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LOUPING-ILL VIRUS SEROSURVEY OF WILLOW PTARMIGAN (*LAGOPUS LAGOPUS LAGOPUS*) IN NORWAY

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ABSTRACT: In Norway, the Willow Ptarmigan (*Lagopus lagopus lagopus*) is experiencing population declines and is nationally Red Listed as Near Threatened. Although disease has not generally been regarded as an important factor behind population fluctuations for Willow Ptarmigan in Norway, disease occurrence has been poorly investigated. Both louping-ill virus (LIV) and the closely related tick-borne encephalitis virus are found along the southern part of the Norwegian coast. We assessed whether and where Norwegian Willow Ptarmigan populations have been infected with LIV. We expected to find infected individuals in populations in the southernmost part of the country. We did not expect to find infected individuals in populations further north and at higher altitudes because of the absence of the main vector, the sheep tick (*Ixodes ricinus*). We collected serum samples on Nobuto filter paper and used a hemagglutination inhibition assay for antibodies against LIV. We collected data at both local and country-wide levels. For local sampling, we collected and analyzed 87 hunter-collected samples from one of the southernmost Willow Ptarmigan populations in Norway. Of these birds, only three positives (3.4%) were found. For the country-wide sampling, we collected serum samples from 163 Willow Ptarmigan carcasses submitted from selected locations all over the country. Of these birds, 32% (53) were seropositive for LIV or a cross-reacting virus. Surprisingly, we found seropositive individuals from locations across the whole country, including outside the known distribution of the sheep tick. These results suggest that either LIV or a cross-reacting virus infects ptarmigan in large parts of Norway, including at high altitudes and latitudes.

Key words: *Lagopus lagopus*, louping-ill virus, ptarmigan, serosurvey, tick-borne viruses.

INTRODUCTION

Louping-ill virus (LIV) is a tick-borne flavivirus known to circulate among sheep (*Ovis aries*), mountain hares (*Lepus timidus*), and Red Grouse (*Lagopus lagopus scoticus*) in the uplands of Great Britain and Ireland (McGuire et al. 1998; Jeffries et al. 2014). Historically, LIV has only been found on the British Islands, but in recent decades the virus has also been detected in Norway (Gao et al. 1993) and on the Danish island Bornholm in the Baltic sea (Jensen et al. 2004). Additionally, a virus with 94% homology to a British LIV strain has caused louping-ill in sheep and goats (*Capra aegagrus hircus*) in Spain (Balseiro et al. 2012).

Louping-ill virus is closely related to tick-borne encephalitis virus (TBEV), which is present in foci over large parts of southern Scandinavia, central and eastern Europe, and Russia (Jeffries et al. 2014). Tick-borne encephalitis virus has until recently been assumed to be absent from the British Isles (Mansfield et al. 2009; Jeffries et al. 2014), but recent studies suggest that the virus is maintained through enzootic cycles in some forest habitats in England (Holding et al. 2019, 2020; Kreusch et al. 2019). Current understanding of viral phenology suggests that LIV and TBEV are the same viral species, with LIV characterized as a sheep-adapted type of TBEV, and that both viruses evolved from a common caprine or ovine encephalitis virus ancestor (Uzcátegui et al. 2012).

The only known tick vector for LIV is the sheep tick (*Ixodes ricinus*), and the most important hosts are sheep, mountain hares, and Red Grouse (Gilbert 2016). Although hares appear to act as healthy transmission hosts (i.e., they are capable of transmitting the virus without experiencing debilitating disease

or long-term viremia), LIV infection can cause severe disease in both sheep and grouse. Furthermore, LIV has been regarded as a major decisive factor for grouse chick survival in tick-infested areas, as experimental infection is linked to high mortality (Reid 1975; Reid et al. 1978; Gilbert et al. 2004).

