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Enhanced environmental surveillance for avian influenza A/H5, H7 and H9 viruses in Guangxi, China, 2017–2019

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Abstract

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Ethics statement

This study was reviewed and approved by the Institutional Review Board (IRB) of the Chinese Center for Disease Control and Prevention (China CDC) and the United States (U.S.) Centers for Disease Control and Prevention relied upon the China CDC IRB (IVDC2018-017).

Conflict of interest statement

The authors declare that there are no conflicts of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Supplementary data

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We conducted environmental surveillance to detect avian influenza viruses circulating at live poultry markets (LPMs) and poultry farms in Guangxi Autonomous Region, China, where near the China-Vietnam border. From November through April 2017–2018 and 2018–2019, we collected environmental samples from 14 LPMs, 4 poultry farms, and 5 households with backyard poultry in two counties of Guangxi and tested for avian influenza A, H5, H7, and H9 by real-time reverse transcription-polymerase chain reaction (rRT-PCR). In addition, we conducted four cross-sectional questionnaire surveys among stall owners on biosecurity practices in LPMs of two study sites. Among 16,713 environmental specimens collected and tested, the median weekly positive rate for avian influenza A was 53.6% (range = 33.5% − 66.0%), including 25.2% for H9, 4.9% for H5, and 21.2% for other avian influenza viruses A subtypes, whereas a total of two H7 positive samples were detected. Among the 189 LPM stalls investigated, most stall owners (73.0%) sold chickens and ducks. Therefore, continued surveillance of the avian influenza virus is necessary for detecting and responding to emerging trends in avian influenza virus epidemiology.

Keywords

Avian influenza virus; Environmental surveillance; Live-poultry-market

1. Introduction

China has experienced annual low pathogenetic avian influenza A (H7N9) virus epidemics in poultry, with sporadic human infections since 2013[1]. However, during the fifth epidemic in 2016–2017, the number of human cases spiked, the geographic distribution of low pathogenic avian influenza A(H7N9) increased, and a novel highly pathogenic avian influenza (HPAI) A(H7N9) virus was detected in chickens and humans[1–4]. It was reported, the prevalence rate of H7 subtype virus was 0.03%[5]. The isolation rate of H7N9 avian influenza in live poultry markets or farms from 2013 to 2017 was 1.03%, 3.03%, 3.31%, 4.21% and 4.19%, respectively[6], indicating the prevalence of the H7 subtype of avian influenza virus had increased during the study period. In response, in the fall of 2017, China initiated a nationwide poultry vaccination program targeting poultry breeders and commercial farms using a bivalent avian influenza A H5/H7 recombinant inactivated vaccine[7,8]. Following the H5/H7 poultry vaccination program, few cases of human infection of H7N9 have been reported in China, and detection of H7N9 in poultry has decreased significantly[3,9].

Guangxi Autonomous Region, located in southwest China, shares a border with Vietnam. Residents in that region are known to visit live poultry markets (LPMs) due to dietary preferences for consuming fresh poultry meat[10]. LPMs can promote the amplification and dissemination of avian influenza viruses and serve as ideal settings for virus transmission at the animal-human interface[11]. Most H7N9 human infections reported exposure to infected poultry or contaminated environments through LPMs[12,13]. In February 2017, the detection of the H7N9 virus in poultry in LPMs peaked[14]. From 2016 to 2017, Guangxi reported 27 H7N9 human infections and HPAI H7N9 virus in poultry[15]. Guangxi Autonomous Region introduced a bivalent avian influenza A H5/H7 poultry vaccine in July 2017 as a pilot for the national immunization program in the fall of 2017. In Guangxi

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border area with various subtypes of avian influenza virus coexistenting, the average virus isolation rate in poultry samples was 15.95%, in environmental samples were as high as 23.65%, significantly higher than the poultry samples[5]. A post-vaccination serologic survey from October 2017 to September 2018 in Chongzuo, Guangxi Autonomous Region (the prefecture city of our study site) reported that 90.40% and 84.90% of sampled poultry had H5 and H7 antibody titers detected that met the Ministry of Agriculture's standard for an effective immunological response to the vaccine[9].

