One Health 18 (2024) 100760

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Assessing avian influenza surveillance intensity in wild birds using a One Health lens

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ARTICLE INFO

Keywords:
Avian influenza virus
Wildlife disease surveillance
Migratory birds
One health
Wild birds
Poultry
Zoonotic pathogen surveillance

ABSTRACT

Wildlife disease surveillance, particularly for pathogens with zoonotic potential such as Highly Pathogenic Avian Influenza Virus (HPAIV), is critical to facilitate situational awareness, inform risk, and guide communication and response efforts within a One Health framework. This study evaluates the intensity of avian influenza virus (AIV) surveillance in Ontario's wild bird population following the 2021 H5N1 incursion into Canada. Analyzing 2562 samples collected between November 1, 2021, and October 31, 2022, in Ontario, Canada, we identify spatial variations in surveillance intensity relative to human population density, poultry facility density, and wild mallard abundance. Using the spatial scan statistic, we pinpoint areas where public engagement, collaborations with Indigenous and non-Indigenous hunter/harvesters, and working with poultry producers, could augment Ontario's AIV wild bird surveillance program. Enhanced surveillance at these human-domestic animal-wildlife interfaces is a crucial element of a One Health approach to AIV surveillance. Ongoing assessment of our wild bird surveillance programs is essential for strategic planning and will allow us to refine approaches and generate results that continue to support the program's overarching objective of safeguarding the health of people, animals, and ecosystems.

1. Introduction

Wildlife disease surveillance systems can support early detection of emerging pathogens, promote preparedness, and provide the contextual basis to inform timely and effective response in the event of an outbreak. Zoonotic pathogen surveillance in particular warrants an integrated approach that maps and evaluates the system within a One Health framework in recognition of the inextricable linkages that exist at the human-animal-environmental interface. One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems [1].

Highly pathogenic avian influenza virus (HPAIV), a virus of global significance, is widely recognized as an important threat to agricultural biosecurity [2,3], and increasingly for wild bird conservation [4,5] and public health [6]. In Canada, there have been significant impacts on commercial, non-commercial, and other captive poultry facilities with millions of domestic poultry culled [7], due to the incursion of the HPAIV H5N1 (clade 2.3.4.4b) to North America starting in late 2021 [8]. HPAIV in wild bird species, including species at risk in Canada, has also resulted in mortality events with the potential for population level impacts causing conservation concern in some cases [9]. Available evidence points to shifting dynamics in the epidemiology of HPAIVs in wild bird populations, with migratory populations implicated in the repeated incursion and maintenance of HPAIVs [10]. Locations and time periods of increased wild bird abundance are associated with peaks in HPAIV detections in wild birds [5,11] and in poultry facilities [12]. The density

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https://doi.org/10.1016/j.onehlt.2024.100760
Received 16 February 2024; Accepted 14 May 2024

of poultry facilities has also been associated with increased risk of HPAIV outbreaks on premises [13] and between farm transmission [14].

Reports of human HPAIV infection and mortality [15] underscore the responsibility of government officials to communicate public health risks, address public concerns, and provide data-driven guidance. Significant media attention on this issue has emphasized the need for government officials to have the information necessary to serve as early and credible sources. Consequently, there is a requirement for due diligence to conduct HPAIV surveillance in areas with high human population density and at other human-wild bird interfaces where negative impacts are possible [16,17], to generate the necessary data to fulfill this responsibility. The emergence of H5N1 HPAIV in North America has intensified the need for a robust wild bird AIV surveillance program to monitor the presence, distribution, and genetic evolution of this zoonotic virus across human, agricultural, and wild bird populations.

This study aimed to evaluate the current strategy of wild bird AIV surveillance in Ontario using a One Health lens across gradients of human, agricultural, and wild bird populations to: 1) identify spatial clusters of high and low wild bird AIV surveillance intensity in relation to poultry facility density, human population density, and wild bird abundance; 2) describe the spatial and temporal distribution of HPAIV wild bird detections and HPAIV infected domestic premises in relation to human population density, poultry facility density, and wild bird abundance; and 3) use a One Health approach to identify opportunities to enhance wild bird AIV surveillance.

2. Methods

2.1. Wild bird surveillance data

Provincial-level data, collected as part of Canada's Interagency Surveillance Program for Avian Influenza Viruses in Wild Birds, were collated for all live, harvested, sick, and dead wild birds sampled between November 1, 2021, and October 31, 2022, in Ontario, Canada. The data presented here were exported from the Program's database on July 4, 2023, and include a subset of the data presented in [5]. Submissions with missing dates, locations, or AIV PCR results were excluded.

Live birds were sampled opportunistically in conjunction with waterfowl capture and banding programs led by Environment and Climate Change Canada (ECCC) and the Ontario Ministry of Natural Resources and Forestry (OMNRF) [18]. Samples from hunter harvested birds were collected in collaboration with ECCC programs. Wild bird carcasses were submitted to the Ontario-Nunavut (ON/NU) node of the Canadian Wildlife Health Cooperative (CWHC) by members of the public, wildlife rehabilitators, or government agencies. A description of Canada's Interagency Surveillance Program for Avian Influenza Viruses in Wild Birds and a detailed sampling protocol has been previously published [5]. Briefly, an oropharyngeal and cloacal swab were obtained from each bird using sterile synthetic swabs. For each bird, the oropharyngeal and cloacal swab were pooled into a vial containing virus transport media. Vials were stored at -20 °C until testing. Pre-screening was carried out at the Animal Health Laboratory (AHL) in Guelph, Ontario, Canada and confirmatory testing at the National Centre for Foreign Animal Disease (NCFAD) in Winnipeg, Manitoba, Canada following standard procedures that have been previously described [5]. Briefly, real-time RT-PCR was used to test for the presence of the avian influenza matrix gene, followed by H5- and H7- specific real-time RT-PCR. All samples that were non-negative (CT <40) for H5 or H7 were sent to the NCFAD to confirm HPAIV and perform virus isolation and sequencing.

2.2. Human population density

Human population density (number of people per square kilometre) was extracted from the Statistics Canada 2021 Census Profile at the level of census subdivision (CSD) [19]. Census subdivision is the general term for municipalities (as determined by provincial/territorial legislation), or areas treated as municipal equivalents for statistical purposes; there are 577 CSDs in Ontario [20].

2.3. Poultry density

The number of poultry and egg facilities, turkey facilities, and ‘all other poultry production’ facilities were each extracted from the Statistics Canada 2021 Census of Agriculture at the level of census consolidated subdivision (CCS) [21] and density of facilities per square km was calculated. These data include both commercial and non-commercial facilities. The data associated with poultry and egg facilities was used for statistical analysis. Census consolidated subdivision is a group of adjacent census subdivisions within the same census division. Generally, smaller, more densely populated census subdivisions (e.g., towns, villages) are combined with the surrounding larger, more rural census subdivision. This is the smallest standard geographic level for which census of agriculture data are available; there are 273 CCSs in Ontario and 205 have at least one poultry facility [21].

2.4. Wild bird abundance

Estimates of weekly wild mallard abundance were sourced from eBird [22] using the ebirdst package in R (4.2.2 (2022-10-31 ucrt)) at low resolution (i.e., for a regular grid of 26.7 × 26.7 km). The relative abundance estimates for mallards were selected for analysis because they are among the most abundant wild bird species in Ontario, are known to be a low pathogenicity avian influenza virus (LPAIV) reservoir species [23,24], and had the highest apparent prevalence of HPAIV among live and harvested bird samples tested between November 1, 2021, and October 31, 2022. EBird raster data for mallards were cropped to the province of Ontario using the rnaturalearth package. Weekly abundance for each grid cell was averaged according to season: overwinter (December–February, weeks 1–8 and 48–52), pre-breeding/breeding (March–May, weeks 9–21), brood rearing (June–August, weeks 22–34), and post-fledging/migration movements (September–November, weeks 35–47), omitting cells where predictions were not made. Seasons roughly correspond with the mallard annual cycle available on Birds of the World [25] with adjustments made, in collaboration with regional waterfowl biologists (C. Sharp), for latitudinal gradients in Ontario. We focused on the pre-breeding and post-fledging seasons for analyses because these are periods of increased wild bird movement and when all the poultry facility detections and majority of wild bird detections occurred in Ontario.

