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Influenza A virus surveillance, infection and antibody persistence in snow geese (*Anser caerulescens*)

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Abstract

Some snow geese (*Anser caerulescens*) migrate between Eurasia and North America and exhibit high seroprevalence for influenza A viruses (IAVs). Hence, these birds might be expected to play a role in intercontinental dispersal of IAVs. Our objective in this manuscript was to characterize basic incidence and infection characteristics for snow geese to assess whether these birds are likely to significantly contribute to circulation of IAVs. Thus, we 1) estimated snow goose infection prevalence by summarizing > 5,000 snow goose surveillance records, 2) experimentally infected snow geese with a low pathogenic IAV (H4N6) to assess susceptibility and infection dynamics and 3) characterized long-term antibody kinetics. Infection prevalence based on surveillance data for snow geese was 7.88%, higher than the infection rates found in other common North American goose species. In the experimental infection study, only 4 of 7 snow geese shed viral RNA. Shedding in infected birds peaked at moderate levels (mean peak $10^{2.62}$ EID_{50} equivalents/mL) and was exclusively associated with the oral cavity. Serological testing across a year post-exposure showed all inoculated birds seroconverted regardless of detectable shedding. Antibody levels peaked at 10 days post-exposure and then waned to undetectable levels by 6 months. In sum, while broad-scale surveillance results showed comparatively high infection prevalence, the experimental infection study showed only moderate susceptibility and shedding. Consequently, additional work is needed to assess whether snow geese might exhibit higher levels of susceptibility and shedding rates when exposed to other IAV strains.

KEYWORDS

Anser caerulescens, experimental infection, influenza A virus, serology, snow goose, surveillance

1 | INTRODUCTION

Avian influenza A viruses (IAVs) pose a potential threat to public, livestock and wildlife health and can cause severe economic harm to the poultry industry (Davison et al., 1999; Koopmans et al.,2004; McQuiston et al.,2005; Swayne et al., 2017; Thompson & Seitzinger, 2019). IAVs circulate naturally in many aquatic wild bird species with limited impact on those populations (Olsen et al., 2006; Webster et al., 1992). However, when H5 and H7 IAV subtypes spillover into poultry, they can evolve into highly pathogenic (HP) strains (Pantin-Jackwood & Swayne, 2009; Ramey et al., 2018). HP strains can then spillback into wildlife and sometimes cause

severe morbidity and mortality in those populations (e.g. Kleyheeg et al., 2017). Because a number of HP and high consequence IAVs have emerged in Asia (Ramey et al., 2018) and because North American wild birds may be less likely to have cross-protection for Asian strain viruses, identifying potential IAV introduction pathways from Eurasia to North America is a high priority for understanding and reducing IAV outbreak risks. Information on basic incidence and infection dynamics of lesser studied species is a first step in evaluating whether these species might contribute to the spread of high consequence IAVs.

In late 2014, a HP Eurasian H5 IAV was detected in the United States (US; Ip et al., 2015; Shriner, Root, et al., 2016). This introduction and associated outbreaks in commercial poultry resulted in the death or culling of more than 50 million birds and caused significant economic harm (Hillberg Seitzinger & Paarlberg, 2016; Ramos et al., 2017), highlighting the importance of identifying IAV dispersal pathways between Eurasia and the United States (Shriner, Root, et al., 2016). Because some snow geese (*Anser caerulescens*) migrate between Eurasia and North America or intermix with intercontinental migrants during migratory staging and because snow geese are highly gregarious and exhibit high IAV seroprevalence rates (Pepin et al., 2017; Samuel et al., 2015), this species might be expected to play a role in intercontinental spread of IAVs. Nonetheless, few studies have focused on IAV epidemiology and infection dynamics in this species to evaluate their role in the natural ecology of IAVs.

In general, most wild goose species are considered to pose a relatively low risk of IAV transmission to poultry and humans, primarily based on low infection prevalence for studied goose species and in some cases high mortality from infection with HP IAVs (Elmberg et al., 2017). In the continental United States, most studies that have evaluated IAV prevalence in geese have focused on either wild geese and swans as a broad taxonomic group (e.g. Bevins et al., 2014) or have primarily or exclusively studied Canada geese (*Branta canadensis*, e.g. Harris et al., 2010; Kistler et al., 2012; Kistler et al., 2015). Several studies focused on IAV surveillance in Alaska, United States, found low infection prevalence in several goose species, but higher infection rates for emperor geese (*Chen canagica*, Ely et al., 2013; Ramey et al., 2016; Reeves et al., 2018). Ramey et al. (2019) showed that emperor geese have very high IAV exposure rates for multiple subtypes, can harbour intercontinental reassortant viruses and exhibit high infection prevalence, especially during fall migratory staging. However, emperor geese are primarily distributed in Beringia and are only infrequently observed in the contiguous United States. In contrast, western breeding snow geese migrate through Alaska and winter in large swaths of the continental United States.

Snow geese are medium-sized, long-lived geese that breed in large colonies along the Arctic and sub-Arctic coasts from far eastern Russia through western Greenland (Mowbray et al., 2000). Western Arctic snow geese are potentially exposed to Eurasian IAVs during breeding and migration which may provide an opportunity for this species to play a role in the intercontinental movement of IAVs (Samuel et al., 2015). Snow geese primarily feed on plant material; during migration and winter, they frequently gather in large flocks in agricultural fields to take advantage of waste grain (Mowbray et al., 2000). The substantial availability of agricultural fields as a resource in the United States during migration and overwintering has led to significant population increases in the species and concomitant degradation of breeding habitat due to over forage, especially for Midcontinent and Western Arctic lesser snow geese (Hupp et al., 2017). The frequent use of pastures and agricultural fields by snow geese has led some researchers to suggest that these geese may play a role in IAV spillover to poultry (Bergervoet et al., 2019; Eriksson et al., 2019; Wong et al., 2016).