To monitor the change in the circulation of avian influenza A, H5, H7, and H9 viruses after introducing the bivalent H5/H7 vaccine, we conducted enhanced environmental surveillance for two consecutive winter and spring seasons in Guangxi, and four cross-sectional questionnaire surveys were conducted among stall owners on biosecurity practices in LPMs of two study sites. Through the monitoring of environmental samples, analysis of the variation of avian influenza virus and infection in occupationally exposed people, laid a foundation for better prevention and control of avian influenza.

2. Materials and methods

2.1. Study sites

Among the eight counties where Guangxi borders Vietnam, the study team selected Pingxiang and Longzhou cities of Chongzuo prefecture city as surveillance sites based on: a) the magnitude of existing cross-border total goods traded between Guangxi and Vietnam; b) local CDC capacity for implementing environmental surveillance; and c) local public health department willingness for collaboration. Both cities have border entry-exit points with Vietnam, and Pingxiang has the most human border crossings and the most significant total trade volume in Guangxi[16].

The study LPMs were defined as fixed wholesale or retail markets that sell live domestic poultry such as chickens, ducks, and geese. The poultry farms were registered commercially and household farms that raised backyard poultry for commercial operations or personal consumption during the study period. Within the two cities, the study team selected 14 LPMs in all townships that shared a border with Vietnam and the LPMs in the downtown area. Seven LPMs were chosen in Pingxiang city, and 7 LPMs were chosen in Longzhou city. In addition, the study team selected 4 registered commercial farm and a convenience sample of 5 household farms. In Pingxiang, the 4 registered commercial poultry farm was selected in addition to 2 household farms. In Longzhou, 3 household farms were selected, and there were no registered poultry farms. At each LPM, the study team selected a convenience sample of 5 poultry stalls as sampling sites. If there were fewer than five stalls at one LPM, all stalls were selected for sampling. The team selected two locations at each poultry farm to collect specimens. Before sampling, oral consent was obtained from stall operators and poultry farm owners.

2.2. Environmental specimen collection

Trained staff collected environmental specimens weekly from November to April during 2017–2018 and 2018–19. The surveillance periods were defined as the months with high

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transmission of avian influenza virus in poultry based on local surveillance data and the period covering the Chinese Spring Festival when production, transport, and consumption of fresh poultry meat increases[10,17].

In LPMs, the study team collected six types of specimens from each stall, including poultry drinking water, bloody sewage on the floor, swabs from fecal droppings, the feather-removal machine, the chopping board, and cages, following China's environmental surveillance protocol[18]. A total of 30 specimens from up to 5 stalls were collected in each LPM. When the booths were selected, the mixed sampling method of targeted sampling and subjective sampling was adopted, mainly for the purpose of disease detection and discovery. Each collection tube for one type of specimen included pooled specimens of up to three swabs from different locations in each stall (i.e., swabs from the surfaces of three cages in one stall to increase representativeness). In the 2017–2018 season, the study team prioritized chicken-related environmental specimens; choosing cages contained more significant numbers of chickens, cages with mixed poultry species; or sites with visibly dirty surfaces to increase the sampling sensitivity. In the 2018–2019 season, the study team collected 40 duck-related environmental specimens each week in each city. In poultry farms, the study team collected poultry drinking water and swabs from fecal droppings, cage, and egg surfaces, for 16 specimens from each farm/household following the same procedure as in LPMs.

2.3. Laboratory testing

Specimens were transported and stored according to the national environmental surveillance protocol[16]. Sterile cotton-tipped swabs were used to collect specimens, and pooled specimens were placed in viral transport media and transported immediately to Pingxiang and Longzhou Center for Disease Control and Prevention (CDC) laboratories on frozen gel packs. The specimens were stored at 4°C and transported to Chongzuo prefecture CDC, the national influenza surveillance network laboratory, within 24 h to be tested for influenza A virus by rRT-PCR. Influenza A positive specimens were further tested for subtypes H5, H7, and H9 using subtype-specific primers and probes. If specimens were positive for influenza A while negative for subtypes H5, H7, and H9, the test result was defined as "Other." RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and rRT-PCR was conducted as described in the national protocol[19].

2.4. LPM survey

Four cross-sectional surveys were conducted in March and December 2018 and February and April 2019. Trained staff interviewed stall owners using a structured questionnaire and collected information on poultry species, volume, poultry trade, and market biosecurity practices. Stall owners were selected by convenience sampling and provided oral consent. The respondents may have differed for each survey.