2.5. Spatial analysis

Geographic coordinates of wild bird samples were used to visualize the distribution of wild bird surveillance intensity and HPAIV detections, and approximate geographic coordinates, based on publicly available location information, were used to visualize the distribution of HPAIV positive infected domestic premises [26] in relation to human density, poultry density, and wild bird abundance. ArcMap Pro (v 3.0.02023) was used for mapping.

To identify spatial clusters of wild bird surveillance intensity, we performed a series of purely spatial 2-tailed discrete Poisson scans in SaTScan v10.1.2 [27]. Case counts used for the spatial scans included the number of sick and dead or live and harvest surveillance samples per strata (i.e., CCS, CSD, or grid cell depending on the population count). Population counts included human population density for each CSD, poultry and egg production density for each CCS, and estimated wild bird abundance for each 26.7 × 26.7 grid cell. Geographic coordinates were assigned as the centroid of the relevant geographic boundary. To overcome issues related to zero population counts for the purposes of spatial analysis, a value was assigned equivalent to 50% of the lowest value for boundaries in which population values were zero (e.g., no poultry facilities). The maximum size of the circular scanning window was set to 50% of the total population and estimates were based on 999 Monte Carlo replications. We report non-overlapping high and low spatial clusters with a *p*-value <0.05 for all analyses.

3. Results

3.1. Wild bird surveillance

During November 1, 2021, to October 31, 2022, a total of 2562 wild bird surveillance samples were submitted from across Ontario (Supplementary Table 1): 1591 live and harvest samples from 74 unique geographic coordinates, and 969 sick and dead samples from 715 unique geographic coordinates were collected. Of the 104 species of wild birds submitted, eight are species at risk in Ontario (Supplementary Table 1). The highest number of surveillance samples and H5N1 HPAIV detections were from Canada geese (*Branta canadensis*) and mallards (*Anas platyrhynchos*; Table 1).

3.2. Human population density, poultry density, and wild mallard abundance

The geographic regions with the highest human population density, poultry density, and wild mallard abundance lack complete spatial congruence (Figs. 1a, 2a, 3a). The distribution of poultry facilities, predominantly concentrated in Southwestern Ontario, varies by production type (Supplemental Fig. 1). Turkey production density and ‘all other poultry production’ density, including ducks, appear to be concentrated in more localized geographic regions of the province (Supplemental Fig. 1). Wild mallard abundance demonstrates spatiotemporal variance between seasons (Fig. 3a).

3.3. Choropleth maps

Based on visual examination, there is more spatial congruence with human population density and surveillance intensity for sick and dead bird sampling compared to live and harvest sampling (Fig. 1b and c). Specifically, there are more areas of overlapping high human population density and high surveillance intensity (i.e., dark purple areas) observed for sick and dead bird sampling. Several major cities and regions in Ontario (e.g., Toronto, Ottawa, Hamilton) are well represented (i.e., surveillance samples per CSD) in both surveillance components. In relation to poultry and egg production facility density, there was a low density of surveillance samples in the Southwestern region of Ontario where poultry density is the highest (Fig. 2b and c). However, there were some areas of high surveillance intensity in this region of high poultry density (i.e., dark purple areas). During the pre-breeding and post-fledging seasons, there are areas with a high abundance of mallards in Ontario where there was no sampling (Fig. 3b and c).

3.4. Spatial scans

Using human density as the population metric, the spatial scan detected six spatial clusters with increased live and harvest wild bird surveillance intensity relative to human population density (Table 2, Fig. 1D). These clusters were located in southern (HD1, HD3), eastern (HD6, HD7), and northern Ontario (HD2, HD5), which includes areas of both high human population density (e.g., Ottawa, Hamilton), and low human population density (northern Ontario; Fig. 1D). Additionally, a single low spatial cluster was detected centered on the Adjala-Tosorontio CSD (HD4), which includes a large portion of the Greater Toronto Area (GTA; i.e., Halton, Peel, City of Toronto, and Durham regions) and is an area of high human population density (Fig. 1D). Whereas for sick and dead wild bird surveillance data, the spatial scan detected five spatial clusters of high surveillance intensity, primarily located around major cities in southern and eastern Ontario, including Toronto (HD13), Hamilton (HD8) and Ottawa (HD9; Fig. 1E). An additional three low clusters (HD10, HD14, HD15) were detected including between three and 329 CSDs (Table 2; Fig. 1E).

Using poultry production density as the population metric, the spatial scan detected six spatial clusters with increased live and harvest wild bird surveillance intensity (Table 2; Fig. 2D). All but one high cluster consisted of a single CCS, with half of the clusters centred around areas of high poultry facility density (PD3, PD4, PD8; Fig. 2D). An additional three low clusters (PD2, PD6, PD9) were detected, all consisting of multiple CCSs in southwestern Ontario in areas of relatively high poultry facility density (Table 2; Fig. 2D). Whereas for sick and dead wild bird surveillance data, four high clusters were detected involving one to five CCSs and two low clusters involving five and 41 CCSs (Table 2; Fig. 2E). There are both high clusters (PD13, PD15) and low clusters (PD11, PD14) in areas of Ontario with high poultry facility density (Fig. 2E).