We took a multi-faceted approach to evaluate IAV epidemiology and infection dynamics in snow geese to better understand their role in the natural ecology of IAVs. We investigated broad-scale infection prevalence, infection characteristics and immunity, by evaluating national-scale surveillance data (APHIS, 2017) across seasons and years to assess the relative prevalence of IAV infections in snow geese. We then experimentally inoculated snow geese with a North American endemic H4N6 virus to assess susceptibility and replication competence. Finally, we tracked long-term antibody kinetics of exposed individuals to improve interpretation of serological data collected from field settings.

2 | METHODS

2.1 | Surveillance

Methods for the US IAV wild bird surveillance based on a US Department of Agriculture and US Department of Interior interagency sampling regime have been described in detail elsewhere (APHIS, 2017; Bevins et al., 2014; Deliberto et al., 2009; Pedersen et al., 2010). In brief, wild bird samples were collected from a variety of avian species from across the United States from 2007–2011 and again in 2016. The majority of samples were collected between October and March except in 2016 when sample collection was limited to January and February. Sampling years were generally defined as beginning in October of a year and lasting through March of the following year. For example, the 2007 sampling year began in October 2007 and continued through March 2008. These sampling seasons reflect the biology of the geese arriving in the continental United States during fall migration, overwintering and then flying north during spring migration.

Samples were collected from 1) morbidity and mortality events, 2) hunter-harvested birds and 3) live bird sampling, with the majority (>98%) of snow goose samples coming from hunter-harvested and live bird sampling. Oropharyngeal and cloacal swabs were collected from each sampled bird, combined in a single cryovial containing brain–heart infusion (BHI) media and shipped to a National Animal Health Laboratory Network (NAHLN) laboratory. NAHLN laboratories are certified by the US National Veterinary Services Laboratory, the World Organization for Animal Health (OIE) reference laboratory for IAV diagnostics in the United States. Surveillance samples were again collected in 2016 using similar methods.

All surveillance samples were tested for IAV matrix (M) gene viral RNA using real-time reverse transcriptase polymerase chain reaction (RRT-PCR) and previously developed primers (Spackman et al., 2002). Samples defined as positive for the M gene by the NAHLN laboratories were further tested by RRT-PCR using H5 and H7 specific primers (Spackman et al., 2002, 2008).

For each sampling year (2007–2010, 2016), we identified all samples collected from snow geese and calculated the number of M gene positive, H5 positive and H7 positive samples. We also assembled associated location information and mapped sample locations to assess broad-scale trends and to visualize the geographic distribution of sample collection compared to snow goose distribution and migratory pathways in the United States. We tested for potential differences in spatial and temporal incidence using logistic regression to compare sampling years, months and the four administrative migratory flyways (Pacific, Central, Mississippi and Atlantic as defined by the US Fish and Wildlife Service), as well as interactions between sampling year (coded as a factor) and flyway and sampling year and month (Model: Incidence ~ Flyway +Sampling Year + Month +Sampling Year*Flyway + Sampling Year*Month). Three models (one with month specified as a continuous variable, one with month specified as a continuous variable plus its squared term, and one with month specified as a categorical variable) were compared using likelihood ratio tests to identify the best fitting model. Models were estimated in R (version 3.5.3) via Rstudio (version 1.2.5033) using the 'glm' function with a logit link (R Core Team, 2019).

2.2 | Experimental infection

We purchased seven approximately one-year-old snow geese from Double 'T' Farm (Glenwood, IA). All birds received a Certificate of Veterinary Inspection prior to purchase. Upon arrival at the National Wildlife Research Center (NWRC), each goose was given an additional health evaluation by the NWRC attending veterinarian and was screened for IAV infection and for antibodies reactive to IAV (see laboratory methods below). All birds were confirmed to be healthy and clear of infection or exposure to IAVs. Birds were individually housed in 2.13 m x 2.13 m x 2.44 m pens constructed with 7.63 cm x 1.27 cm PVC-coated wire mesh in a Biosafety Level 2 (BSL-2) indoor aviary. Each pen was equipped with a shallow water bowl, a food bowl and a small pool (24" x 36" x 8") for swimming and preening.

Following a one-week quarantine period, each goose was orochoanally inoculated with one mL 10^5 Egg Infectious $Dose_{50}$ (EID_{50})/mL of A/mallard/CO/P66F1-5/08 (H4N6) IAV. The virus was collected from a wild bird environmental sample (A/environment/Pennsylvania/NWRC/185996-06/2007 (H4N6)) and then passaged through a mallard prior to virus propagation in hen eggs. An H4N6 virus was selected because it is one of the most commonly isolated subtypes from North American waterfowl (Krauss et al., 2004; Piaggio et al., 2012). Orochoanal, cloacal and faecal swabs were collected daily for 10 days and then again on day 17. All swabs were placed in one mL viral transport media (BA-1: M199-Hank's salts, 1% bovine serum albumin, 350 mg/L sodium bicarbonate, 2.5 mg/ml amphotericin B in 0.05 M Tris, 100 units/mL penicillin, 100 mg/ml streptomycin, pH 7.6) and stored at −80°C prior to laboratory testing.

