SUSCEPTIBILITY OF LAUGHING GULLS (LEUCOPHAEUS ATRICILLA) AND MALLARDS (ANAS PLATYRHYNCHOS) TO RUDDY TURNSTONE (ARENARIA INTERPRES MORINELLA) ORIGIN TYPE A INFLUENZA VIRUSES

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Journal of Wildlife Diseases. 2020;56(1):167–174 DOI: 10.7589/2019-03-065

ABSTRACT: Delaware Bay, US is the only documented location where influenza A virus (IAV) is consistently detected in a shorebird species, the Ruddy Turnstone (RUTU; Arenaria interpres morinella). Although IAV in shorebirds has been well studied at this site for decades, the importance of other species in the avian community as potential sources for the IAVs that infect RUTUs each spring remains unclear. We determined the susceptibility of Mallards (Anas platyrhynchos) and Laughing Gulls (Leucophaeus atricilla), to IAVs isolated from RUTUs in order to gain insight into the potential host range of these viruses. **Captive-reared gulls were challenged with RUTU-origin H6N1, H10N7, H11N9, H12N4, and H13N6 IAV, as well as Mallard-origin H6N1 and H11N9. We challenged captive reared Mallards with the same viruses, except for H13N6**. At a biologically plausible challenge dose (104 50% embryo infective doses/0.1 mL), one of five gulls challenged with both H6N1 IAVs shed virus. The remaining gulls were resistant to infection with all viruses. In contrast, all Mallards were infected and shed virus. The H12N4 Mallard challenge group was an exception with no birds infected. These results indicated that Mallards are permissive to infection with viruses originating from a shorebird host and that interspecies transmission could occur. In contrast, host adaptation of IAVs to RUTUs may compromise their ability to be transmitted back to gulls.

Background

* High prevalence of influenza A virus (IAV) at Delaware Bay, NJ in ruddy turnstones with no clear source
* Suggested origins are gulls or dabbling ducks, both have documented IAV infections in the area

Key Points

* Challenged laughing gulls and mallards with ruddy turnstone and mallard-origin IAVs to assess potential for virus to be shared with ruddy turnstones.
* Cloacal and oropharyngeal swabs taken for virus isolation and qRT-PCR
* Serum antibodies measured
* No birds displayed clinical signs
* Mallards seroconverted and shed ruddy turnstone-origin IAVs
* Laughing gulls were very resistant to ruddy turnstone and mallard-origin IAVs

Conclusions

* Influenza A virus may pass readily between ruddy turnstones and mallards
* Host adaptation of ruddy turnstone-origin IAVs may limit transmission back to gulls

## GENETIC CHARACTERIZATION OF H13 AND H16 INFLUENZA A VIRUSES IN GULLS (LARUS SPP.) WITH CLINICALLY SEVERE DISEASE AND CONCURRENT CIRCOVIRUS INFECTION

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J Wildl Dis. 2017 Jul;53(3):561-571.

**Taxonomy:** Aves → Charadriiformes (order) → Laridae (family) → Larinae (subfamily) → Larus (genus)

**Abstract**: Influenza A viruses (IAVs) of the subtypes H13 and H16 are primarily found in gulls (Larus spp., order Charadriiformes). Although the gull-adapted subtypes replicate efficiently during infection, gulls usually remain apparently healthy during infection. Avian influenza virus isolates are generally separated into two distinct populations, North American and Eurasian, because of the limited gene flow between the continents. Reassortment between these lineages does occur occasionally; however, direct intercontinental transmission of all eight gene segments is rare. Extensive research has been done to understand the ecology of IAV subtypes that naturally circulate in ducks (order Anseriformes), but the ecology of H13 and H16 IAVs in gulls remains far less studied. In Finland, gulls were screened for IAVs for passive (dead and diseased gulls) and active (clinically healthy gulls) surveillance purposes during the years 2005–10. During that period, 11 H13, two H16 viruses, and one H3N8 IAV were detected. We sequenced partial and full-length hemagglutinin genes of these gull-origin IAVs for phylogenetic assessments. All but one of the H13 genes clustered together with northern European and northeastern Asian viruses, whereas one virus clustered with North American viruses. Interestingly, a high rate (10/ 14) of these low-pathogenic IAVs was detected in dead or diseased gulls. The atypical clinical status of the IAV-positive gulls and previous observations of circovirus-like inclusion bodies in diseased gulls during autopsies, led us to screen for concurrent circovirus infections in our samples. The DNA of circovirus, an immunosuppressive pathogen of both birds and mammals, was detected in 54% (7/13) of the tested IAV-positive gulls, whereas only 25% (14/56) of our panel of IAV-negative gulls tested positive by circovirus PCR.