Ticks can become infected by feeding on an infected host (typically sheep or Red Grouse) or by cofeeding, whereby an uninfected tick becomes infected when feeding in close proximity to another infected tick on the host, typically mountain hares (Labuda et al. 1993; Hudson et al. 1995; Jones et al. 1997). Hosts become infected when bitten by an infected tick. Red Grouse chicks can also become infected by eating infected ticks (Gilbert et al. 2004).

The presence or prevalence of LIV in the tick vector depends both on the density of ticks and on the density of hosts that can facilitate transmission of the virus from one tick to another (Gilbert 2016). Although Red Grouse are effective hosts for LIV, viral infection can cause rapid and high mortality (Reid 1975; Reid et al. 1978). Maintenance of the virus life cycle is probably dependent on the presence of other hosts such as sheep, which experience a far lower mortality after infection, as well as mountain hares, which may not experience viremia after infection (Hudson et al. 1995; Jones et al. 1997; Gilbert 2016). High density of sheep ticks is supported by high densities of suitable hosts (Ytrehus and Vikøren 2010). Survival, development, and reproduction of sheep tick is dependent on suitable microclimate (i.e., relatively warm temperatures and high humidity) for a sufficiently long period. Tick abundance decreases with elevation, even after correcting for the decline in maintenance host densities with increasing elevation (Gilbert 2010).

Populations of Willow Ptarmigan (*Lagopus lagopus lagopus*) and rock ptarmigan (*Lagopus muta*) in Norway are currently declining (Lehikoinen et al. 2014; Eriksen et al. 2018), and both species were listed as Near Threatened in the *National Red List for Species* (Henriksen and Hilmo 2015). The factors underlying the declines are not fully understood, but so far the effects of harvest (Eriksen et al. 2018) and habitat change (Henden et al. 2011), as well as the direct and indirect effects of climate change in causing alterations in the trophic interactions between ptarmigan and their predators (Kvasnes et al. 2014; Fuglei et al. 2020), have received the most attention. The potential role of disease and other health factors in the population declines have not been investigated.

The distribution of the only known LIV vector, the sheep tick, has been expanding in Norway, with recent investigations reporting the tick at an elevation >580 m in the mountains of southern Norway and as far north as 69°N (Jore et al. 2011, 2014; Andersen et al. 2019). Because sheep tick distribution is partly determined by climatic conditions, specifically mild and humid climates (Jore et al. 2011, 2014), ongoing climate change is predicted to increase distribution and thereby increase the probability of the presence of tick-borne diseases. Consistent with this prediction, an expansion of the distribution of TBEV has been observed in the Nordic countries (Jaenson et al. 2012; Tonteri et al. 2016; Andersen et al. 2019). From multifactorial models and predictions (Randolph and Rogers 2000), TBEV will be present along the southeastern coast of Norway and will increase in range there, whereas the more western, northern, and alpine parts of the country will be protected by their colder climates. This hypothesis has been challenged recently by the findings of both TBEV seropositive animals and ticks positive for TBEV RNA in these parts of the country (Ytrehus et al. 2013; Paulsen et al. 2015, 2019; Soleng et al. 2018), indicating that TBEV is focally present in large areas along the northwestern and northern coastline of Norway, at least up to 65.1°N.

Although LIV infection has been detected in sheep and wild cervids in Norway (Gao et al. 1993; Ytrehus et al. 2013), its distribution is not well delineated. In addition to an expanding sheep tick distribution, the presence of important transmission hosts (mountain hares and sheep) and a climate and landscape

resembling British and Irish moorlands (particularly along the coast), suggests a high potential for the presence of LIV in Norwegian Ptarmigan. If present, an effect on chick survival similar to the reported high mortality in Red Grouse chicks (Duncan et al. 1978; Reid et al. 1978) could occur. Our study aimed to determine whether and where in Norway Willow Ptarmigan have been infected by LIV. Serum samples were collected through country-wide surveillance, as well as by targeting one of the southernmost ptarmigan populations close to the coastal areas where TBEV and LIV circulate. We expected to find a low seroprevalence of LIV in the south, with few to no infected ptarmigan in the higher latitudes and altitudes where louping-ill has never been reported in sheep, no screening studies indicate that the virus is present, the sheep tick is absent or rare, and climate predicts that cofeeding transmission is unlikely to occur.