2.3 | Long-term antibody kinetics

After confirming that all viral shedding had ceased, the birds were removed from BSL-2 testing pens and housed in a large outdoor field pen (approximately 0.25 hectares; Figure 1) for the remainder of the study. We collected serum samples at regular intervals to confirm seroconversion and to evaluate the pattern of detectable antibodies reactive to IAV over time. In addition to the pre-screen when the birds arrived, we also collected serum samples on days 2, 4, 7, 10, 14, 21, 29, 42 and 57 days post-infection (dpi) and then every 4 weeks through 365 dpi. In general, 200–400 μL blood was collected from a peripheral vein, usually the medial metatarsal, into serum separator microtubes, mixed by inverting several times, centrifuged at 12,000 RCF for five minutes and then stored at −80°C until testing.

2.4 | Laboratory methods

All swab samples were tested for the presence of IAV RNA by quantitative PCR (RT-qPCR). Viral RNA was extracted per manufacturer's instructions using MagMax-96 AI/ND Viral RNA Isolation Kits (Life Technologies, Carlsbad, CA USA). RNA extracts were tested in duplicate using primers and a probe specific for the IAV M gene (Spackman et al 2003), iTaq Universal Probes One-Step Kits (Bio-Rad, Hercules, CA USA) and CFX96 Touch Thermo Cyclers (Bio-Rad). Thermocycler conditions were as follows: 50°C for 10 min, 95°C for 3 min and 40 cycles of 95°C for 15 s and 55°C for 30 s. Calibrated controls with known viral titres (10^2, 10^3, 10^4 and 10^5 EID_{50}/mL) were used to construct four-point standard curves. Sample viral RNA quantities were extrapolated from the standard curves and are reported as PCR EID_{50} equivalents/mL (VanDalen et al., 2010). Positive samples were defined as those yielding a two-well positive amplification with a C_q (quantification cycle) value ≤ 38.

Serum samples were analysed by ELISA with the FlockCheck® Avian Influenza MultiS-Screen Antibody Test Kit (IDEXX Laboratories, Inc, Westbrook, ME) as described by the manufacturer, except a sample-to-negative ratio [*S*/*N*] threshold of < 0.7 was applied to optimize correct classification for wild waterfowl (Brown et al., 2009; Shriner et al., 2016).

2.5 | Ethics statement

All experimental procedures complied with the ethical standards of the journal and institutional guides on the care and use of laboratory animals. Wildlife surveillance activities were carried out in accordance with permitting agencies and, if applicable, with the permission

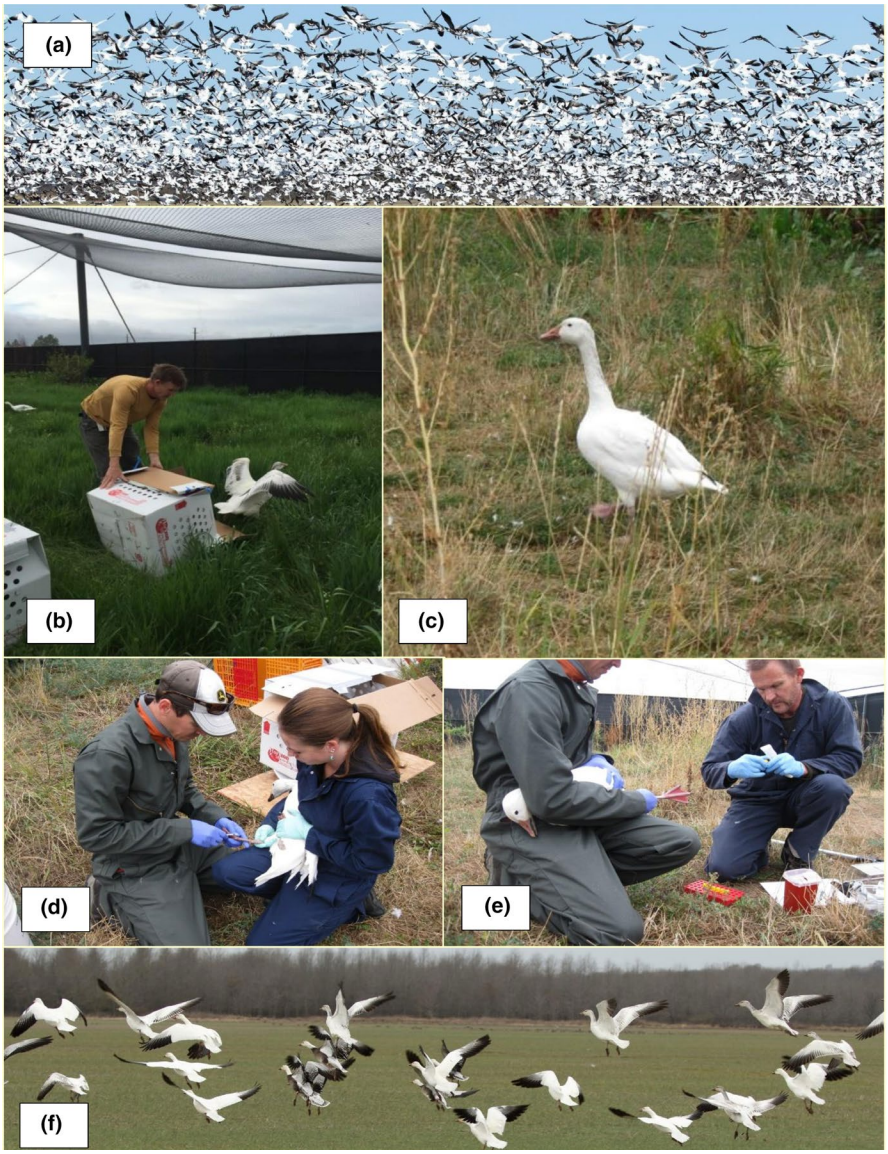

FIGURE 1 Snow geese images captured during surveillance and long-term antibody sampling. (a) shows the very high population densities that occur in many snow goose populations, (b) shows the release of a snow goose into the large flight pen which housed the geese during long-term antibody persistence testing, (c) shows an adult white morph snow goose, (d) and (e) show blood collection for the long-term antibody kinetics study, and (f) shows a flock of snow geese taking off from an agricultural field