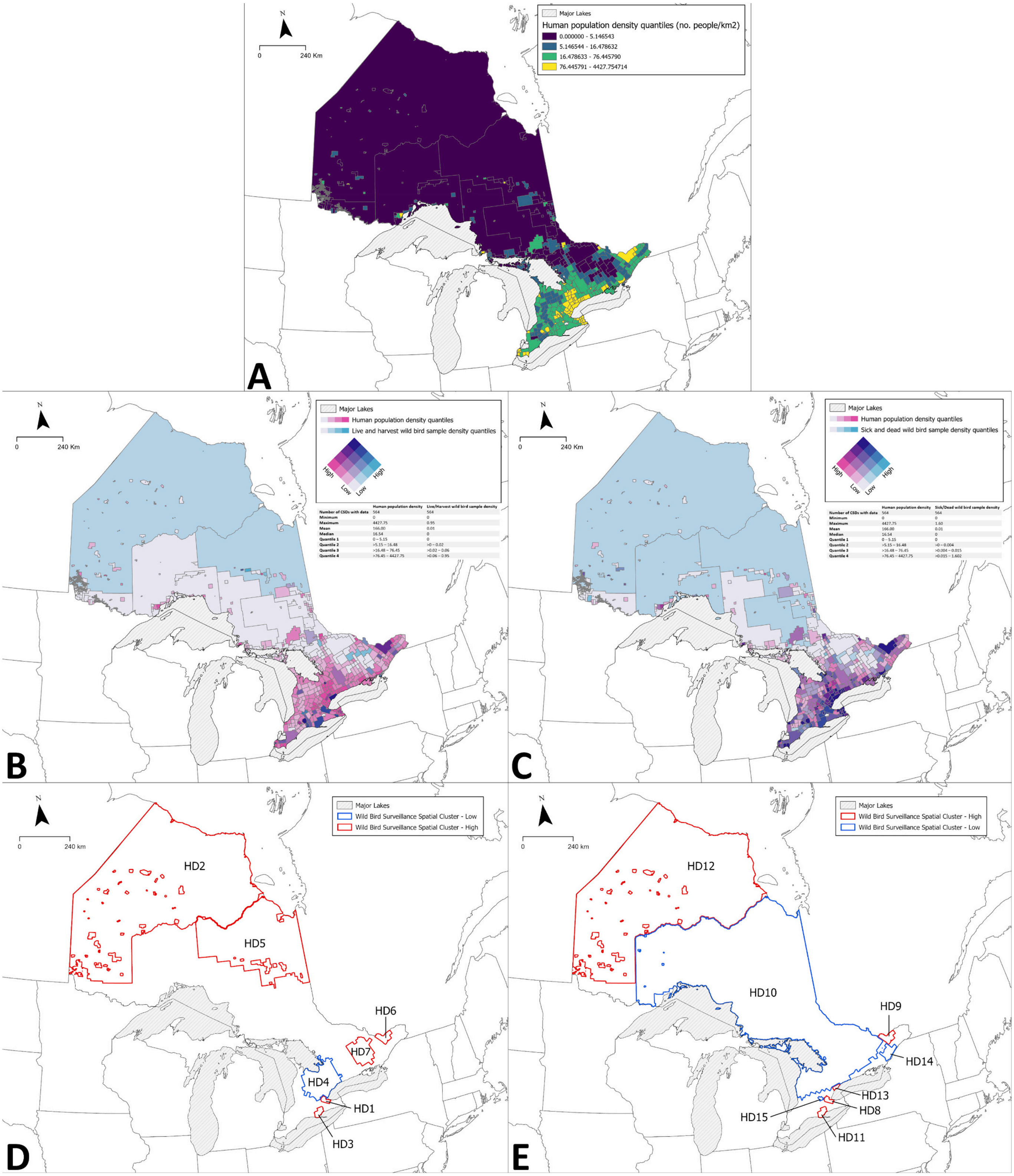
Using mallard abundance during both the pre-breeding (Panel 1, Fig. 3D and E) and the post-fledging (Panel 2; Fig. 3D and E) seasons as the population metric and for both live and harvest and sick and dead wild bird surveillance, the spatial scans detected one cluster of high surveillance intensity in southern Ontario (WM1, WM3, WM5 and WM7, respectively), and one cluster of low surveillance intensity (WM2, WM4, WM6, and WM8, respectively) in northern Ontario. Clusters of low surveillance intensity in northern Ontario were relatively large ranging from 912 to 1334 grid cells with a radius of 744.80–1230.21 km. Whereas clusters of high surveillance intensity in southern Ontario were more focal ranging from 1 to 345 grid cells with a maximum radius of 398.22 km.

3.5. H5 HPAIV-infected premises and wild bird detections

Figs. 4–6 display H5 HPAIV-infected domestic premises and H5 HPAIV detections in wild birds relative to human population density, poultry facility density, and wild mallard abundance during pre-breeding and post-fledging time periods. Poultry facility detections occurred in areas with the highest poultry facility density, but not exclusively (Fig. 5A). Additionally, there were poultry facility detections in areas with high human population density and in locations and during time periods when wild birds were in high abundance (Figs. 4A and 6A), including near the GTA and Ottawa. However, conversely, there were areas with high wild mallard abundance and no poultry facility detections (Fig. 6A). Poultry facility detections occurred during the pre-breeding and post-fledging seasons (Fig. 6A), with no detections during the brood rearing period or overwinter although the virus was not detected in Ontario until March 2022. The distribution of wild bird detections varied across quantiles of human density (Fig. 4b and c), poultry facility density (Fig. 5b and c), and wild mallard abundance (Fig. 6b and c). Most wild bird detections occurred during the pre-breeding and post-fledging season (Figured 6b and 6c), with some detections during the brood rearing season (Supplemental Fig. 2). There were no detections during the overwinter period; however, the virus was not detected in Ontario until March 2022.

4. Discussion

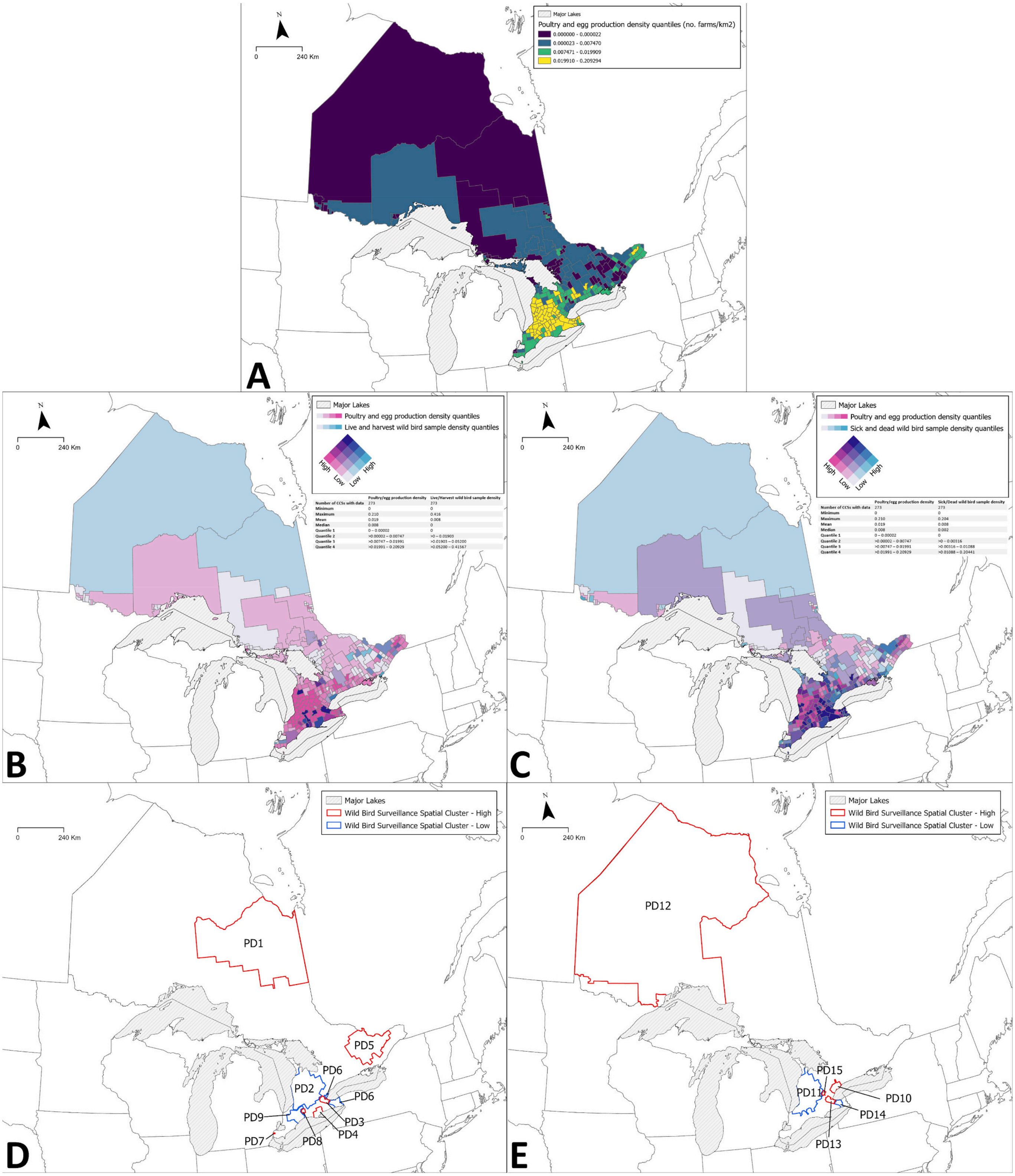
A One Health approach to the design and evaluation of AIV wild bird surveillance recognizes the importance of human, agricultural, and wild bird domains. A targeted and adaptive AIV surveillance strategy acknowledges the capabilities and limitations of each surveillance method, to enable strategic resource allocation and the prioritization of sampling opportunities to maximize coverage. Through an analysis of AIV surveillance intensity in Ontario, Canada during the first year following the 2021 H5N1 HPAIV incursion, we identified specific opportunities to enhance Ontario AIV wild bird surveillance inputs across human, poultry, and wild bird domains in alignment with the overarching goals