2.5. Data analysis

We calculated the median weekly positive rates of influenza A, H5, H7, and H9 viruses by dividing the number of positive pooled specimens per week by the total number of pooled specimens collected that week. We then estimated the total and subtype median weekly positive rates for the observation period and 95% confidence intervals (CI). The frequency

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of positive detections of influenza A virus and subtype were compared by year, surveillance location, poultry species, and specimen category using Chi-Square or Fischer exact tests.

Since the LPM stall owners changed over time, we assumed the selected stalls in each survey were a series of independent samples and, for the analysis, merged the data from the four cross-sectional LPM surveys. We described the following market variables: poultry species, volume, source, cleaning practices, nights of poultry stay market, and separation procedures. Since chickens were the most common poultry species, we created scatter plots using the volume of chickens and the overall influenza positivity. We observed that the data had no clear linear relationship, so it did not satisfy the conditions for Pearson's correlation coefficient. We used Spearman's rank correlation analysis to determine the relationship between each market variable and influenza A, H5, or H9 virus percent positive within the same week the market survey was conducted. H7 was not analyzed because of few detections. Market variables used in the correlation analysis included total poultry volume, chicken volume, duck volume, and the scores of the market biosecurity behaviors, including frequency of stall-cleaning, frequency of cage-cleaning, number of nights poultry stayed in the market and reported practices such as sharing cages, poultry drinking water, and food between species. A higher score meant a higher level of biosecurity practice (i.e., ordinal variable). We then performed a sub-analysis using the same methods described above that stratified the results by the location of sampled environmental specimens (i.e., the relationship among each market variable and influenza positive on chopping board surfaces, depilation machines, etc.). All tests were two-sided, and a $P < 0.05$ was considered statistically significant.

3. Results

3.1. Positive rate of avian influenza viruses in pooled environmental specimens

From November 2017 to April 2018 and November 2018 to April 2019, 16,713 environmental specimens were collected and tested from 14 LPMs, 4 poultry farms, and 5 household farms. The overall median weekly positive rate for influenza A virus was 53.6% (33.5%–66.0%). H9 was the most frequently detected subtype, with a median weekly positive rate of 25.2% (17.1% − 42.8%). The median weekly positive rate of H5 was 4.9% (1.3% − 18.4%) and 21.2% for another influenza A subtypes (range = 3.1% – 40.0%). In addition, a total of two H7-positive samples were detected.

In 2017–2018, among 7,467 environmental specimens, 3,589 (48.1%) were positive for influenza A viruses, including 1,804 (24.2%) for H9, 456 (6.1%) for H5, two (0.027%) for H7, and 1,327 (17.8%) for others. Of the H7 positive specimens, one was from Pingxiang, and one was from Longzhou. In 2018–2019, among 9,246 environmental specimens, 5,316 (57.5%) were positive for influenza A viruses, including 2,626 (28.4%) for H9, 466 (5.0%) for H5, and none for H7. The median weekly positive rates for the two study periods were significantly different and increased in 2018–2019 for influenza A viruses overall (48.1% vs 57.5%, $P < 0.001$) and for H9 (24.2% vs 28.4%, $P < 0.001$) and declined for H5 (6.1% vs 5.0%, $P = 0.003$) (Table 1). The positive weekly detections over the study periods were shown (Fig. 1).

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The positive rates detected in Pingxiang were higher than those in Longzhou for influenza A viruses (59.6% vs 47.2%), H9 (31.2% vs 22.0%), and H5 (7.0% vs 4.0%) ($P < 0.001$). In addition, positive detections of influenza A viruses from LPMs were more frequent than detections from poultry farms (56.7% vs 16.3%), H9 (27.9% vs 11.1%), and H5 (6.0% vs 0.2%) ($P < 0.001$) (Table 1).

In bloody sewage specimens, the influenza A virus, H5, and H9 positive rates were the highest (73.6%, 15.4%, 38.7%). The two H7 positives were detected from a chopping board specimen and a fecal dropping specimen, respectively (Table 1).