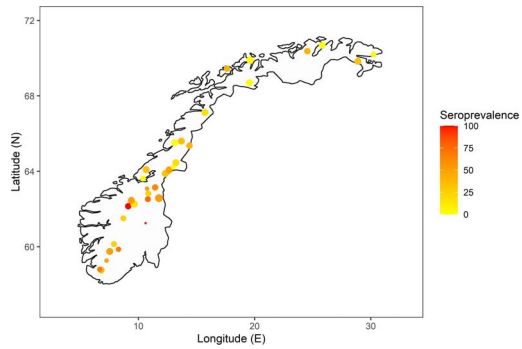

FIGURE 1. Overview of sample locations in the Norway-wide sampling of Willow Ptarmigan (*Lagopus lagopus lagopus*), and seroprevalence of louping-ill virus in the different locations. Note that sample sizes are relatively small within locations (range, 1–13). Seroprevalence is here defined as proportion of positive samples out of the total number of samples (i.e., treating equivocal samples as negative). The size of the dot represents the sample size for the location. Local sampling in Njardarheim in 2017 is not shown in this map.

MATERIALS AND METHODS

Sample collection

Blood samples were collected from Willow Ptarmigan harvested during the annual autumn harvest following two data collection schemes, local and countrywide, outlined in the upcoming text. For both schemes, blood was sampled with Nobuto filter paper strips (Advantec MFS, Dublin, California, USA).

For the local sampling in autumn 2017, Willow Ptarmigan were collected from Njardarheim, an 800-km^2 state-owned property (approximately 59°N, 7°E, 500–1,400 m elevation) in southern Norway. The mountain area was chosen because it is heavily used as summer pasture for sheep from farms along the nearby coast, which has a high abundance of sheep tick (Jore et al. 2014), clinical cases of TBEV infection in humans (Skarpaas et al. 2004), and cases of LIV infection in sheep (Ulvund et al. 1983; Ulvund 1987; Gao et al. 1993; Stuen et al. 1996). It could hence be expected that infected ticks would be transported into the area each year. Through collaboration with local wildlife managers, registered ptarmigan hunters were contacted by email to explain the purpose of the project and to ask them to register online if they were willing to participate. Volunteers received sampling sets consisting of four filter paper strips mounted in a cardboard holder (Curry et al. 2014a, b), a protocol outlining the blood collection procedure, and envelopes with prepaid postage and address labels. The hunters were instructed to examine the harvested Willow Ptarmigan for hemorrhages to sample from. If no such hemorrhages were found, the hunters were told to incise the brachial vein. The filter paper strips were to be dipped in visible blood until the strips were saturated up to the indicated line. The cardboard holder would then be marked with the name and telephone number of the hunter, the date and time of the bird's death, and whether the hunter regarded it to be a juvenile (<1 yr) or adult bird on the basis of the appearance of the primary flight feathers of the wings. The strips were air-dried before being transferred to an envelope and posted to the Norwegian Institute for Nature Research, Trondheim, Norway. The filter paper sets were stored at –80 C until elution in May–June 2018.

For country-wide sampling, as part of the Norwegian Monitoring Programme for Terrestrial Ecosystems, appointed hunters submitted complete Willow Ptarmigan carcasses harvested during the hunting seasons of 2014–16. Each hunter was requested to submit six adult birds from their hunting area, thus providing samples from 33 selected locations throughout the country (Fig. 1 and see Supplementary Material Table S1). Carcasses were stored at –20 C until postmortem examination in March–April 2017, when three filter paper strips mounted in a cardboard holder were used to sample blood from the large veins entering the heart. If it was not possible to fill the

filter paper strips completely from the heart vessels, blood was also sampled from the cut lungs. The strips were air dried at room temperature then stored at –80 C until elution in May–June 2018.