	Human population density	Live/Harvest wild bird sample density
Number of CSDs with data	564	564
Minimum	0	0
Maximum	4427.75	0.95
Mean	166.00	0.01
Median	16.54	0
Quantile 1	0 – 5.15	0
Quantile 2	>5.15 – 16.48	>0 – 0.02
Quantile 3	>16.48 – 76.45	>0.02 – 0.06
Quantile 4	>76.45 – 4427.75	>0.06 – 0.95

	Human population density	Sick/Dead wild bird sample density
Number of CSDs with data	564	564
Minimum	0	0
Maximum	4427.75	1.60
Mean	166.00	0.01
Median	16.54	0
Quantile 1	0 – 5.15	0
Quantile 2	>5.15 – 16.48	>0 – 0.004
Quantile 3	>16.48 – 76.45	>0.004 – 0.015
Quantile 4	>76.45 – 4427.75	>0.015 – 1.602

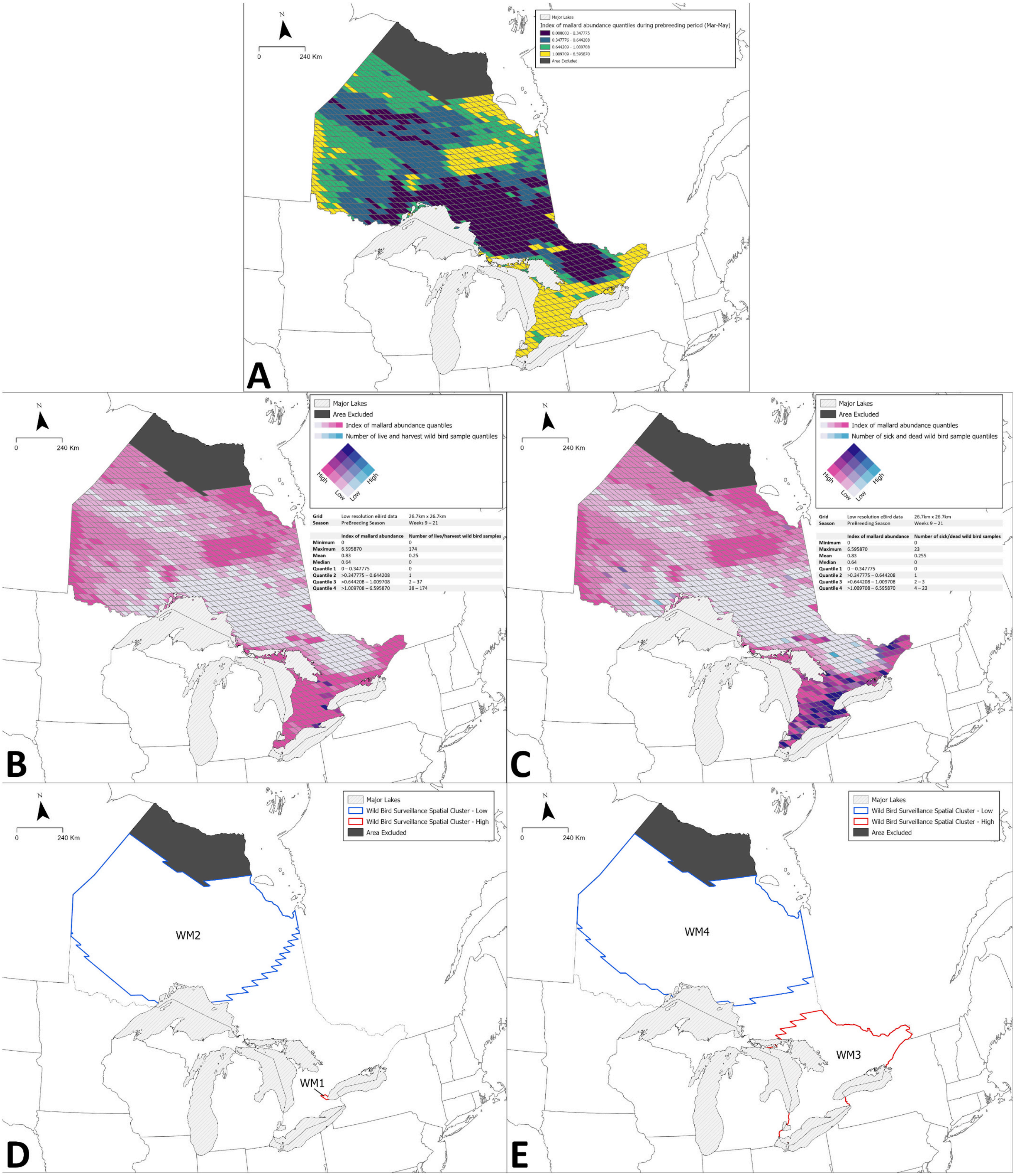
Fig. 1. (A) Choropleth map of human population density (number of people per square kilometre, categorized into quantiles) across 577 census subdivisions (CSD). Bivariate choropleth maps of human population density and B) live and harvest and C) sick and dead wild bird sample density (number of wild bird samples collected between November 2021–November 2022 per square kilometre, categorized into quantiles). Locations of spatial clusters of low (blue) and high (red) wild bird surveillance, based on a purely spatial discrete Poisson probability model, shown for D) live and harvest and E) sick and dead wild bird surveillance (number of wild bird samples submitted per CSD between November 2021–November 2022). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



	Poultry/egg production density	Live/Harvest wild bird sample density
Number of CCSs with data	273	273
Minimum	0	0
Maximum	0.210	0.416
Mean	0.019	0.008
Median	0.008	0
Quantile 1	0 – 0.00002	0
Quantile 2	>0.00002 – 0.00747	>0 – 0.01903
Quantile 3	>0.00747 – 0.01991	>0.01903 – 0.05200
Quantile 4	>0.01991 – 0.20929	>0.05200 – 0.41567

	Poultry/egg production density	Sick/Dead wild bird sample density
Number of CCSs with data	273	273
Minimum	0	0
Maximum	0.210	0.204
Mean	0.019	0.008
Median	0.008	0.002
Quantile 1	0 – 0.00002	0
Quantile 2	>0.00002 – 0.00747	>0 – 0.00316
Quantile 3	>0.00747 – 0.01991	>0.00316 – 0.01088
Quantile 4	>0.01991 – 0.20929	>0.01088 – 0.20441

Fig. 2. (A) Choropleth map of poultry and egg facility density (number of poultry and egg facilities per square kilometre, categorized into quantiles) across 273 census consolidated subdivisions (CCS) in Ontario, Canada. Bivariate choropleth maps of poultry facility density and B) live and harvest and C) sick and dead wild bird sample density (number of wild bird samples collected between November 2021–November 2022 per square kilometre, categorized into quantiles). Locations of spatial clusters of low (blue) and high (red) wild bird surveillance, based on a purely spatial discrete Poisson probability model, shown for D) live and harvest and E) sick and dead wild bird surveillance (number of wild bird samples submitted per CSD between November 2021–November 2022). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