3.2. LPM surveys

All 14 LPMs evaluated were retail markets, and 189 stalls were investigated. The stalls held a total of 13,480 poultry, including 10,678 (79.2%) chickens, 2,704 (20.1%) ducks, 98 (0.7%) geese, and 98 (0.7%) pigeons. In most stalls, 138 (73.0%) sold chickens and ducks, 33.6% of stalls were solely purchased from wholesale LPMs in Nanning (the capital of Guangxi Autonomous Region), 31.8% were purchased from local markets, and 32.7% from both Nanning and local markets. Three stalls purchased poultry from Vietnam. Ninety (42.0%) of the LPM stalls had a poultry volume of <50 birds. Most (67.3%) stalls kept live poultry in the market for 2–3 nights, and 14 (11.2%) kept poultry for one week. Less than half of the owners, 89 (41.6%), reported total separation of species by cages without sharing drinking water or food. In addition, 52.3% and 48.6% of stall owners reported daily cleaning of the stall and the cages, respectively. No stall owner reported sick or dead poultry in the previous month before the investigation (Table 2).

The Spearman's rank correlation analysis indicated that as the volume of chickens increased in the market, the H9 positive rates in environmental samples increased (correlation coefficient |r_s| of 0.31, $P = 0.045$). No correlations were identified between other LPM variables with influenza A, H5, or H9 positive rates. The results of our sub-analysis are shown in supplementary table 1. Our sub-analysis identified the following statistically significant findings: For cage specimens, influenza A positivity increased as the frequency of stall and cage cleanings decreased (|r_s|= −0.41 with $P = 0.007$ and |r_s|= −0.37 with $P = 0.015$). For chopping board surface specimens, positives for influenza A (|r_s|= −0.43 with $P = 0.006$ and |r_s|= −0.40 with $P = 0.011$) and H5 (|r_s|= −0.40 with $P = 0.011$ and |r_s|= −0.39 with $P = 0.014$) increased while the frequency of stall and cage cleanings decreased. For bloody sewage specimens, H9 positive rates increased as the duration of poultry increased in the market increased (|r_s|= 0.36, $P = 0.019$). Finally, for poultry drinking water specimens, influenza A (|r_s|= 0.33, $P = 0.036$) and H9 (|r_s|= 0.38, $P = 0.014$) positive rates increased as the frequency of sharing cages, drinking water, and food by different bird species increased.

4. Discussion

In 2017–2019, environmental samples from LPM and poultry farms in Southern China near the border of Vietnam were frequently (~25%) positive for avian influenza A(H9) and rarely for H5 (~5%) or H7 (< 1%). We also identified several weaknesses in biosecurity measures and correlations with increased detection of avian influenza viruses in LPMs.

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This study was initiated after China's bivalent H5/H7 poultry vaccination program. One of the purpose of our study was to monitor the effect of the vaccination program on the detection rate of the H5, H7, and H9. The positive rate of avian influenza A/H7 virus was 0.012% in LPM, lower than the rates observed in Guangxi before the introduction of poultry vaccination: 3.90% (385/9,628, unpublished data) through local environmental surveillance in Guangxi Autonomous Region, from January to July 2017 and 0.11% (2/1,855) from March to April 2017 in Fangchenggang, a neighboring city of our study cities[20]. The findings of decreased detection of H7 were consistent with the results of national poultry surveillance and environmental surveillance in Zhejiang and Guangdong Provinces[21,22]. During the 2017–2018 study period, we only sampled environmental specimens from chickens as fecal dropping and poultry drinking water. However, limited bloody sewage and chopping board swabs involving poultry other than chicken might not be excluded. In response to the concerns for heightened H7N9 and H7N2 virulence in ducks following the poultry vaccination introduction[3], we added a sampling of duck-related environmental specimens in 2018–2019 but did not identify any H7 in the second year of the study. Despite identifying other avian influenza viruses while conducting our environmental surveillance, it is notable that we had few H7 detections and that there were also no reports of human infections with the H7 virus in Guangxi during the study period in contrast to the 27 infections reported in 2016–2017 prior to the initiation of the national poultry vaccination program [15]. Our findings suggest that implementing the H5/H7 poultry vaccination program may have reduced the presence of the H7 virus in the environments of LPMs and poultry farms in our study sites, likely through the reduction of the virus in the birds themselves. This apparent successful use of a poultry vaccination program to control emerging avian influenza virus helps provide an alternative to the use of effective depopulation methods used for controlling poultry outbreaks of avian influenza virus in many other countries[23,24]. Although our results do not establish causality, they suggest that poultry vaccination campaigns can be essential in controlling emerging avian influenza viruses of animal health or public health concern.

