Avian Influenza Virus Detection Rates in Poultry and Environment at Live Poultry Markets, Guangdong, China

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We report the use of environmental samples to assess avian influenza virus activity in chickens at live poultry markets in China. Results of environmental and chicken samples correlate moderately well. However, collection of multiple environmental samples from holding, processing, and selling areas is recommended to detect viruses expected to have low prevalence.

Live poultry markets (LPMs) can serve as hubs for avian influenza virus (AIV) amplification in poultry and pose a risk for human zoonotic infections (*1*–*4*). Adopting efficient sampling strategies to monitor AIVs with human zoonotic potential at LPMs is essential for zoonotic disease prevention and pandemic preparedness. Recommendations regarding routine surveillance that would robustly and efficiently inform AIV activity at LPMs have been limited (*5*).

Handling of live poultry interrupts the vending process; moreover, such routine surveillance is difficult to implement. Environmental samples have been collected to monitor AIV activity at LPMs (*5*–*9*). There have been limited parallel comparisons of AIV detection rates among poultry and environmental samples (*7*,*10*). Without frequent cleaning, the environment often permits AIV accumulation; environmental samples may thus overestimate AIV prevalence in poultry. Subtype-specific detection rates among different environmental samples may also vary. To inform the development of effective sampling strategies for AIV surveillance, we compared monthly detection rates for AIV subtypes H5, H7, and H9 in chickens and various environmental samples at LPMs in Guangzhou, China.

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The Study

During December 2015–July 2018, we performed sampling twice per month at 1 wholesale (52 stalls) and 1 retail (8 stalls) LPM, from 2 randomly selected stalls per sampling event. We collected paired oropharyngeal and cloacal swab samples (n = 3,119 chickens) and environmental samples (n = 3,008) in viral transport medium at the LPMs (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/26/3/19-0888-App1.pdf). We randomly collected samples from all chickens at the selected stalls. We rarely observed sick chickens but we sampled them when identified. We also collected environmental samples from 3 key activity areas: poultry holding zones (fecal droppings, drinking water, and poultry feed), slaughtering zones (defeathering machines and surrounding defeathering working areas), and selling zones (chopping boards and display tables) near the selected chickens whenever possible (*5*–*9*). (Stalls sampled at the wholesale LPM [wLPM] have only poultry holding zones.) We sampled air using BC-251 cyclone-based NIOSH bioaerosol samplers that fractionate airborne particles into >4 μm, 1–4 μm, and <1 μm size fractions (*11*). We applied quantitative real-time reverse transcription PCR to detect the matrix gene segment of AIV; we analyzed positive samples by the hemagglutinin gene to determine the AIV subtype (H5, H7, or H9) using specific primers and probes (*12*,*13*).

H5, H7, and H9 detection rates in environmental samples (median monthly difference 6.2% for H5,

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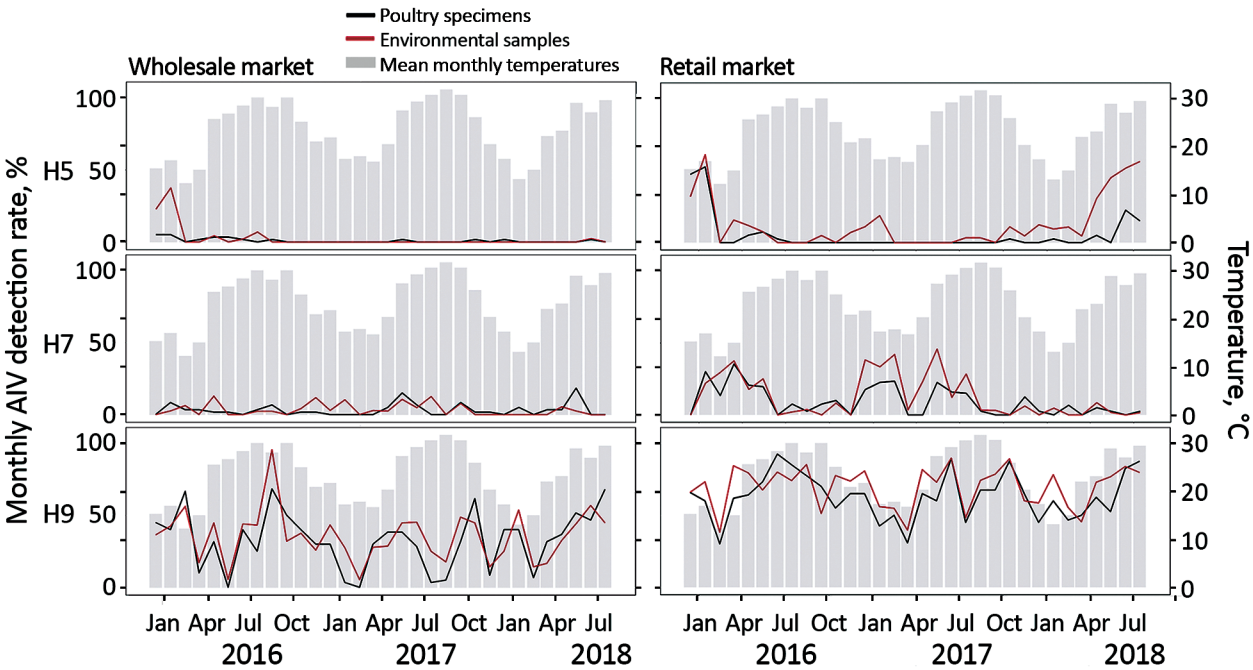

Figure 1. Monthly AIV H5, H7, and H9 positivity rates detected in poultry and environmental samples at live poultry markets (LPMs), Guangdong, China, December 2015–July 2018. Chicken (oropharyngeal and cloacal swab specimens) and environmental (swab specimens and air samples) samples were collected monthly from 1 retail and 1 wholesale LPM in Guangzhou and tested for H5, H7, and H9 AIV by real-time RT-PCR. Gray bars indicate mean temperatures recorded on the sampling date in Guangzhou. AIV, avian influenza virus.