**Background:**

* Influenza A viruses (IAV) are maintained by Anseriformes and Charadriiformes (no clinical signs)
  + Typically shed from GI tract
* IAV surface glycoproteins : Hemagglutinins (H) = 16 and neuraminidase (N) =9
* H13 and H16 are found almost exclusively in gulls and terns
  + H13 is specific to gulls because of the host cell sialic acid receptor
  + Gulls shed H13 from oropharynx and intestines; H16 from GI epithelium
* Migration, molting, and wintering allow sharing of disease and reassortment
* Circoviruses = small non-enveloped, circular/ssDNA
  + Some strains cause immunosuppression
  + Typically a narrow host range (e.g. psittacine beak and feather disease)
  + Target organ is bursa of fabricius (where B lymphocytes develop)

**Key Points:**

* Majority of positive IAV gulls were European Herring gull (11/14)
* Circovirus found in 13% of healthy birds and 40% of dead or diseased birds
* IAV-positive gulls (54%) were more likely to be positive for circovirus than IAV-negative birds (25%)
* Low pathogenic IAVs generally do not cause disease, but most of IAV came from dead or disease birds

**Conclusion:**  Gulls carry H13 and H16 strains of influenza A virus, and sick gulls appear to have co-infection with circovirus.

Ballard, Jennifer R., et al. "Prevalence and distribution of Wellfleet Bay virus exposure in the common eider (Somateria mollissima)." *Journal of wildlife diseases* 53.1 (2017): 81-90.

Abstract: Between 1998 and 2014, recurrent mortality events were reported in the Dresser's subspecies of the Common Eider (*Somateria mollissima dresseri*) on Cape Cod, Massachusetts, US near Wellfleet Harbor. The **early die-offs were attributed to parasitism and emaciation, but beginning in 2006 a suite of distinct lesions was observed concomitant with the isolation of a previously unknown RNA virus. This novel pathogen was identified as an orthomyxovirus in the genus *Quaranjavirus* and was named Wellfleet Bay virus (WFBV).** To assess evidence of exposure to this virus in Common Eiders, we **conducted a longitudinal study of the prevalence of WFBV antibodies at multiple locations from 2004–14;** we collected 2,258 serum samples from six locations and analyzed each using a microneutralization assay. Results corroborate the emergence of WFBV in 2006 based on the first detection of antibodies in that year. **Significantly higher prevalence was detected in Common Eiders sampled in Massachusetts compared to those in Maine, Nova Scotia, and Québec.** For birds breeding and wintering in Massachusetss, **viral exposure varied by age, sex, and season of sampling, and prevalence by season and sex were highly interrelated with greater numbers of antibody-positive males in the autumn and females in the spring.** No evidence of viral exposure was detected in the Northern subspecies (*Somateria mollissima borealis*). Among the locations sampled, **Massachusetts appears to be the epicenter of Common Eider exposure to WFBV.** Further research is warranted to understand the factors controlling the epidemiology of WFBV in Massachussetts, including those that may be limiting geographic expansion of this virus.