The positive rate of H5 in LPMs was 5.5% in the study period, and higher than the local surveillance result of 3.8% (368/9,628) in Guangxi overall from January to July 2017 (unpublished) and 1.5% (28/1,855) from March to April 2017 in Fangchenggang, a neighboring city of our study cities[20]. Our study observed a slight increase in H5, while Zhejiang and Guangdong Provinces detected no significant change in H5 prevalence after the introduction of bivalent vaccination[21,22]. In addition, compared to the 2017–2018 study period, we observed fewer H5 detections (5.0% vs 6.1%, $P = 0.003$) in the 2018–2019 study season. In the absence of complete subtyping or clade information, we do not know if there was a shift in the predominant H5 virus species. Nevertheless, it is notable that in the 2018–2019 season, the Chinese Ministry of Agriculture updated the H5 component of the H5/H7 poultry vaccine from re-8:2.3.4.4d to re-11:2.3.4.4d[22,25]. Since other conditions in the market were similar in both study periods, this change in the vaccine composition may have affected the H5 ecology in Guangxi in 2018–2019.

Among influenza A virus-positive environmental samples, H9 was the most frequently detected subtype (26.5%). The rates found in our study were significantly higher than those found in local surveillance: 15.4% (1,497/9,628) in Guangxi (unpublished) from

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January to July 2017 and 10.7% (198/1,855) from March to April 2017 in Fangchenggang, a neighboring city of our study sites[20]. The increased frequency of H9 detections was consistent with findings in Zhejiang and Guangdong Provinces[21,22]. H9 viruses are endemic in poultry populations in China, and H9N2 has caused sporadic cases of the human infection since 1998[26,27]. Although China implemented an H9 vaccination program in chickens in 1998, H9 has persisted even in vaccinated flocks[28]. Similar to other studies, we found that chicken-related environmental samples had a higher detection percentage for H9 than those from duck-related specimens [29]. The high levels of detections of H9 in our results, particularly compared with those from before the H5/H7 bivalent vaccine program, are notable because they may suggest that widespread vaccine use is putting ecological pressure on circulating avian influenza viruses in poultry populations in Guangxi and driving a shift in the predominant strain. This finding of high levels of H9 in poultry populations is significant because of the broad host range of H9 and the role that H9 gene reassortments have played in the emergence of novel influenza viruses such as H5N1, H7N9 and H5N6 viruses[29,30]. Continued surveillance for avian influenza viruses in LPMs and poultry farms is warranted for the early detection of novel viruses that may emerge in poultry or humans, particularly in the setting of the evolutionary pressures on the viral ecology that may occur as a result of the continuous use of a virus-specific poultry vaccination such as the H5/H7 bivalent poultry vaccine.

Our LPM evaluation revealed several biosecurity measure vulnerabilities related to the periodic cleaning of stalls and cages, poultry remaining in markets for multiple nights, and the use of shared cages and drinking water among multiple species. These observations are consistent with findings of limited biosecurity measures in LPMs in a previous study in Guangxi[5]. In addition, only half of the stall owners reported cleaning their stalls and cages every day, and as expected, the risk factor analysis found greater avian influenza A and H5 virus-positive rates detected on cages and chopping block surfaces that were less frequently cleaned. Furthermore, most stalls kept live poultry in the market for multiple nights, some for as long as one week, and the H9 positives in bloody sewage increased as poultry stayed longer in the market. In March 2018, the Chinese Ministry of Agriculture officially issued the “1101” policy, which refers to the requirement to perform daily cage cleaning in markets, weekly market cleaning and disinfection, monthly market closure, and zero overnight poultry storage in the market[16]. However, our findings suggest that the “1101” policy was not consistently followed during the study period and that continued gaps in the implementation and monitoring of LPM biosecurity policies may contribute to ineffective control of avian influenza viruses in LPMs. Therefore, biosecurity measures targeting poultry farming and production facilities may be necessary as controlling avian influenza viruses upstream may help overcome the deficiencies in implementing control measures in LPMs.

Our study has several strengths. First, we collected environmental surveillance specimens weekly, increasing our confidence that the lack of detections was real rather than the result of positive samples missed during infrequent surveillance. However, other studies similarly described the few detections of H7 in LPMs and poultry farms following the implementation of the H5/H7 bivalent poultry vaccination program using routine monthly surveillance

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data[21,22]. In addition, unlike other studies which only evaluated surveillance data from a single season after vaccine introduction[21,22], our study described data from two seasons.