Serologic analysis

Filter paper strips were thawed at room temperature and processed according to Curry et al. (2014a, b). For each sample, the absorbent part of three fully filled filter paper strips were each cut in five to six pieces inside a 2.0-mL Eppendorf tube (Eppendorf AG, Hamburg, Germany). Between samples, the scissors used for cutting were washed with soap and tap water, disinfected in 10% household bleach (Klorin®, Lilleborg AS, Oslo, Norway, 4% sodium hypochlorite) for at least 10 min, rinsed in distilled water, put into 98% ethanol for 2–5 min, and air dried to remove ethanol residues completely. If strips were not completely filled with blood and more than three strips were available, blood-absorbed parts were cut from the fourth strip to compensate for the part not filled, to approximate the size of three filled strips. In cases where the total amount of blood-filled filter paper constituted less than three completely filled strips, all the blood-absorbed portions and some unabsorbed portion of the strips were cut to approximate the size of three strips. Filter strips were rated as having good, fair, or suboptimal quality, based on the color and proportion of filled filter paper. Good strips were uniformly dark, fair strips had lighter color or were not completely filled, and suboptimal strips had lighter color, were only partly filled, or both.

A stock solution containing Dulbeccos's phosphate-buffered saline with calcium chloride and magnesium chloride (Biowest, Nuaillé, France) and an antibiotic (penicillin-streptomycin) solution (Biowest, final concentrations of 100 U/mL and 100 µg/mL, respectively) were prepared and stored at 4 C for a maximum of 24 hr (Curry et al. 2014a, b). A total of 1.2 mL Dulbeccos's phosphate-buffered saline–antibiotic solution (0.4 mL/strip) was added to each Eppendorf tube. The tubes were finger-flicked to ensure that all parts of the strips were in contact with the fluid and incubated at 4 C for 16 hr. After incubation, the supernatant (~750–800 µL) was transferred to a new 1.5-mL Eppendorf tube and prepared for storage by centrifuging for 10 s at 20.817 × G. This eluate was stored at –80 C for 1–2 mo until transportation by express mail on ice blocks to the Moredun Research Institute (Midlothian, Scotland).

The presence of antibodies to LIV was evaluated by a hemagglutination inhibition (HI) assay as described previously (Clarke and Casals 1958; Grist et al. 1966). Briefly, goose erythrocytes were collected in Alsever's solution (Clarke and Casals 1958; Grist 1966), and used to titrate the eluted samples in the presence of constant-titer virus antigen in a microtiter plate. This assay format has been validated at Moredun Research Institute in routine diagnostic use for many species, including birds (Ytrehus et al. 2013). Nonspecific inhibitors and goose erythrocyte agglutinins were removed by kaolin and by goose erythrocyte absorption (Reid et al. 1978). Positive and negative controls (ovine sera of known LIV serostatus) were included in each test batch to confirm assay performance. The first dilution was 1:10 and the endpoint titer was 1:640. Samples with a titer >1:20 were considered positive, and titers above 1:640 would have been reported as >1:640. Samples with a titer of 1:10 were considered equivocal, and titers of <10 were considered negative. To assess to what extent seroprevalence (i.e., the proportion of samples considered positive out of the total number of analyzed samples, treating equivocal samples as negative) was related to longitude in the national surveillance data set, we used a binomial mixed effects model, with location identification fitted as a random intercept, implemented in the R statistical language (R Development Core Team 2020) add-on library *lme4* (Bates et al. 2015) by the *glmer* function.