of private landowners. Migratory bird capture and sampling were approved by the US Fish and Wildlife Service (Permit Number MB124992) for HP avian influenza surveillance. Samples collected at hunter-check stations were collected through state and local officials and with the permission of participating hunters. Experiments were approved by the Institutional Animal Care and Use Committee of the US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, NWRC, Fort Collins, CO, USA (Approval NWRC 2442).

3 | RESULTS

3.1 | Surveillance

Across 2007 to 2011 and in 2016, 5,178 swab samples were collected from wild snow geese during the US surveillance for the detection of avian influenza viruses in wild birds (Figure 2). The overwhelming majority of samples were collected between October through March, with additional samples collected opportunistically between July and September. These additional samples comprised less than one per cent of the data set. The number of samples tested in each sampling year varied with a minimum of 424 samples collected in the 2010–2011 sampling year and a high of 1,570 samples collected in the 2008–2009 season. The majority of samples were collected from hunter-harvested geese.

The overall incidence of influenza detections was 7.88% with a low of 4.99% during 2016 and a high of 11.32% during the 2010–2011 sampling year. Incidence varied seasonally, peaking over winter (6.04%; Table 1). H5 and H7 detections were relatively rare with 6.37% of the positive samples identified as harbouring H5 viruses (i.e. 0.50% of all samples tested) and only 1.72% of the positive samples identified as harbouring H7 viruses (i.e. 0.14% of all samples tested; Figure 2).

Surveillance samples were collected from a broad geographic area across the contiguous United States, representing a high

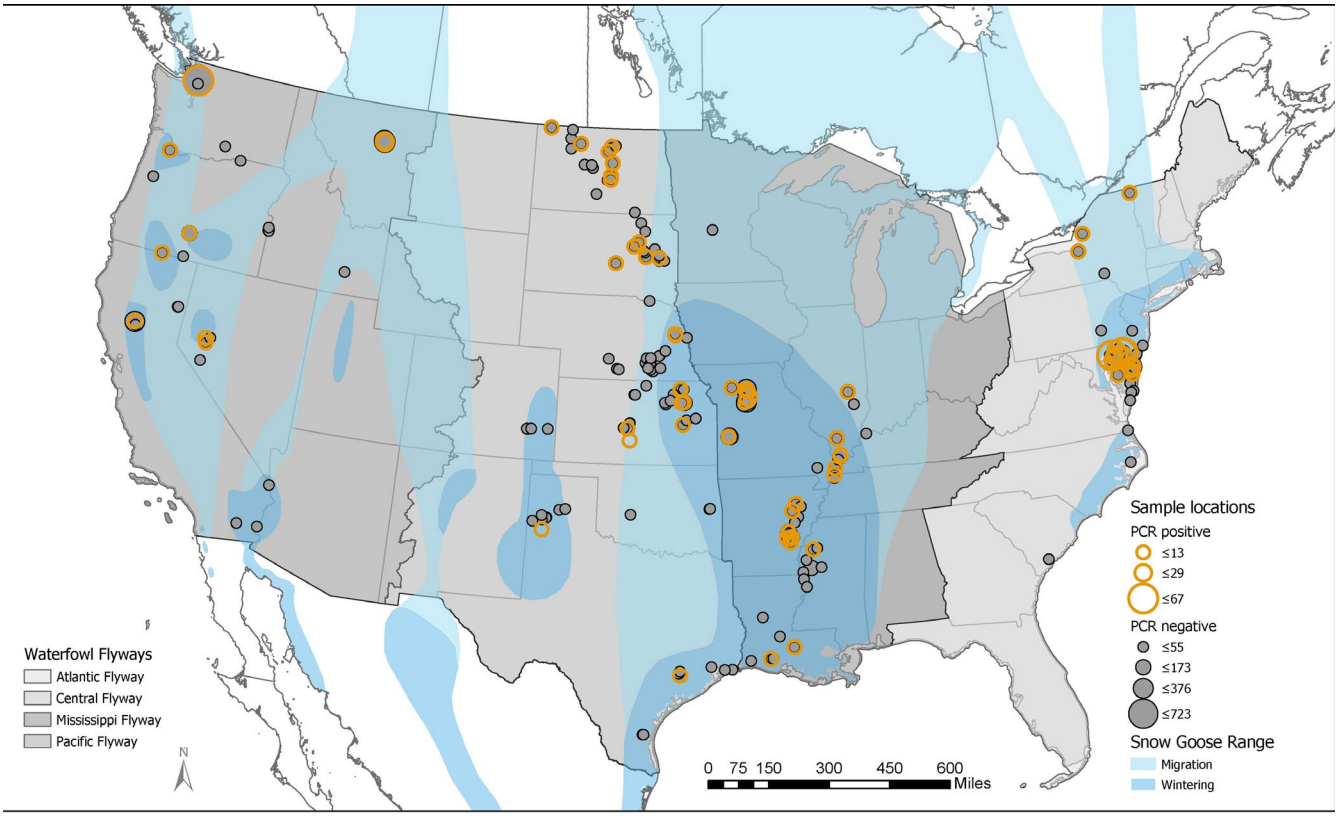

FIGURE 2 Map of the distribution of avian influenza A virus surveillance samples collected from snow geese in the United States, 2007–2011, 2016. Grey dots are negative samples and orange dots are positive samples. Dot sizes increase with the number of samples collected at a site. Snow goose winter distribution is illustrated in darker blue and migration range is illustrated in lighter blue. US Fish and Wildlife Service administrative migratory flyways are illustrated in different shades of grey. The snow goose range map was kindly provided by BirdLife International and Handbook of the Birds of the World (2019)