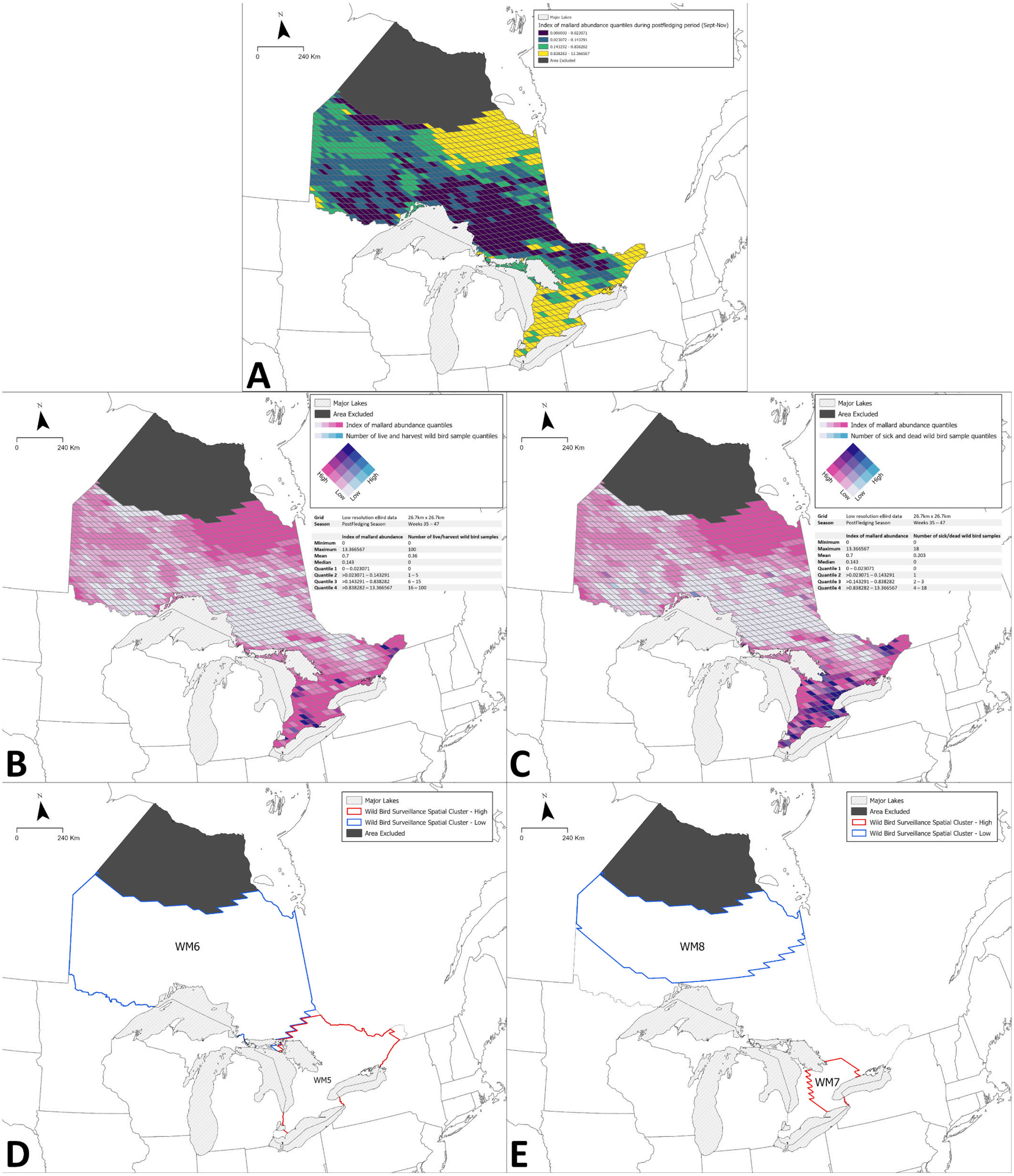
Grid	Low resolution eBird data	26.7km x 26.7km
Season	PreBreeding Season	Weeks 9 – 21

	Index of mallard abundance	**Number of live/harvest wild bird samples**
Minimum	0	0
Maximum	6.595870	174
Mean	0.83	0.25
Median	0.64	0
Quantile 1	0 – 0.347775	0
Quantile 2	>0.347775 – 0.644208	1
Quantile 3	>0.644208 – 1.009708	2 – 37
Quantile 4	>1.009708 – 6.595870	38 – 174

Grid	Low resolution eBird data	26.7km x 26.7km
Season	PreBreeding Season	Weeks 9 – 21

	Index of mallard abundance	**Number of sick/dead wild bird samples**
Minimum	0	0
Maximum	6.595870	23
Mean	0.83	0.255
Median	0.64	0
Quantile 1	0 – 0.347775	0
Quantile 2	>0.347775 – 0.644208	1
Quantile 3	>0.644208 – 1.009708	2 – 3
Quantile 4	>1.009708 – 6.595870	4 – 23

Fig. 3. (A) Choropleth maps of pre-breeding (March–May; Panel 1) and post-fledging (September–November; Panel 2) seasonal abundance for wild mallards in Ontario, Canada using EBird 2021 predictions (Fink et al., 2022), categorized into quantiles. Bivariate choropleth maps of wild mallard seasonal abundance and B) number of live and harvest wild bird samples and C) number of sick and dead wild bird samples submitted over the relevant time period in 2022. Locations of spatial clusters of low (blue) and high (red) wild bird surveillance, based on a purely spatial discrete Poisson model, shown for D) live and harvest and E) sick and dead wild bird surveillance conducted over the relevant time-period. Abundance was not available for areas within the Ontario boundary shown in dark grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Grid	Low resolution eBird data	26.7km x 26.7km
Season	PostFledging Season	Weeks 35 – 47

	Index of mallard abundance	Number of live/harvest wild bird samples
Minimum	0	0
Maximum	13.366567	100
Mean	0.7	0.36
Median	0.143	0
Quantile 1	0 – 0.023071	0
Quantile 2	>0.023071 – 0.143291	1 – 5
Quantile 3	>0.143291 – 0.838282	6 – 15
Quantile 4	>0.838282 – 13.366567	16 – 100

Grid	Low resolution eBird data	26.7km x 26.7km
Season	PostFledging Season	Weeks 35 – 47

	Index of mallard abundance	Number of sick/dead wild bird samples
Minimum	0	0
Maximum	13.366567	18
Mean	0.7	0.203
Median	0.143	0
Quantile 1	0 – 0.023071	0
Quantile 2	>0.023071 – 0.143291	1
Quantile 3	>0.143291 – 0.838282	2 – 3
Quantile 4	>0.838282 – 13.366567	4 – 18

Fig. 3. (*continued*).

of One Health surveillance to safeguard the health of people, animals, and ecosystems.

The detection of HPAIV in wild birds in densely populated areas of Ontario (e.g., GTA) underscores the importance of surveillance in these areas for generating situational awareness and informing risk at an important One Health interface. There was a strong connection between sick and dead bird surveillance intensity and human population density, which is not surprising given that the CWHC sick and dead bird surveillance program relies on people detecting and submitting carcasses that they find in the environment. However, despite this connection, there are areas with high human population density that received a low number of submissions. Previous work has shown that