* Introduction:
  + **1998-2014, recurrent mortality events in Dresser’s subspecies of Common Eider. Hepatocellular, pancreatic, splenic necrosis with isolation of unknown RNA virus later determined to be a novel orthomyxovirus – Wellfleet Bay virus.**
  + 6 spp of Common Eider globally:
    - Dresser’s ssp – Labrador to MA, winters south of Gulf of St. Lawerence.
    - Northern ssp – Northeastern Arctic CA, Greenland, Iceland, winters in CA, S Greenland, Iceland.
    - Hudson Bay ssp – Breeds and winters in James and Hudson Bays, some overlap with N ssp.
  + **Two inoculation trials showed seroconversion by 5d postinoculation and could be detected through day 30.**
    - **Detection of WFBV Ab from free-ranging Common Eiders should be a useful indicator of viral exposure, though temporal interpretation is limited.**
* M+M:
  + **Serum samples from banked samples (2004-2011), targeted sampling of healthy, sick, recently dead birds (2011-2014), and apparently healthy CE collected during normal banding/other activities in Iceland, Maine, Nova Scotia, Nunavut, Quebec, Rhode Island.**
  + **Two microneutralization assays** used for WFBV antibody detection. Samples positive by the first assay were tested by the second assay.
  + Evaluated relationship between demographic characteristics and WFBV exposure in the MA subpopulation.
* Results:
  + 2,259 serum samples.
  + First WFBV exposure in three eiders in St. Lawrence Estuary 2006.
  + **Prevalence highest for Massachusetts (16.3%) and then RI. Ab also detected in nesting colonies in ME, NS, and Quebec.**
  + Ab prevalence differed between N ssp (0%) and **Desser’s ssp (6.2%)**. Within Dresser’s ssp, did not vary significantly by sampling location.
    - Prevalence independent of sex, age, and season.
  + **MA subpopulation – prevalence higher in breeding season, adult birds. Females in breeding season, males in nonbreeding season higher prevalence.**
* Discussion:
  + Apparent WFBV emergence in 2006. Early samples positive from Quebec.
  + Massachusetts most likely important than Quebec for epidemiologic importance.
  + Females only spend extended periods on land during the breeding season, this is also the period most-often studied.
  + Virus sources and routes of transmission unknown.

Leclerc, Antoine, et al. "Stress influence on plasma protein electrophoresis in the red-breasted goose (branta ruficollis) and hawaiian goose (branta sandvicensis)." *Journal of Zoo and Wildlife Medicine* 48.1 (2017): 136-143.

Abstract: The objective of this study was to evaluate the effects of stress (as measured by total heterophil þ eosinophil counts [THECs] and plasma corticosterone [PC] levels) on plasma protein electrophoresis (PPE) in two Anseriform species. Ten red-breasted geese (Branta ruficollis) and eight Hawaiian geese (Branta sandvicensis) were received into quarantine at Beauval Zoo and housed together with other Anseriformes. Two days later, all were examined as part of routine quarantine procedures, and blood was collected. THECs were performed using a Malassez hemocytometer after a 1 : 200 dilution with an eosinophil dilution liquid containing phloxine B. PPE, as well as total protein and PC assays, was performed on lithium heparin plasma. Twenty days after arrival, all birds were similarly restrained, examined, and sampled. Pododermatitis lesions were identified on four geese that were excluded from statistical analysis. For each goose, THECs and PC values were sorted by value (lower or higher) independently from the day of sampling. A Wilcoxon signed rank test showed no significant differences between lower and higher values of THECs for any of the PPE fractions. Higher values of corticosterone were associated with higher values of prealbumin, but none of the other fractions were significantly different. A Spearman rank correlation coefficient showed that THECs and PC were not correlated; this suggested differences in kinetics between these stress markers. Results did not show significant alterations of electrophoresis patterns associated with stress as measured with THECs and PC. The results also highlight the complexity of precisely assessing acute and chronic stress in avian species

Intro

* Plasma protein electrophoresis (PPE) is a valuable diagnostic tool in avian medicine
* Changes in different PPE fractions occur in response to chronic or acute inflammatory processes
* However, reliability is controversial and variation exists between species or in response to physiologic and metabolic phenomena such as molting or egg-laying
* The objective of this study was to evaluate the effects of stress, as measured by total heterophil and eosinophil counts (THECs) and plasma corticosterone (PC), on PPE in two Anseriform species: the redbreasted goose (Branta ruficollis) and the Hawaiian goose (Branta sandvicensis).