TABLE 1 Seasonal incidence of influenza A virus for snow geese, United States, 2007–2011, 2016

Season	Swab Samples (*n*)	Positives (*n*)	Positives (%)
Fall Migration October	895	51	6.04
Over Winter November-January	3,780	339	9.85
Spring Migration February-March	464	18	4.04
Summer June-September	39	0	0

proportion of snow goose migration and winter range. Positive samples were broadly distributed across the sampled regions (Figure 3). However, sampling effort varied significantly across years, months and geographic range (Figure 4). For example, in 2007, samples were collected throughout October-March in the Atlantic Flyway, only in December and January in the Mississippi Flyway, and from October-December in the Central Flyway.

The logistic regression model assessing the impact of administrative flyway, sampling year and month that included month as a categorical variable was selected as the model that best fit the data so we report those results here. However, all three models provided qualitatively similar results. The model showed that incidence varied as a function of administrative flyway, sampling year and sampling month with significant interactions between sampling year and flyway and sampling year and month. Incidence was highest in the Atlantic Flyway, primarily driven by a high proportion of positives in the Delmarva Peninsula, but was not significantly different from the Central and Pacific Flyways. Incidence in the Mississippi Flyway (p =.001), however, was significantly lower compared to the other three flyways. Incidence also varied by sampling year with the 2009–2010 and the 2016 sampling years having significantly lower infection prevalence compared to the other years (p =.041 and p =.040, respectively). The interaction terms between sampling year and flyway and sampling year and month captured potential epizootic peaks (Figure 4), e.g. in January 2008 in the Atlantic Flyway), December 2009 in the Pacific Flyway, and December-January 2010 in the Mississippi Flyway (Figure 4).

3.2 | Experimental inoculation

Only four of seven geese shed viral RNA for more than one day at greater than $10^{1.00}$ EID_{50} equivalents/mL (Figure 5; Supplementary material S1). Two of the geese did not have a single positive swab,

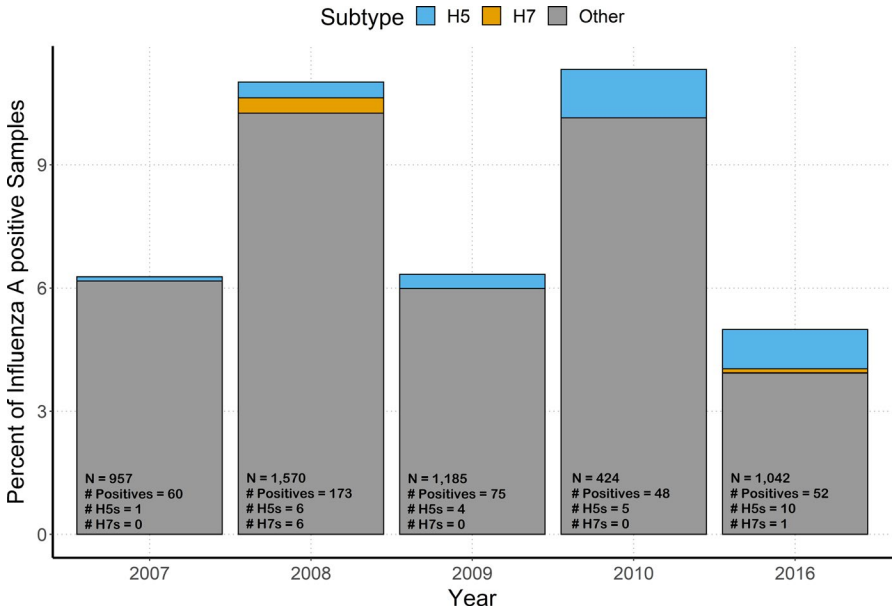

FIGURE 3 Per cent of snow geese infected with influenza A virus by sampling year (October of sampling year through September of the following year), United States, 2007–2011, 2016. Column heights indicate the overall per cent of positive samples for each year while the blue bar (top) represents H5 infections and the orange (middle) indicates H7 infections. Each sampling year started in October and continued through March of the following year (with opportunistic samples from April to September) except for 2016 which only included sampling from January and February