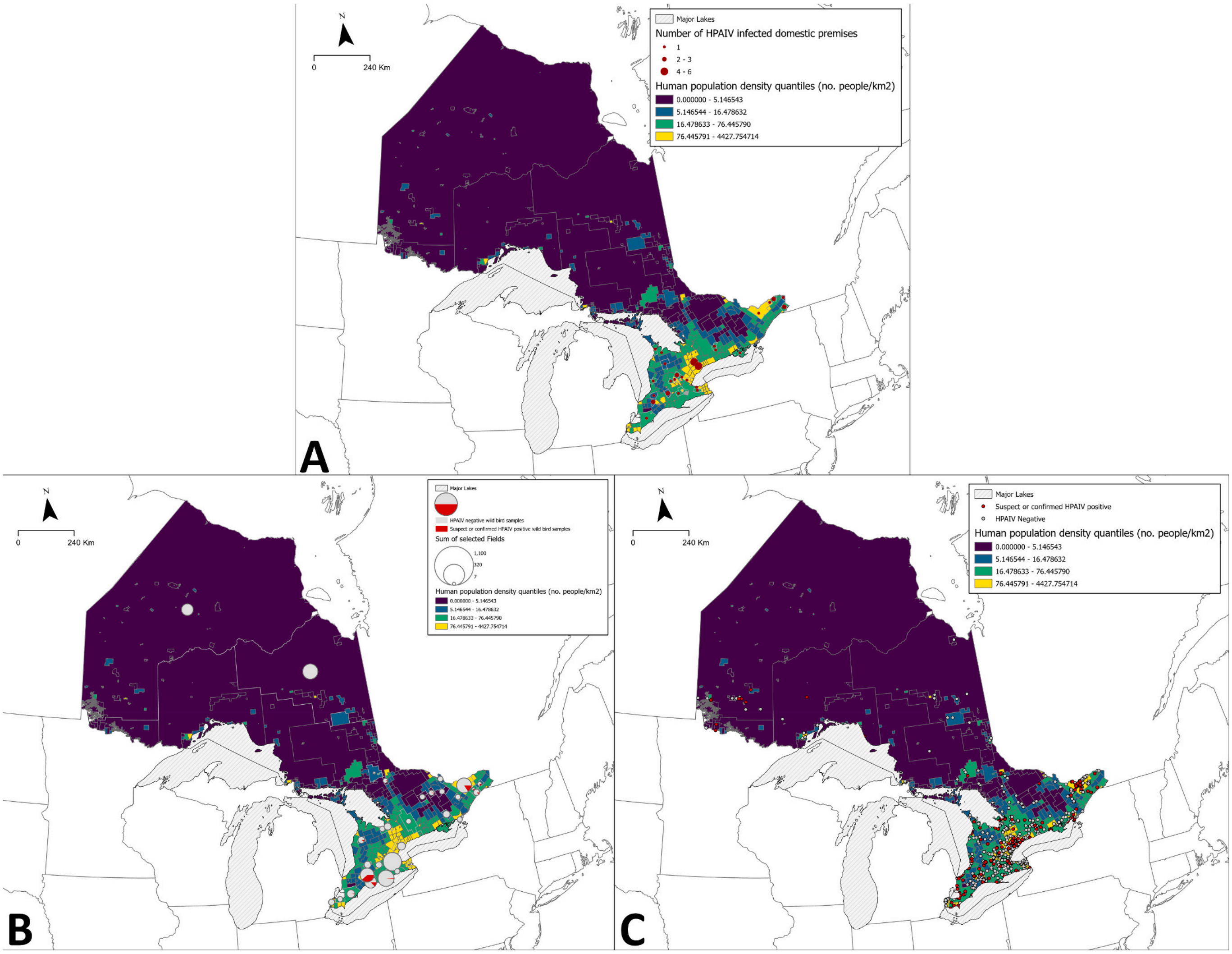


Fig. 4. Choropleth maps of human population density (number of people per square kilometre, categorized into quantiles) across 577 census subdivisions (CSD) in Ontario, Canada and A) highly pathogenic avian influenza virus (HPAIV) infected domestic premises, B) live and harvest and C) sick and dead wild bird surveillance data collected between November 2021–November 2022.

sociodemographic factors may impact citizen engagement in, and awareness of, surveillance programs [28,29], and these complex relationships need to be considered as we develop surveillance programs, interpret the data, and communicate results [28]. Surveillance gaps in areas identified as having lower surveillance intensity may be overcome by increasing awareness and support for the sick and dead bird surveillance program and – where possible – extending live bird surveillance to these areas. Increased awareness of all ongoing surveillance efforts provides opportunities for community engagement and education, which are key components of a One Health approach [30].

It is important to note that human population density does not consider all aspects of the human-wild bird interface. The harvest and consumption of wild birds is also a key interface where the results of AIV wild bird surveillance can facilitate informed guidance. Targeted surveillance at this interface is therefore a critical component of a One Health approach to AIV surveillance. Migratory bird harvest occurs in areas of both high and low human population densities, but is disproportionately important for rural and Indigenous communities, and largely targets species that are of key relevance to the maintenance and transmission of AIV. Therefore, submissions from Indigenous and non-Indigenous hunters and harvesters can also enhance the geographic and taxonomic coverage of wild bird surveillance more efficiently than other surveillance methods. We recommend a focus on dedicated and sustained relationship-building with these partners and stakeholder groups to foster collaboration and facilitate two-way communication. This will allow us to integrate the priorities and insights from these groups into surveillance planning, and to build a shared understanding of the important role that surveillance plays in protecting public health and wildlife populations while increasing engagement and participation in surveillance.

In northern Ontario, surveillance intensity was notably limited despite areas of elevated wild bird abundance. Surveillance results may provide early warning regarding the presence, prevalence, and genetic change in the virus prior to southward movement of birds through areas of high human and poultry density, during fall migration. Given the relatively low human population densities in northern Ontario, passive sick and dead bird surveillance methods are unlikely to result in adequate sampling intensity. The introduction of entirely new live bird field sampling programs in this expansive and remote geographic region of Ontario presents a considerable financial and logistical challenge. The most pragmatic and cost-effective strategy is to maximize the integration of AIV sampling into existing field programs and to engage with hunters and harvesters that are active in these regions as discussed above.

We have identified an area in Ontario with concentrated poultry operations and limited AIV surveillance. To strategically address this,