Our study has several limitations. First, the study was observational and descriptive; it was not designed to assess the effectiveness of the H5/H7 bivalent poultry vaccination program. Second, we reported influenza virus detections by PCR rather than virus isolation, so some detections may not represent viable viruses. Third, the results cannot be generalized to LPMs and poultry farms in other geographical areas of China. Fourth, we do not have clade information for A(H5) specimens, and therefore we do not know if there were any changes in the H5 viruses in the environment over the study period. Finally, the stall owners self-reported the LPM biosecurity practices, which were not independently verified.

5. Conclusions

From November 2017-April 2019, environmental surveillance at LPMs and poultry farms in Guangxi Autonomous Region, near the China-Vietnam border, detected only two avian influenzas A/H7 virus-positive samples, while H5 and H9 viruses continued to circulate. There are persistent gaps in the implementation and monitoring of LPM biosecurity policies. Therefore, in addition to better enforcement of the current 1101 policy, other measures targeting poultry farming and production facilities upstream may be necessary. Poultry vaccination campaigns can be essential in controlling emerging avian influenza viruses that potentially threaten animal and human health. Continued surveillance for avian influenza viruses is necessary to detect and respond to emerging trends in avian influenza virus epidemiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

Scientific question

Live poultry markets can promote the amplification and dissemination of avian influenza viruses and serve as ideal settings for influenza virus transmission at the animal-human interface. Therefore, it is essential for the detection and response to avian influenza viruses surveillance in live poultry markets.

Evidence before this study

During the fifth epidemic in 2016–2017, the number of human cases spiked, geographic distribution of low pathogenic avian influenza A(H7N9) increased. Moreover, a novel highly pathogenic avian influenza (HPAI) A(H7N9) virus was detected in chickens and humans.

New findings

We conducted environmental surveillance to detect avian influenza viruses circulating at live poultry markets (LPMs) as well as poultry farms at Guangxi, China, where is near China-Vietnam border. Four cross-sectional questionnaire surveys among stall owners on biosecurity practices in LPMs of two study sites were conducted. Among 16,713 environmental specimens were collected and detected, with the median weekly positive rate for avian influenza A 53.6% (range = 33.5% − 66.0%), including 25.2% for H9, 4.9% for H5, and 21.2% for other avian influenza virus A subtypes, whereas a total of two H7 positive samples were detected. Among the 189 LPM stalls were contained in the investigation, in which most stall owners (73.0%) sold both chicken and ducks.

Significance of the study

Continued surveillance for avian influenza virus is necessary for detecting and responding to emerging trends in avian influenza virus epidemiology.