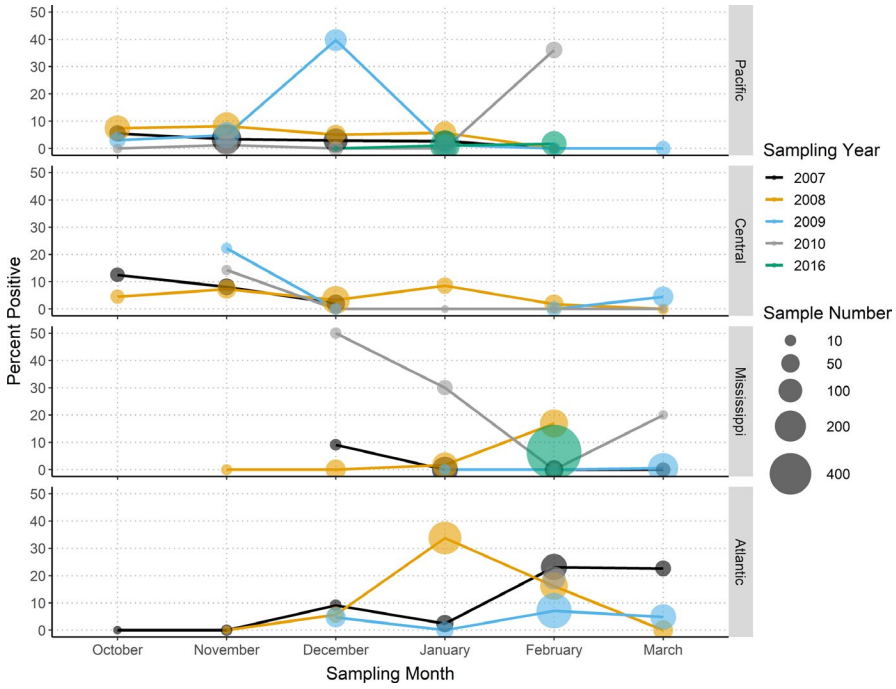

FIGURE 4 Monthly infection prevalence for the four US Fish and Wildlife Service administrative flyways and five sampling years. Dot sizes scale to the number of samples collected for a particular month, sampling year and flyway

and a third goose only had a single suspect positive swab ($10^{0.84}$ EID_{50} equivalents/mL). For the four geese that shed viral RNA, detections were exclusively associated with oral swabs and all cloacal and faecal swabs were negative for viral RNA. For these four birds, the mean peak viral load was $10^{2.93}$ EID_{50} equivalents/mL (range: $10^{2.79}$–$10^{3.20}$), the mean peak day post-infection was 2.75 (range 2–4) and shedding lasted for an average of 5.25 days (range 2–8 dpi).

3.3 | Long-term antibody kinetics

Six of the seven snow geese were positive for antibodies against IAV on 7 dpi and all geese were positive on 10 dpi. The peak median response occurred on 10 dpi and was followed by a gradual decline over time (Figure 6; Supplementary material S2). The median response dropped below the threshold for a positive sample on approximately 141 dpi (20 weeks), rose above the threshold 4 weeks later and then stayed below the threshold for the remainder of the year. The mean response on 365 dpi was the same as on 0 dpi (0.8 *S/N*) and fell to approximately that level on 309 dpi. One of the geese died prior to our six-month antibody sampling unrelated to experimentation based on gross pathology at necropsy. The remaining six snow geese remained in good health throughout the rest of the study.

While all of the geese showed a similar pattern of antibody kinetics—a sharp rise between days 4 and 7 post-exposure, a peak

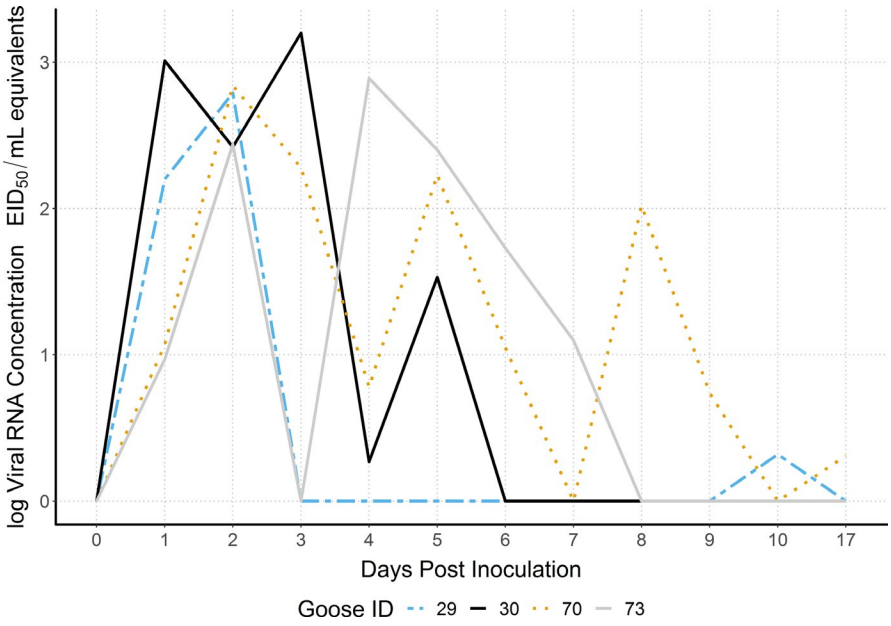

FIGURE 5 Viral RNA concentrations (EID_{50}/mL equivalents) for oral swabs collected from snow geese inoculated with an H4N6 influenza A virus. Only 4 of 7 inoculated geese shed viral RNA above 10 EID_{50}/mL for more than one day post-inoculation