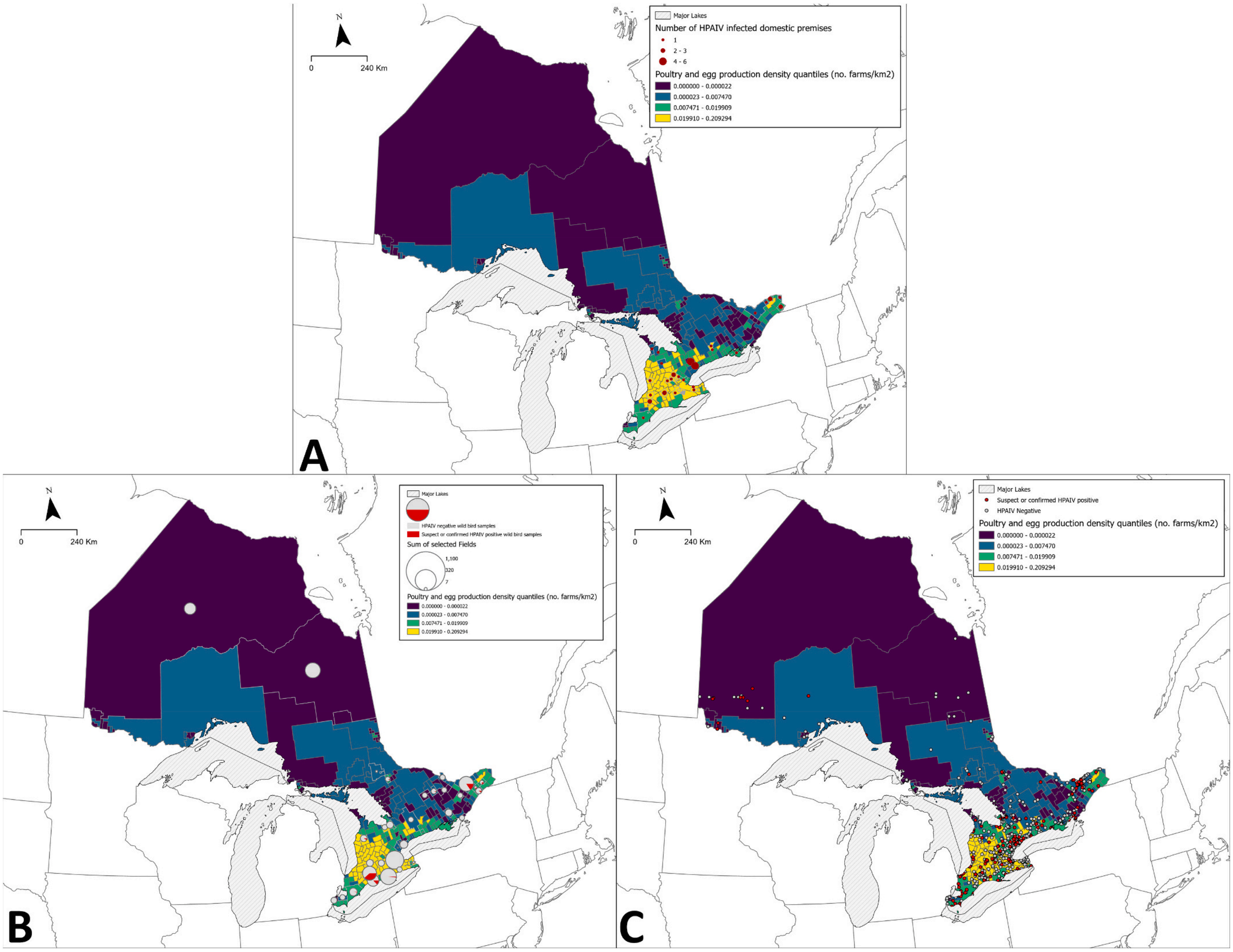


Fig. 5. Choropleth maps of poultry and egg facility density (number of poultry and egg facilities per square kilometre, categorized into quantiles) across 273 census consolidated subdivisions (CCS) in Ontario, Canada and A) highly pathogenic avian influenza virus (HPAIV) infected domestic premises, B) live and harvest and C) sick and dead wild bird surveillance data collected between November 2021–November 2022.

we propose targeted initiatives to increase awareness of AIV surveillance among poultry producers. This includes encouraging submissions of sick and dead wild birds found on or near premises and seeking permission to conduct live wild bird surveillance in these areas during periods of increased wild bird abundance. However, poultry facilities are not evenly distributed across consolidated census subdivisions (the current spatial scale of available data) and the risk of AIV introduction varies according to production type, among other variables [31]. Therefore, access to higher resolution poultry production data would facilitate more informed and effective targeted surveillance strategies at this interface. Furthermore, these data would facilitate landscape-level analyses to identify environmental risk factors associated with AIV, pivotal for understanding transmission and spillover dynamics at the wild-bird poultry interface.

There are several limitations associated with the present analyses. Ebird species abundance predictions are based on statistical models that use semi-structured citizen science data and environmental covariates. While the results of these models are only made available after expert-review [32], there are limitations associated with citizen science-based data. Data collected through standardized surveys will produce more robust estimates of abundance, but they are only conducted during specific time periods. The extent and frequency of available estimates is an advantage of Ebird data for evaluating a surveillance program that operates year-round across large geographic areas. Therefore, for the purposes of this analysis EBird relative abundance predictions for a commonly observed and easily identified species in Ontario, and for which validation (albeit limited) against targeted monitoring efforts has been undertaken [32], were considered informative. Furthermore, the maps produced in this study were validated by a review with regional waterfowl biologists with knowledge of mallard presence and abundance on the Ontario landscape (C. Sharp; R. Wood). Second, while we focused on mallard presence and abundance as a proxy for wild migratory bird species, this assumption will not apply universally. Future efforts will benefit from assessing surveillance intensity relative to presence and abundance estimates for other potential reservoir species (e.g., gulls), as well as species at risk, where HPAIV could pose potential conservation concerns [4,33].

The spatial scan statistic with a discrete Poisson probability model was used to identify areas of high and low intensity of surveillance. The background population concerned populations impacted by HPAIV while the numerator concerned the number of specimens tested for a particular surveillance component. Typically, spatial scan statistics with a Poisson model are applied to examine rates of disease within a specific population where the numerator represents the cases, and the denominator captures the population from which the cases occurred [34]. Consequently, the software will provide an error message and not run if there is a value greater than zero in the numerator but zero in the denominator. This type of situation was possible with our data since specimens could be collected for HPAIV from areas without poultry farms or the observation of mallards. Our choice of using a low value for zero denominators allowed us to maintain a relatively high resolution for cluster detection, avoid selection biases from removing locations, and avoid arbitrary decisions concerning the joining of neighbouring regions that might limit the reproducibility of our analyses. Any potential bias resulting from replacing a zero denominator with a small value would have likely impacted our ability to detect high intensity clusters involving a single census subdivision, but based on our results this type of cluster was readily detected.

To optimize a One Health approach, it is important to regularly review and adapt surveillance strategies in response to evolving ecological and epidemiological contexts. Our analysis underscores the importance of both live, through capture or harvest, and sick and dead wild bird surveillance, and suggest that both programs could benefit from expansion and enhanced support.

Our findings have identified several strategic opportunities to enhance wild bird AIV surveillance:

1. Expanded Sampling of Hunter-Harvested Birds

- Expand the sampling of hunter-harvested birds through partnerships, particularly targeting northern and rural areas of the province. This strategy aims to broaden geographic coverage, enhance understanding of AIV risk at crucial human-wild bird interfaces, and enable early detection of virus evolution among key migratory reservoir species as they return south from northern breeding grounds.

2. Targeted Live Bird Sampling

- Implement targeted live bird sampling focused on identified reservoir species, such as dabbling ducks [5], and in areas with high density of commercial poultry operations. This approach will maximize virus detection in apparently healthy wild birds and provide important data on risk at the domestic-wild bird interface.

3. Expanded Coverage of Sick and Dead Bird Surveillance

- Continue the surveillance of sick and dead birds in a broad range of species. Resources to support enhanced public engagement can facilitate submissions more broadly across densely populated CSDs to inform risk at the human-wild bird interface, generate baseline

