mange lesions is not uncommon (Gilardi et al. 2001, Rettenmund et al. 2015), the absence of characteristic features of chronic infestation, like epidermal hyperplasia, was unexpected. All the adults had been classified with mild and moderate mange, but seven of the juveniles had been classified with severe mange. This suggests that the determination of mild and moderate mange is error-prone and especially so for adult birds.

Urinary system

Calcium oxalate crystal deposits in the kidneys of mammals can lead to oxalate nephrosis and renal failure when the rate of accumulation is higher than the excretory capacity (Bargagli et al. 2020). Oxalate nephrosis can be primarily due to genetic defects in glyoxylate metabolism, or secondary. Secondary oxalate nephrosis has various causes, including ingestion of oxalate-containing plants or ethylene glycol (Cianciolo & Mohr 2016, Stegelmeier et al. 2020). There are a few reports on oxalate nephrosis in birds due to ethylene glycol poisoning (Özcan et al. 2007, Radi et al. 2003) and only one case involving ingestion of oxalate-containing plants (Orlandini 1933).

Glomerular lipidosis with secondary renal oxalate nephrosis has been reported in Japanese ptarmigan (Murai et al. 2011). Although frothy material and mild lipid deposits in the glomeruli were seen in some of the Icelandic ptarmigan, there was no obvious correlation between renal tubular oxalosis and glomerular lesions.

The ptarmigan in our study likely acquired oxalate from their diet, as many plants produce oxalate, including species of the Polygonaceae family, four of which were consumed by the study population (Dépré & Nielsen 2023, Miyazaki et al. 2003, Costan et al, 2023). Renal tubular oxalosis was quite common among the ptarmigan, but with significant variation among years. There was also a notable age-related difference in the severity of renal oxalosis, with juveniles exhibiting grades 2 and 3 more frequently than adults.

Studies have shown that mammals have microbiota in their intestines that metabolize oxalate, thereby maintaining the balance of oxalate in the body (Miller & Dearing 2013). Variation in diet can influence microbial communities, as observed in wild versus captive ptarmigan (Salgado-Flores et al. 2019). For example, the caecal bacterial flora of wild Japanese ptarmigan differed significantly from that of captive Svalbard ptarmigan (Ushida et al. 2016), making the latter more prone to various diseases, including possibly renal tubular oxalosis.

Half of the 319 ptarmigan with oxalate crystals in their kidneys showed moderate to severe tubular oxalosis. The reason for individual differences in the extent of renal tubular oxalosis is unknown, but an imbalance in intestinal bacterial flora of unknown cause might contribute. It is also unclear whether oxalate deposits affected kidney function and overall health. Renal tubular oxalosis, unlike mange, did not appear to cause significant physiological costs related to fat reserves in the study population. When controlling for age, sex, and year, the presence or absence of oxalate deposits did not significantly explain fat reserve levels. Additionally, there was no link between the severity of oxalate deposits and fat reserves (unpublished data).

Alimentary system

In poultry, coccidiosis is a self-limiting disease that, depending on the infective dose and on the *Eimeria* species involved, can be subclinical or clinical. Subclinical infection can, however, result in reduced weight gain due to malabsorption (Cervantes et al. 2020).

The ptarmigan in the present study showed no obvious signs of enteric disease at the time of autopsy, and histopathological lesions were negligible, irrespective of the OPG values. However, villous atrophy that could cause malabsorption (Flores et al. 2022) could not be ruled out. It can be presumed that the absence of overt signs of enteric disease and histopathological lesions was due to the timing of bird harvesting. The primary impact of *Eimeria* infection likely occurred earlier, in late summer or early autumn. Research into the seasonal changes in endoparasite burden of Icelandic ptarmigan over a 12-month period showed a peak in oocyst count of *E. muta* in September, with a rapid decline in October (Þórarinsdóttir et al. 2010). The study by Stenkewitz et al. (2016) on the potential role of parasites in the dynamics of the Icelandic ptarmigan population did reveal a correlation between *E. muta* infection and body condition, as well as an association between *E. muta* infection and ptarmigan density.

Endogenous stages of coccidia in the present study were primarily observed in enterocytes throughout the intestinal tract, indicating that specific predilection for certain segments of the intestine is not apparent for *E. muta*, the main species infecting the ptarmigan. Recent Japanese studies assessing the pathogenicity of two *Eimeria* species in both caged birds and in experimentally infected Svalbard ptarmigan showed similar results (Matsubayashi et al. 2020, Matsubayashi et al. 2023). A recent phylogenetic analysis confirmed that one of those *Eimeria* species, *E. uekii*, is identical to *E. muta* and *Eimeria sp. A*, which infect ptarmigan in Iceland and Svalbard-Norway, respectively (Matsubayashi et al. 2024).