RESULTS

For local sampling, we received filter paper samples from 88 individual Willow Ptarmigan shot by 19 hunters in Njardarheim in 2017. One sample was damaged during handling and therefore could not be included, resulting in 87 samples available for analysis. Of these samples, 42 were from birds classified (by the hunters) as juveniles, 25 from birds classified as adults, and 20 samples from birds where age was not classified. Of the 87 filter paper sets, 32 were of good, 27 of fair, and 28 of suboptimal quality with regards to saturation and color. Three (3.4%) of the 87 analyzed serum samples from Njardarheim were seropositive for LIV: one juvenile, one adult female, and one adult male. The titers were low, at 1:80, 1:40, and 1:20, respectively. The quality of the filter strips from these individuals were evaluated as good, good, and fair, respectively.

In the country-wide sampling, we collected 163 samples, all of good quality, from Willow Ptarmigan carcasses sent from throughout

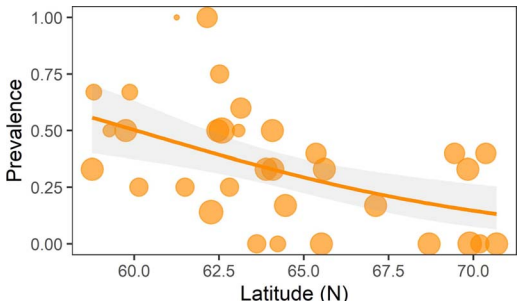

FIGURE 2. Proportion of Willow Ptarmigan (*Lagopus lagopus lagopus*) seropositive for louping-ill virus in the Norway-wide sampling relative to latitude in geographic degrees. The size of the circles is proportional to the number of samples in each location. The proportion of seropositive birds generally is higher in the southern than in the northern locations. Results from local sampling in Njardarheim in 2017 are not included.

Norway. Of these samples, 53 (33%) were classified as seropositive for LIV, although showing low titers (1:20–1:80); 36 (22%) were classified as equivocal, having titers of 1:10; and 74 (45%) were classified as negative. One additional sample was excluded from the laboratory analysis because of the presence of agglutination in the control well (red blood cells and serum only). Positive and equivocal samples were detected in birds throughout the country (Fig. 1 and see Supplementary Table S1), including locations north of 69°N and from inland and high-altitude areas in southern Norway (e.g., Røros, Tolga, and Lierne in Supplementary Table S1), which are outside the range of the sheep tick according to the most recent tick distribution investigation (Jore et al. 2011). Study sites with the lowest proportion of positive birds were located in northern parts of the country, with a clear pattern of lower seroprevalence at higher latitudes (binomial mixed effects model with location identification as random effect; slope=–0.65 [SE 0.21], P=0.002 [Fig. 2]).

DISCUSSION

The finding of three LIV-seropositive Willow Ptarmigan with low titers in the local sampling from Njardarheim in the southernmost mountains of Norway is not surprising. Louping-ill virus is regarded to be present along the southern coast of Norway, and the mountain areas are used as summer pastures for thousands of sheep from these coastal areas. Some of these sheep presumably carry infected *I. ricinus* ticks when they are moved up to the mountains in the spring, and some fed ticks that have fallen from sheep could potentially be ingested by ptarmigan chicks, which have been shown to be infected in this manner (Gilbert et al. 2004; Gilbert 2016). The low seroprevalence suggests, however, that permanent, LIV-infected populations of sheep tick are not established in the environments used by Willow Ptarmigan in these areas, despite the recent increases of sheep tick abundance and range (Jore et al. 2011, 2014). The lower proportion of seropositive birds among juveniles (2.4%) compared with adults (8.0%) could simply be sampling error caused by low sample sizes and few positive birds overall. Alternative explanations are that the difference could be caused by the longer period of risk of exposure for the adults, or that a higher case fatality rate (Reid et al. 1978) among chicks compared with adults contributes to this difference.