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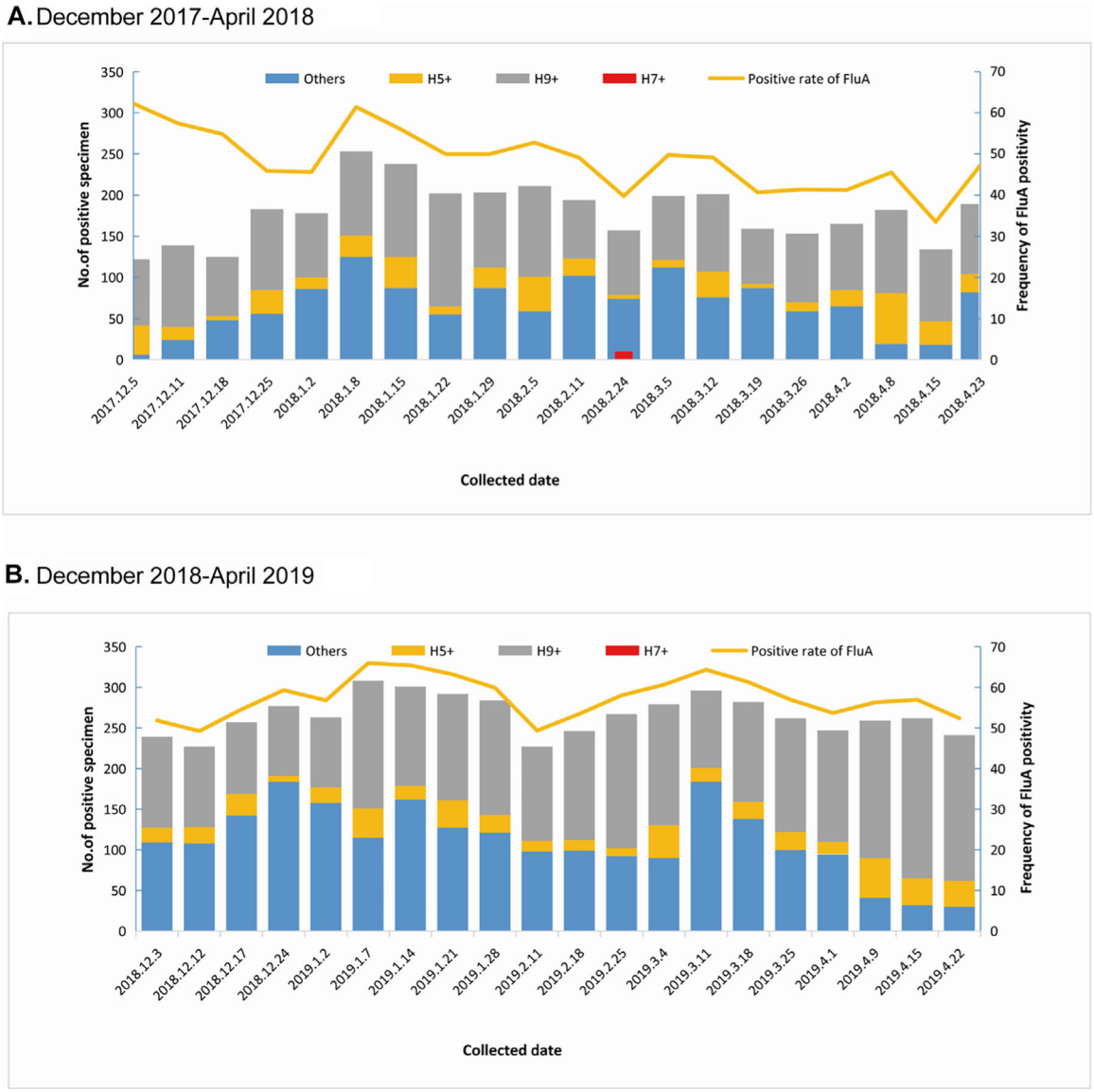

Fig. 1.

Weekly avian influenza virus positives by subtype in pooled environmental specimens, Longzhou and Pingxiang of Guangxi Autonomous Region, December 2017 to April 2018 and December 2018-April 2019. A) December 2017 to April 2018; B) December 2018 to April 2019.

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Table 1

Average weekly positivity of Avian Influenza A (H5), A(H7), and A(H9) viruses in pooled environmental specimens, Longzhou and Pingxiang Cities of Guangxi Autonomous Region, China, 2017–2019.

Specimen	Influenza A virus positive %(N/ total number)	*P* Value[a]	A(H5) positive % (N/total number)	*P* Value[b]	A(H9) positive % (N/total number)	*P* Value[c]	A(H7) positive % (N/total number)
	53.3 (8,905/16,713)		5.5 (922/16,713)		26.5 (4,430/16,713)		0.012 (2/16,713)
Year							
2017–2018	48.1 (3,589/7,467)	<0.001	6.1 (456/7,467)	0.003	24.2 (1,804/7,467)	<0.001	0.027 (2/7,467)
2018–2019	57.5 (5,316/9,246)	Reference	5.0 (466/9,246)		28.4 (2,626/9,246)		0.0 (0/9,246)
City							
Pingxiang	59.6 (4,905/8,230)	<0.001	7.0 (579/8,230)	<0.001	31.2 (2,567/8,230)	<0.001	0.012 (1/8,230)
Longzhou	47.2 (4,000/8,483)		4.0 (343/8,483)		22.0 (1,863/8,483)		0.012 (1/8,483)
Study site							
LPMs	56.7 (8,674/15,295)	<0.001	6.0 (919/15,295)	<0.001	27.9 (4,273/15,295)	<0.001	0.013 (2/15,295)
Poultry farms	16.3 (231/1,418)		0.2 (3/1,418)		11.1 (157/1,418)		0.0 (0/1,418)
Related poultry							
Chicken-related	52.2 (8,016/15,356)	<0.001	5.4 (824/15,356)	0.004	27.6 (4,238/15,356)	<0.001	0.0 (2/15,356)
Duck-related	65.6 (886/1,350)		7.3 (98/1,350)		14.1 (191/1,350)		0.0 (0/1,350)
NA	42.9 (3/7)		0.0 (0/7)		14.3 (1/7)		0.0 (0/7)
Specimen category							
Bloody sewage	73.6 (1,389/1,887)	<0.001	15.4 (290/1,887)	<0.001	38.7 (730/1,887)	<0.001	0.0 (0/1,887)
Poultry drinking water	64.3 (2,017/3,135)		7.9 (249/3,135)		36.6 (1,148/3,135)		0.0 (0/3,135)
Cage surface	45.9 (1,598/3,479)		1.0 (36/3,479)		23.9 (830/3,479)		0.0 (0/3,479)
Fecal dropping	46.0 (1,603/3,484)		1.8 (61/3,484)		18.2 (635/3,484)		0.029 (1/3,484)
Depilation machine	60.2 (1,417/2,355)		6.4 (151/2,355)		30.4 (716/2,355)		0.0 (0/2,355)
Chopping board	47.9 (844/1,761)		7.7 (135/1,761)		20.1 (354/1,761)		0.057 (1/1,761)
Egg surface	6.0 (37/612)		0.0 (0/612)		2.8 (17/612)		0.0 (0/612)