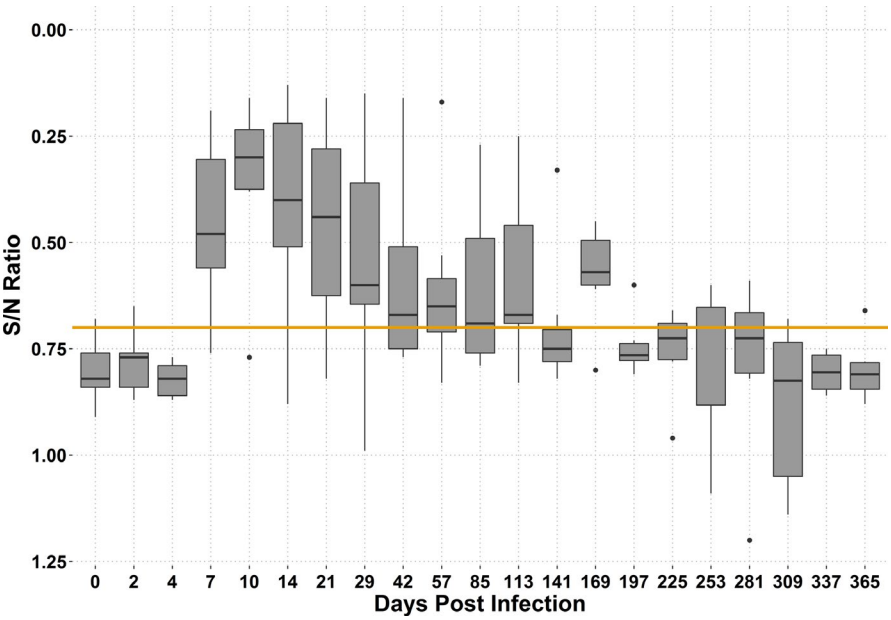

FIGURE 6 Antibody persistence for snow geese exposed to an H4N6 influenza A virus. Sample-to-negative (*S/N*) ratios < 0.7 (orange line) were considered positive for antibodies to influenza A virus. All 7 exposed geese exhibited a positive antibody response by 10 days post-inoculation

around 10 dpi and then a general waning to undetectable levels around six months—individual results showed substantial individual heterogeneity and variability between sampling periods. One of the three geese that did not show evidence of viral shedding had consistently high antibody levels (i.e. *S/N*s consistently below the median), but the other two RNA negative birds had antibody levels generally near the median response. The goose that died after six months of antibody testing had antibody levels considerably higher than the median for all sampling periods after 10 dpi, potentially indicating an elevated immune response associated with non-test related underlying disease.

While the FlockCheck® Avian Influenza MultiS-Screen Antibody Test Kit was not optimized for antibody testing in snow geese, the results from this study indicate the test is effective in this species and that a *S/N* threshold of 0.7 is appropriate for discriminating between positive and negative samples (mean *S/N* = 0.78 across the pre-bleed and days 0, 2 and 4 post-infection samples, mean *S/N* = 0.58 across samples from days 7, 10, 14 and 21 post-infection). Because antibody levels for exposed birds dropped below detectable limits by six months post-exposure for most individuals, it is possible that our initial pre-screen may not have identified a prior exposure since the birds were approximately a year old when we acquired them. However, in the event of a previous exposure, we would have expected to see an anamnestic response (a rapid rise in antibodies against a previously encountered pathogen recognized by memory cells), but ELISA results for days 2 and 4 post-inoculation did

not differ from the pre-bleed or day 0 results, and a sharp rise did not manifest until 7 dpi.

4 | DISCUSSION

Overall, the snow goose samples collected during the 2007–2011 and 2016 US interagency wild bird surveillance programme provided a good spatial match to snow goose migratory and winter range in the continental United States. Positive samples were generally evenly spread across the sampled regions, with no obvious spatial patterns emerging (Figure 2). Infection prevalence for snow geese was notably higher at a mean of 7.88% across years (high of 11.32% in 2010) compared to the 4.26% reported by Bevins et al. (2014) for all geese and swans as a group (3.4% if snow geese are excluded) but lower than the 15.8% for dabbling ducks. H5 and H7 subtype IAVs were relatively uncommon in this data set and made up less than 10% of positive samples. In a study focused on North American Canada geese, Harris et al. (2010) estimated a much lower mean prevalence of 0.5% for Canada geese in a review of nine studies based on virus isolation of IAVs. In addition to the expected lower prevalence estimates for studies reporting isolation compared to the PCR detections reported here, this estimate might also be artificially low due to a reliance on cloacal swabs in some of the studies. A surveillance study conducted in the Pacific Flyway as a response to the 2015 HP H5N8 and H5N2 outbreaks in the United States, also found a low infection prevalence (<2.0%) for Canada and cackling geese for PCR-based detections (Bevins et al., 2016).

Consistent with other broad-scale evaluations of IAVs in waterfowl in North America (Bevins et al., 2014; Gorsich et al., 2020), we identified variability in infection prevalence across years, sampling month and flyways. However, the correlations identified by our regression model should be interpreted with some caution because the interagency surveillance programme necessarily had a somewhat unbalanced sampling design, partially due to a reliance on opportunistic sampling (e.g. hunter harvest and morbidity/mortality samples), but also because migratory pulses vary temporally between latitudes. For example, peak fall migration can occur six weeks earlier in North Dakota compared to Louisiana, thus constraining the sampling months available at the different locations (Mowbray et al., 2000). For the surveillance data set analysed here, most of the samples collected from the Mississippi Flyway were collected in southern states rather than broadly throughout the flyway. That bias towards samples from more southern latitudes may explain the relatively lower infection prevalence found for that flyway since infection prevalence in the northern hemisphere (during migration and overwintering) is generally thought to decrease with decreasing latitude (Bevins et al., 2014). Our finding of elevated infection prevalence in winter compared to spring and fall migration may indicate that snow geese are more likely to become infected while overwintering than on the breeding grounds. Nonetheless, mean infection prevalence was 6.04% during fall migration, indicating a not insignificant potential to spread IAVs from the breeding grounds and Alaska to the continental United States.