M&M

* 10 red-breasted geese and 8 Hawaiian geese
* Blood collected on day 1 and 20 of quarantine
* Heterophil and eosinophil count performed
* PPE performed 5 weeks later (frozen plasma)

Results

* Hawaiian and red-breasted geese electrophoresis patterns appeared to be very similar
* When sorting electrophoresis results by THECs, no significant difference was established regarding the plasma electrophoresis values
* regarding the plasma electrophoresis values. When sorting electrophoresis results by PC, prealbumin values appeared to be significantly higher among high PC blood samples (Wilcoxon test: P ¼ 0.033)
* No significant difference was established regarding the other plasma electrophoresis values. THECs and PC values were not correlated (Spearman rank correlation coefficient: R = 0.095; n =28; P . 0.05

Discussion

* Our results did not show significant alterations of the PPE patterns associated with stress as assessed by the two markers studied; only the prealbumin fraction was significantly more elevated in high PC value samples but not in high THECs value samples
* The prealbumin fraction of avian PPE is known to be markedly variable between species and seasons and therefore is considered of little diagnostic value
* In the present study, prealbumin increased with PC. This finding is similar to reported increases in prealbumin values in humans receiving exogenous steroids
* However due to low reproducibility of this fraction, the authors consider that the difference obtained for this fraction, with only one of the two stress markers, is not clinically relevant
* THECs and PC values were not correlated; this suggests potential differences in kinetics between these stress markers
* Fecal corticosterone could be evaluated as a marker of chronic stress for future studies
* Heterophil:lymphocyte ratio could also have been evaluated as a marker of stress
* It is likely that chronic stress would result in more-profound physiologic changes that could alter PPE patterns whereas acute stress stimuli, assessed by acute markers, might not be sufficient to trigger important physiologic changes.
* Another possibility is that stress levels might not have been sufficient to significantly alter PPE in the present study

GEOGRAPHIC AND ECOLOGIC ASPECTS OF THE COMMUNITY STRUCTURE OF TREMATODES OF MALLARDS (ANAS PLATYRHYNCHOS) IN NORTHERN POLAND AND THE CZECH REPUBLIC

JWD 2020 56(3) 576-587

Abstract:

Trematode infestation of Mallards (Anas platyrhynchos) varies between northern Poland and the Czech Republic. We determined the trematode fauna of juvenile and adult Mallards from Poland in 2010–16 (n=79 Mallards) and from the Czech Republic in 1964–2001 (n=296). Infracommunity diversity of trematodes from juvenile Mallards, defined by the Brillouin index and mean number of species, was significantly greater in Poland than it was in the Czech Republic. There were more species and greater biodiversity in the trematode communities of Mallards from Poland, where the environment is more natural and less altered by human activity than it is in the Czech Republic. In the trematode community of juvenile Mallards, the Simpson’s index of biodiversity was higher in the Czech Republic than it was in Poland. The Berger-Parker dominance index was comparable in both countries. In terms of prevalence, the structure of the trematode fauna was hierarchic, and trematode species had different positions in this hierarchy in the two countries. Statistically significant differences in the epidemiologic indices between infection of juvenile Mallards from Poland and the Czech Republic were noted for Bilharziella polonica, Echinostoma miyagawai, Echinostoma revolutum, Prosthogonimus cuneatus, Prosthogonimus ovatus, Notocotylus attenuatus, Echinoparyphium recurvatum, Australapatemon minor, Apatemon gracilis, Cyathocotyle prussica, Hypoderaeum conoideum, Metorchis xanthosomus, Psilochasmus oxyurus, and Cotylurus cornutus.

Summary:

* Introduction
  + trematodes common in migratory Mallards in Czech Republic and Poland
* M + M
  + trematode burdens and diversity evaluated in juvenile and adult Mallards from Poland in 2010–16 (n = 79) and from Czech Republic in 1964–2001 (n = 296)
  + birds collected via hunting
* Results/Discussion
  + trematode fauna of juveniles more diverse than adults
  + geographic variation exists - variation based on ecosystem origin
  + infracommunity trematode diversity and mean number of trematode species greater in Poland
  + higher faunistic similarity in adult Mallards than in juveniles in Poland and Czech Republic
  + lower abundance of individual helminth species in the Czech cohorts – may be related to degradation of water bodies and decreased environmental naturalness
  + trematodes with highest prevalence co-occur because they require similar environmental conditions in life cycles
  + in US and Canada, non-native trematodes (Cyathocotyle bushiensis and Sphaeriodiotrema spp.) have been cause of die-offs of waterfowl
    - host - invasive Eurasian faucet snail