Different species of *Capillaria* nematodes are found in both domestic and wild birds. *Capillaria* infects the upper digestive system or the intestine and can, in heavy infection, cause severe disease due to inflammation and obstruction (McDougald 2020). *C. caudinflata* infects the small intestine of many gallinaceous birds, but the description of the histopathological lesions is scarce. In the samples examined histologically in the present study, there were obvious reactive changes to the parasite in the duodenum. The impact of these lesions on the overall health of the individual ptarmigan could not be assessed. The ptarmigan examined histologically for lesions due to endoparasitism were mainly juveniles, but a study on the variation in parasitism concerning sex and age in Icelandic ptarmigan showed no sex or age bias due to infection with *C. caudinflata*. (Nielsen et al. 2020).

Inflammation of the crop, ingluvitis, is a well-known lesion of birds that is caused by various infectious agents, especially the protozoan *Trichomonas gallinae* (*T. gallinae*) and *Candida* spp. Trichomoniasis has been diagnosed in many different species of domestic and wild birds and is an emerging disease in finches worldwide (Neimanis 2010, Brunthaler 2022). *T. gallinae* infection causes inflammation of the mucosal surfaces of the upper alimentary tract, including the crop, with ulceration and caseous necrosis that can extend deep into the underlying tissues. The infection leads to emaciation and death. Inflammation due to *Candida* spp. infection primarily affects the mucosal lining of the crop.

The granulomas in or around the crop in the Icelandic ptarmigan were infrequent, occurring in about 1% of the birds examined during autopsy. The pathogenesis behind these lesions is uncertain, but roughly 50% of cases showed a foreign body reaction to plant material. Fungal infection was present in about 50% of the cases, with or without plant material being present. The ptarmigan did not have macroscopic lesions suggestive of *T. gallinae* or *Candida* spp. infection, and the mucosal surfaces of the crops were intact. These crop-related inflammatory lesions may, in some cases, have resulted from trauma to the neck. It is also plausible that an early damage to the mucosal lining of the crop, and possibly the oesophagus, by sharp plant material, allowed plant material, fungi, and/or bacteria to penetrate deeper structures in the crop wall and into the surrounding connective tissue, resulting in granulomatous inflammation.

Musculo-skeletal system

In birds, common causes of trauma include impact with man-made structures such as buildings, vehicles, and powerlines, where mainly wings and legs are affected (Cummings et al. 2022, Garcês et al. 2018, Montesdeoca et al. 2017). Natural causes include trauma to the pectoral girdle in raptors due to impact while hunting (Goodman & Glynn 1988).

The prevalence of trauma in the 1,294 dissected Icelandic ptarmigan was 2.6 %, and the body parts mainly affected were the sternum and pectoral muscle. Based on experience from radio tracking over 200 ptarmigan (unpublished data), this is most likely caused by collisions with man-made objects, such as fences. Trauma elicited an extensive reactive change in bone, with large callus formations that could resemble bone tumours macroscopically. The high trauma rate in the Icelandic ptarmigan is different from the 0.3% trauma rate observed in the dusky grouse (*Dendragapus obscurus*) (Zwickel & Bendell 2004). This is likely due to extensive fencing in areas used for sheep grazing, both in the lowland and the highland of the Iceland study area.

Incidental findings

Incidental pathological findings in a few ptarmigan were examined histologically. In four ptarmigan, lesions were due to infection with tetrathyridia, the second larval stage of the cestode *Mesocestoides canislagopodis*. Coelomic tetrathyridiosis caused minimal inflammatory reaction, whereas in one ptarmigan, infection in the liver parenchyma elicited an extensive, localized, granulomatous response (Skirnisson et al. 2016). Tetrathyridiosis has been reported to cause severe inflammatory lesions in birds (Toplu et al. 2006), but reaction to the infection can also be minimal or non-existent (Kubeĉka et al. 2018).

The pathological lesions in the other four ptarmigan were unlikely to have significantly affected their health, except possibly the one juvenile female that had severe bursal atrophy and bursitis of unknown etiology.

CONCLUSIONS

This study provides an important long-term (13 years) and extensive (2,730 individuals) analysis of pathology in a wild, free-ranging bird. The main objective of the ptarmigan health study in Iceland, which lasted 13 years, was to examine how health-related parameters influence population changes. Histological examination of organ samples complemented this comprehensive research. The main pathological findings in Icelandic ptarmigan included mange (caused by skin mites) and renal tubular oxalosis, together with *E. muta* infection (Stenkewitz et al. 2016). At least *Eimeria* infections and mange have the potential to negatively affect the overall health of the birds. Other notable findings were trauma-related lesions, which were unusually common. The study highlights the importance of histological examination of organs from hunter-collected specimens, as it can provide unique insights into predicting both individual and population health consequences.

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