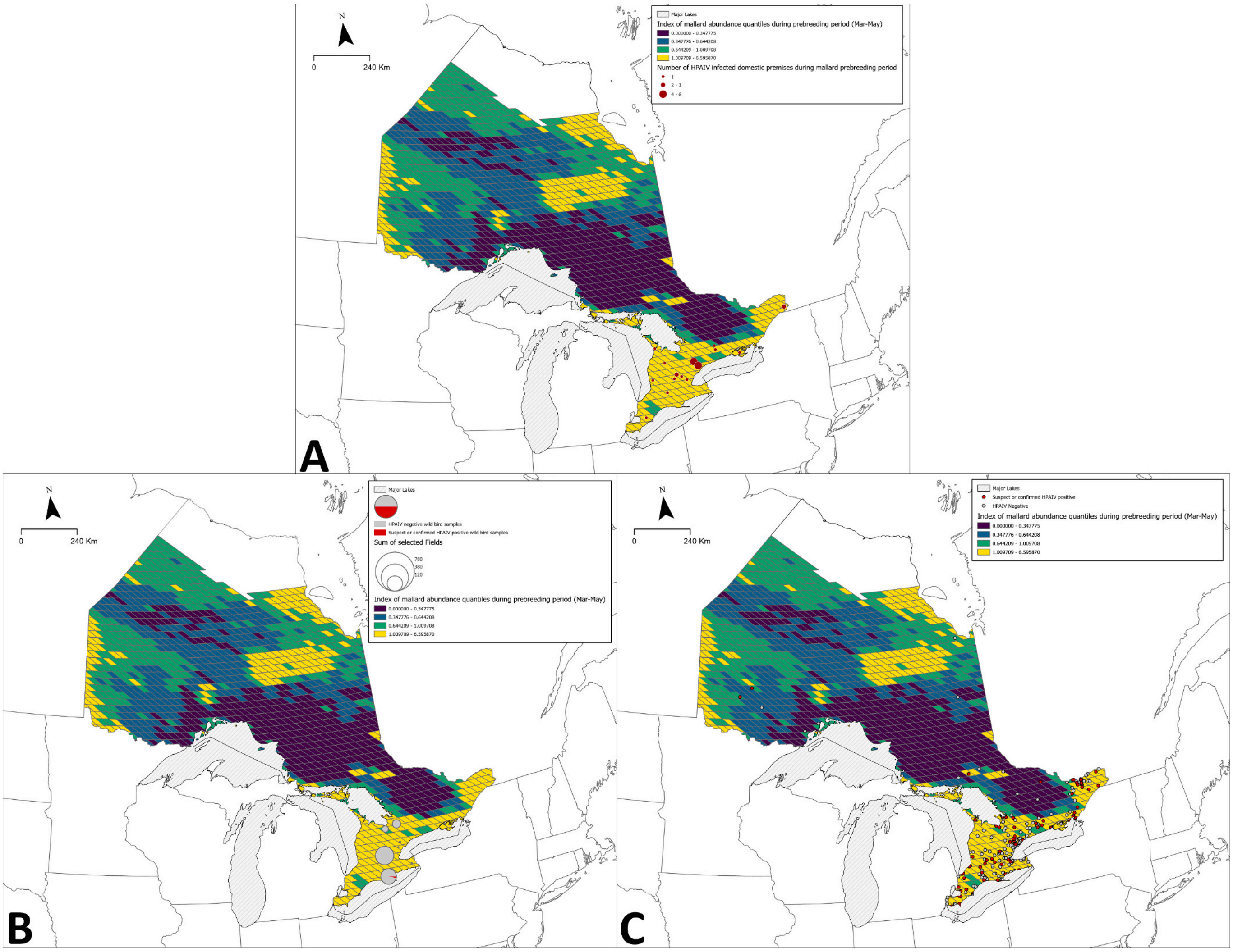


Fig. 6. (A) Choropleth maps of pre-breeding (March–May; Panel 1) and post-fledging (September–November; Panel 2) seasonal abundance for wild mallards in Ontario, Canada using EBird 2021 predictions (Fink et al., 2022), categorized into quantiles with (B) highly pathogenic avian influenza virus (HPAIV) infected domestic premises, (C) live and harvest and (D) sick and dead wild bird surveillance data collected between March and May 2022. Predicted abundance was not available for areas within the Ontario boundary that are white.

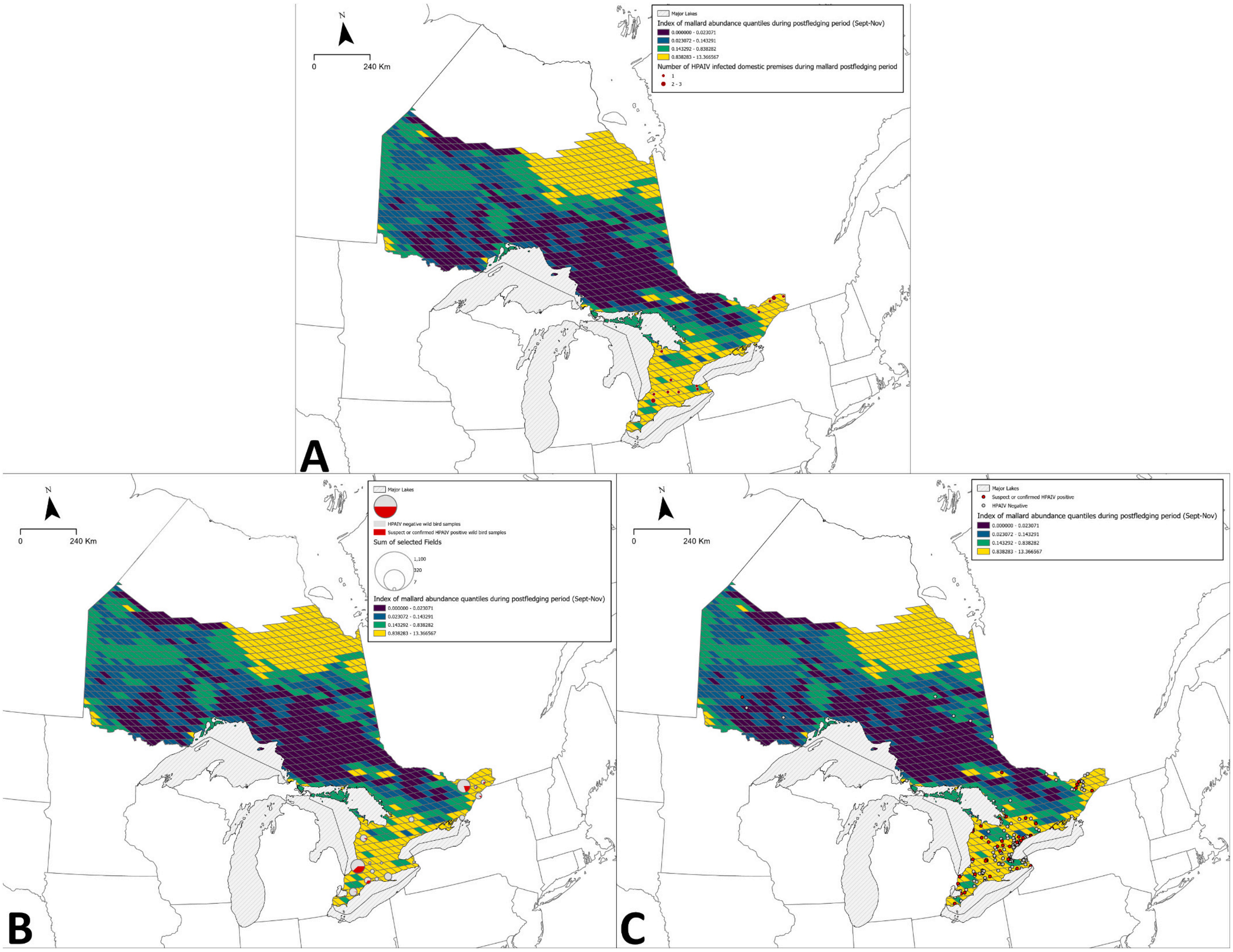


Fig. 6. (*continued*).

wild bird mortality data, signal periods of increased mortality, and monitor viral evolution particularly for genetic changes that heighten mortality risks among wild birds and transmission risk to humans and other mammals.

4. Collaborative and Sustained Engagement

o Strong engagement with the public, poultry producers, and Indigenous and non-Indigenous hunters and harvesters to build a robust surveillance network. This collaborative approach is crucial to a successful One Health framework. Looking ahead, consideration for how to integrate wild bird surveillance planning with monitoring of other wildlife, domestic species, and humans, along with incorporating additional sampling strategies such as blood and sediment sampling, are logical extensions of a successful One Health framework for surveillance.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100760.

CRediT statement

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Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the multi-agency AIV surveillance program in partnership with the Ontario Ministry of Natural Resources and Forestry, the Ontario Ministry of Agriculture, Food, and Rural Affairs, the Ontario Ministry of Health, the Canadian Food Inspection Agency, the Public Health Agency of Canada, Environment and Climate Change Canada, and the Canadian Wildlife Health Cooperative.

We thank Lenny Shirose, Laura Dougherty, and the CWHC ON/NU communications team and all the individuals and groups who submitted samples to the CWHC for testing. We thank the Animal Health Laboratory and the National Centre for Foreign Animal Disease staff for diagnostic testing.

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