Limited surveillance sampling for IAVs in snow geese has been previously reported for the continental United States. One of the largest previously reported data sets is work by Preskenis et al. (2017) who found an infection prevalence of 12% for 656 samples collected between 2007 and 2009 in the Delmarva Peninsula in Delaware based primarily on a subsample of the interagency surveillance programme samples reported herein. Across the three years in which samples were collected from snow geese, they found very high prevalence in the first two years (20 and 21%), but only 1% incidence in the third year, demonstrating significant year-to-year variability. Similarly, Samuel et al. (2015) reported on a subset of snow goose samples from the interagency surveillance programme collected from 2006 to 2010 in the Pacific Flyway. While infection prevalences varied across years and sites, in general they found the highest levels of infection from samples collected from the US state of Washington (2.4% - 17% from a total of 1,007 oral/cloacal samples), much lower levels from samples collected in Alaska (0%-4.6% across 2,920 samples), and no positives for the relatively fewer 325 samples collected in California, Nevada and Idaho. In two small scale studies, testing of 29 faecal samples from snow geese from the Platte River, Nebraska, collected during spring migration were all negative (Vogel et al., 2013) and another 151 combined oral/cloacal swabs from snow geese overwintering on the Gulf Coast of Texas were also negative by virus isolation (Wong et al., 2016).

Three studies have reported results for snow geese sampled in Canada and Alaska. Liberda et al. (2017) reported negative results from 16 cloacal swabs collected from hunter-harvested snow geese in sub-Arctic Ontario during spring and autumn migration and tested by RT-PCR. Reeves et al. (2013) reported two IAV sequences from cloacal swabs collected from snow geese in Alaska. Of note, neither of the isolated viruses included Eurasian lineage genes. Ramey et al. (2016) found a relatively high infection prevalence of 8.06% based on RRT-PCR from 62 combined oral/cloacal swabs collected during May, but none of those detections were positive by virus isolation. The RNA detections may have benefited from testing oral swabs and not just samples collected from faecal or cloacal swabs, especially given the results of our experimental inoculation study that showed shedding was exclusively from the oral cavity.

In a study of nearly 3,000 snow geese from Wrangel Island, Russia, and Banks Islands, Canada, in the Arctic, seroprevalence levels were quite high, ranging from 32.4% to 75.9% (Samuel et al., 2015). While these geese were sampled in the Arctic, the birds overwinter in the continental United States so IAV exposures may have occurred in their breeding, migratory or winter ranges. Wong et al. (2016) also found high seroprevalence rates in their study of overwintering snow geese on the Gulf Coast of TX with an overall seroprevalence of 59% from 147 birds tested. Microneutralization tests indicated that most birds had been exposed to multiple IAV subtypes with H6 and H9 subtypes the most common, followed by H1, H5 and H12. Very few of the snow geese showed evidence for exposure to the H4

subtype. These results are consistent with the isolations of H6 and H1 viruses in snow geese by Preskenis et al. (2017).

We selected an H4N6 IAV for the experimental inoculation study because it is one of the most common subtypes in North American waterfowl and because genetic studies reveal that most H4s in the United States are endemic (Piaggio et al., 2012). Because the H4N6 subtype is widespread in the United States, but was uncommonly observed in the Wong et al. (2016) study, this subtype might only play a minor role in natural snow goose IAV dynamics. Given the relatively high infection rates for snow geese in the wild bird surveillance combined with the very high seroprevalence rates reported by Samuel et al. (2015), we anticipated that we would see high rates of susceptibility and shedding in our experimental inoculation study rather than the moderate susceptibility and shedding that we observed. We hypothesize that other subtypes, such as the H6s commonly observed in snow geese in prior studies (Preskenis et al., 2017; Wong et al., 2016), may produce different results. Our result that shedding was primarily via the oral route is consistent with studies of other goose species that suggest that oral shedding is more common than cloacal shedding (Eriksson et al., 2019; Kleijn et al., 2010).

While only four of the seven snow geese that we experimentally inoculated shed viral RNA for more than a day, our long-term antibody persistence study showed that all birds were exposed and developed an immune response. Antibody responses developed rapidly with most birds showing antibodies reactive to IAV by 7 dpi with a peak on 10 dpi. Antibody levels waned over the next several months, but most birds still had detectable antibodies within six months post-exposure. Antibody levels were undetectable within a year. Wild snow geese are a long-lived species so it is likely that they are exposed to multiple IAVs across their lifespans. Multiple authors who have evaluated long-lived birds have found that IAV seroprevalence increases with age in swans and geese (Lambrecht et al., 2016; Samuel et al., 2015; Wong et al., 2016). Even though antibody levels can wane after a primary exposure, it is likely that geese experience an anamnestic response after a secondary exposure such that antibody levels are higher after the secondary exposure and wane more slowly.

The results of this study indicate that snow geese are commonly exposed to and infected by IAVs in nature. However, more work is needed to determine whether the infection dynamics we observed in the experimental infection study are typical or whether these geese might show higher rates of susceptibility and shedding for other IAV subtypes. Moreover, field studies or surveillance efforts focused on the isolation and sequencing of viruses from naturally infected snow geese in the continental United States would provide subtype and strain information for assessing the risk these geese pose in the movement of high consequence IAVs.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All experimental data that support the findings of this study are available in the supplementary material of this article. Surveillance data are not publicly available due to privacy or ethical restrictions, but may be available upon request from the corresponding author and the implementation of a material transfer agreement.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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