The finding of LIV-seropositive birds in localities throughout Norway (up to 70.4°N) was surprising. Many of these study sites are at higher altitudes or higher latitudes, where the sheep tick is not supposed to be able to maintain populations (Jore et al. 2011; Kjær et al. 2019) and where sheep are not transported from sheep tick–infested areas on a regular basis. Even when taking into account that the distribution of the sheep tick has expanded northwards and further inland during the past decades (Jore et al. 2011) and that several studies have indicated presence of TBEV in areas previously believed to be climatically unsuitable for TBEV virus transmission (Ytrehus et al. 2013; Soleng et al. 2018; Paulsen et al. 2019), it seems implausible that LIV (or TBEV) is circulating at all the locations at the high altitudes and latitudes described in the current study.

We propose five alternative explanations for these results: 1) false positive results, 2) cross-reactivity with other, unknown flaviviruses, 3)

ptarmigan infected with LIV during migration to areas within the range of the sheep tick, 4) sheep tick and LIV found in unrecognized microfoci also at high altitudes and latitudes, 5) alternative vectors transmitting LIV.

Most studies that compare filter paper methods with blood sample collection and centrifugation for serum or plasma separation conclude that they perform as well as or slightly inferior to the latter (Curry et al. 2011, 2014a, b; Smit et al. 2014). In a recent study of West Nile virus in Ruffed Grouse (*Bonasa umbellus*), Nemeth et al. (2017) concluded that there was complete agreement between the results from analyses of filter paper samples and ordinary serum samples, but that titers were lower when using the filter paper method. Consequently, we do not expect that the use of filter paper as a sample collection medium should increase the proportion of false positives, but rather that titer would be underestimated. The modified HI test for antibody to LIV used in this study has been used at the Moredun Research Institute for decades and is validated for routine diagnostic use on Red Grouse serum, with no interference from hemolysis in the samples (M.R. pers. comm.). There is no reason to expect any difference between the subspecies Red Grouse and Willow Ptarmigan in this regard.

The difference in prevalence of positive individuals from the birds sampled as fresh carcasses (local sampling) and the birds sampled postmortem after a period of storage at –20 C (country-wide sampling) is puzzling. The quality of the hunter-collected filter paper strips was highly variable compared with the standardized samples taken at postmortem examination. Some of the filter paper strips from the local sampling may not have been thoroughly air-dried before they were shipped to the laboratory, but this should rather have caused false positive test results (M.R. pers. comm.). We do not know whether, for example, microbial growth in a moist filter paper can affect serology, but we do not disregard this possibility. Sampling from frozen carcasses instead of fresh blood could also affect the results. When Sacks et al. (2002) compared results of an enzyme-linked immunosorbent assay for heartworm (*Dirofilaria immitis*) used on serum samples collected with filter paper immediately after death with samples collected postmortem from frozen and thawed carcasses of the same animals, they did not find any difference in discriminatory power, but did find higher enzyme-linked immunosorbent assay values in samples collected postmortem from noninfected individuals, indicating more nonspecific binding with test antibodies. We are not aware of reports describing similar effects on HI tests, but we cannot disregard the possibility that freezing and thawing increased the number of false positives in samples collected from carcasses. However, 42 of the 77 (62.7%) individuals with an age determined that were collected in the local sampling were juveniles, whereas all the birds in the country-wide sampling were adults. As discussed earlier, a difference in seroprevalence between juveniles and adults is plausible, suggesting that the difference between the two sample populations may at least be attributed partially to their different age composition.

Cross-reactivity between LIV and TBEV can occur (Mansfield et al. 2011; Klaus et al. 2014), and cross-reactivity may also occur with other closely related flaviviruses. The existence of other flaviviruses in wildlife in Norway has been suggested by Traavik (1979) and Traavik et al. (1984), who hypothesized that flaviviruses transmitted by mosquitos could explain seroreactivity against TBEV outside the known range of sheep tick. A candidate could be West Nile virus, a mosquito-borne flavivirus that can cause fatal infections in bird species within the same subfamily (Tetraoninae) as ptarmigan (Clark et al. 2006; Nemeth et al. 2017). West Nile virus was regarded as not present in Norway in 2017 or during previous transmission seasons (European Centre for Disease Prevention and Control 2017). However, two mosquito-borne flaviviruses (Ilomantsi and Lammi viruses) have recently been isolated in Finland (Huhtamo et al. 2009, 2014), suggesting a potential for unknown flaviviruses in Norway.