Note.

a: Comparison of avian influenza A weekly positive rate.

b: Comparison of H5 weekly positive rate.

c: Comparison of H9 weekly positive rate.

Abbreviations: LPMs, live poultry markets; NA, Information not availabe.

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Biosaf Health. Author manuscript; available in PMC 2024 August 28.

Table 2

Avian influenza virus positives in live poultry markets (LPM), LPM characteristics, Longzhou and Pingxiang of Guangxi Autonomous Region, China, 2017–2019.

Block sort	Total of all markets	Median of all markets	Range of all markets
Environmental surveillance			
# of specimen	15,292	1,077	653 – 3,245
% of influenza A+	56.7%	58.9%	28.8% – 70.3%
% of influenza H5+	6.0%	5.8%	0.5% – 10.4%
% of influenza H7+	0.0%	0.0%	0.0% – 0.1%
% of influenza H9+	27.9%	26.0%	15.9% – 40.2%
Market survey			
# of stalls	214	12	5 – 79
# of poultry	13,480	1,050	281 – 3,203
# of chicken	10,678	940	113 – 2,705
# of duck	2,704	168	50 – 539
# of other poultry	98	2	0 – 44
# of stalls by volume of poultry			
< 50 poultry	90 (42.1%)	3	0– 46
50 – 100 poultry	84 (39.3%)	4	0– 33
100 – 150 poultry	28 (13.1%)	1	0– 8
≥150 poultry	12 (5.6%)	0	0– 6
# of stalls by the source of poultry			
Nanning	77 (36.0%)	5	0– 30
Local	62 (29.0%)	6.5	0– 14
Mixed	71 (33.2%)	4	1– 35
Other	4 (1.9%)	0	0– 3
# of stalls by frequency of stall cleaning			
Everyday	112 (52.3%)	8	1– 33
Every 2–3 days	76 (35.5%)	3	1– 33
Once a week	7 (3.3%)	0	0– 3
Once a month	2 (0.9%)	0	0– 2

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Block sort	Total of all markets	Median of all markets	Range of all markets
Seldom/never	1 (0.5%)	0	0– 1
Unclear	16 (7.5%)	0	0– 13
# of stalls by frequency of cage cleaning			
Everyday	104 (48.6%)	8	1– 30
Every 2–3 days	62 (29.0%)	3	0– 36
Once a week	26 (12.1%)	1	0– 8
Once a month	5 (2.3%)	0	0– 3
Seldom/never	1 (0.5%)	0	0– 1
Unclear	16 (7.5%)	0	0– 13
# of stalls by nights poultry stay in the market			
One night	16 (7.5%)	0	0– 10
2–3 nights	144 (67.3%)	9	4– 50
One week	24 (11.2%)	3	0– 5
> One week	8 (3.7%)	0	0– 5
Unclear	22 (10.3%)	0	0– 18
# of stalls by different species holding			
Shared cages	20 (9.3%)	1	0– 6
Separated by cages but share drinking water	74 (34.6%)	2	0– 45
Separated by cage	89 (41.6%)	7	2– 15

Note: #, number.