Ptarmigan visiting areas with TBEV- or LIV-infected sheep tick, or both, before returning back to the localities where they were shot could explain incidental seropositive birds. Willow Ptarmigan show high philopatry in most areas in Norway, although dispersal from natal areas to breeding grounds can occur (Rørvik et al. 1998; Brøseth et al. 2005). Hence, the exposure of ptarmigan to LIV or TBEV in areas with sheep tick is expected to be limited. Therefore, it seems unlikely that this could cause high prevalence in areas very far away from the known distribution of sheep tick.

The existence of small populations of sheep tick with LIV in climatically suitable microhabitats throughout Norway could explain the pattern of seroconversion observed in this study. Ticks infected with TBEV have been found recently in the UK in areas where no LIV is present in livestock, and this finding seems to represent microfoci of virus persistence (Holding et al. 2020). However, the recent mapping efforts (Jore et al. 2011; Hvidsten et al. 2014) and the geographically limited distribution of tick-borne diseases among people and domestic animals indicate that it is unlikely that microfoci of sheep tick and virus persistence are as common and widespread as indicated in our study (Jore et al. 2011; Paulsen et al. 2015). Ticks infected with LIV could be transported to ptarmigan areas with other birds, as occurs with TBEV (Waldenström et al. 2007; Hasle 2013), and thereafter ingested by ptarmigan chicks. It seems unlikely that this would occur to the extent needed to create the patterns found in this study.

Another possibility is that vectors other than sheep tick transmit LIV—for example, other ixodid ticks present in Norway, such as *Ixodes trianguliceps*, *Ixodes hexagonus*, or *Ixodes uriae* (Jore et al. 2011). Still, it seems unlikely that ptarmigan should have frequent contact with these tick species, which are nidicolous and found in or around the nests or hiding places of rodents and shrews, hedgehogs (*Erinaceus europaeus*) and mustelids, and seabirds.

Infectious diseases have not been regarded as important contributors to population fluctuations in Norwegian ptarmigan populations, but Holmstad et al. (2005a, b, 2006, 2008) describe the presence and effect of several parasite species. No high mortality or disease outbreaks in ptarmigan have been reported in Norway during the last century. Given the low population densities of Norwegian ptarmigan and the rugged and remote landscapes the birds live in, it may be that even high disease-related mortality would be difficult to detect or to differentiate from other causes of mortality. The widespread seroconversion to LIV or a cross-reacting virus do, however, warrant more detailed monitoring and studies into the potential effects of flaviviruses on Willow Ptarmigan population dynamics in Scandinavia. Climate warming, increased bush encroachment in alpine areas, and increased population densities of deer have facilitated increased sheep tick distribution and density (Jore et al. 2014), which may increase distributional overlap between the tick and Willow Ptarmigan, potentially increasing exposure of the ptarmigan to LIV in the coming decades.

This study is a typical illustration of the many impediments of wildlife disease research when it comes to sample acquisition and to the performance and validity of diagnostic tests on samples of variable quality (Stallknecht 2007). The results should hence be interpreted with some caution. The study may indicate that Norwegian ptarmigan in many parts of the country are at risk of infection with LIV or with serologically cross-reacting flaviviruses. Further research should focus on molecular detection of flaviviruses in potential vectors, such as mosquitos and ixodid ticks. Further monitoring and research into the ecology of the virus and potential effect on the ptarmigan population is also warranted. Studies of flaviviruses in wild birds might be important not only in the context of wildlife management, but also in a public health context, especially when exploring the potential for recombination between flaviviruses (Norberg et al. 2013; Bertrand et al. 2016).

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SUPPLEMENTARY MATERIAL

Supplementary Material for this article is online at http://dx.doi.org/10.7589/JWD-D-20-00068.

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