3.1% for H7, and 34.1% for H9; all p<0.02 by Mann-Whitney test) were much higher at the retail LPM (rLPM) than that at the wLPM (Figure 1), probably because of poultry mixing, aggregation, and extended stay at retail settings. Human H5 or H7 zoonotic infections clustered in winter, but we observed no correlation (p>0.215 for both) between temperature (*14*) and H5 or H7 detection rates in chickens or environmental samples at both markets. We did not assess other confounding factors, including market interventions and poultry holding duration.

We evaluated correlations between monthly AIV detection rates in chickens and environmental samples (moderate correlation for r_s>0.5, at which point environmental samples are considered useful to monitor AIV in chickens). We observed a positive correlation for H5 (Spearman r_s = 0.569, p<0.001) and H9 viruses (r_s = 0.702, p<0.001) at the wLPM and for H5 (r_s = 0.581, p<0.001), H7 (r_s = 0.760, p<0.001), and H9 viruses (r_s = 0.685, p<0.001) at the rLPM. We examined the use of environmental samples to assess AIV activity in poultry (Table 1). Environmental

Table 1. Sensitivity and specificity of applying environmental samples to assess AIV activity in poultry, based on monthly AIV detection, Guangdong, China, December 2015–July 2018*

Market type	Subtype	Sensitivity, % (95% CI)†	Specificity, % (95% CI) †	Positive predictive value, % (95% CI)†	Negative predictive value, % (95% CI)†	Accuracy, % (95% CI)†
Wholesale	H5	45.5 (16.8–76.6)	95.2 (76.2–99.9)	83.3 (39.9–97.4)	76.9 (65.8–85.2)	78.1 (60.0–90.7)
	H7	68.4 (43.5–87.4)	69.2 (38.6–90.9)	76.5 (57.6–88.6)	60.0 (41.4–76.1)	68.8 (50.0–83.9)
	H9‡	100 (88.4–100)	0 (0–84.2)	93.8	NA	93.8 (79.2–99.2)
Retail	H5	90.0 (55.5–99.8)	45.5 (24.4–67.8)	42.9 (32.7–53.7)	90.9 (59.6–98.6)	59.4 (40.6–76.3)
	H7	87.0 (66.4–97.2)	66.7 (29.9–92.5)	87.0 (72.3–94.5)	66.7 (38.7–86.4)	81.3 (63.6–92.8)
	H9§	100 (89.1–100)	NA	100 (89.1–100)	NA	100 (89.1–100)

*Test results from reverse transcription PCR on bird samples were assumed to be the standard in the analysis. The results may not be applicable to other surveillance systems with more intensive sampling or accurate laboratory testing. AIV, avian influenza virus; NA, not applicable.
†Sensitivity: probability that the environmental samples will test positive when the subtype of AIV is present in chickens on site (true positive rate). Specificity: probability that the environmental samples will test negative when the subtype of AIV is not present (true negative rate). Positive predictive value: probability that the subtype of AIV is present in poultry when environmental samples are tested positive. Negative predictive value: probability that the subtype of AIV is not present in poultry when the environmental samples are tested negative. Accuracy: probability that the presence or absence of AIV in poultry will be correctly determined based on the test results of environmental samples.
‡H9 was detected during every month during the study period in the environmental samples (monthly data can be found in Appendix Table 1, https://wwwnc.cdc.gov/EID/article/26/3/19-0888-App1.pdf).
§H9 was detected during every month during the study period in both the poultry and the environmental samples (monthly data can be found in Appendix Table 2).

samples collected at the rLPM provided higher sensitivity in detecting H5, H7, or H9 viruses in poultry than those from the wLPM. Environmental samples were less likely to detect H5 and H7 viruses in poultry at the wLPM than at the rLPM (Appendix Tables 1, 2), possibly because of the low prevalence of infection in birds, a higher poultry turnover rate, and comparatively thorough daily cleaning practices at the wLPM. The lower specificity for H5 at the rLPM may be the result of carryover contamination at the poultry slaughtering area caused by processing birds of other species. The probabilities of accurately detecting the presence or absence of H5, H7, and H9 subtypes in poultry from environmental samples were comparable for the wLPM (68.8%–93.8%) and the rLPM (59.4%–100%) (Table 1). This finding suggests that environmental samples provided a useful indication of AIV activity in chickens at LPMs. Nevertheless, for H5 and H7 viruses at the wLPM, in only 1 month did all environment samples test positive when bird samples were also positive, demonstrating the need to take a wide range of environment samples.

We investigated correlations between specific environmental samples and monthly H5, H7, and H9 detection rates in chickens (Figure 2; Appendix Figure 2). At the wLPM, positive rates for H5 (r_s = 0.515, p = 0.003), H7 (r_s = 0.514, p = 0.003), and H9 (r_s = 0.508,

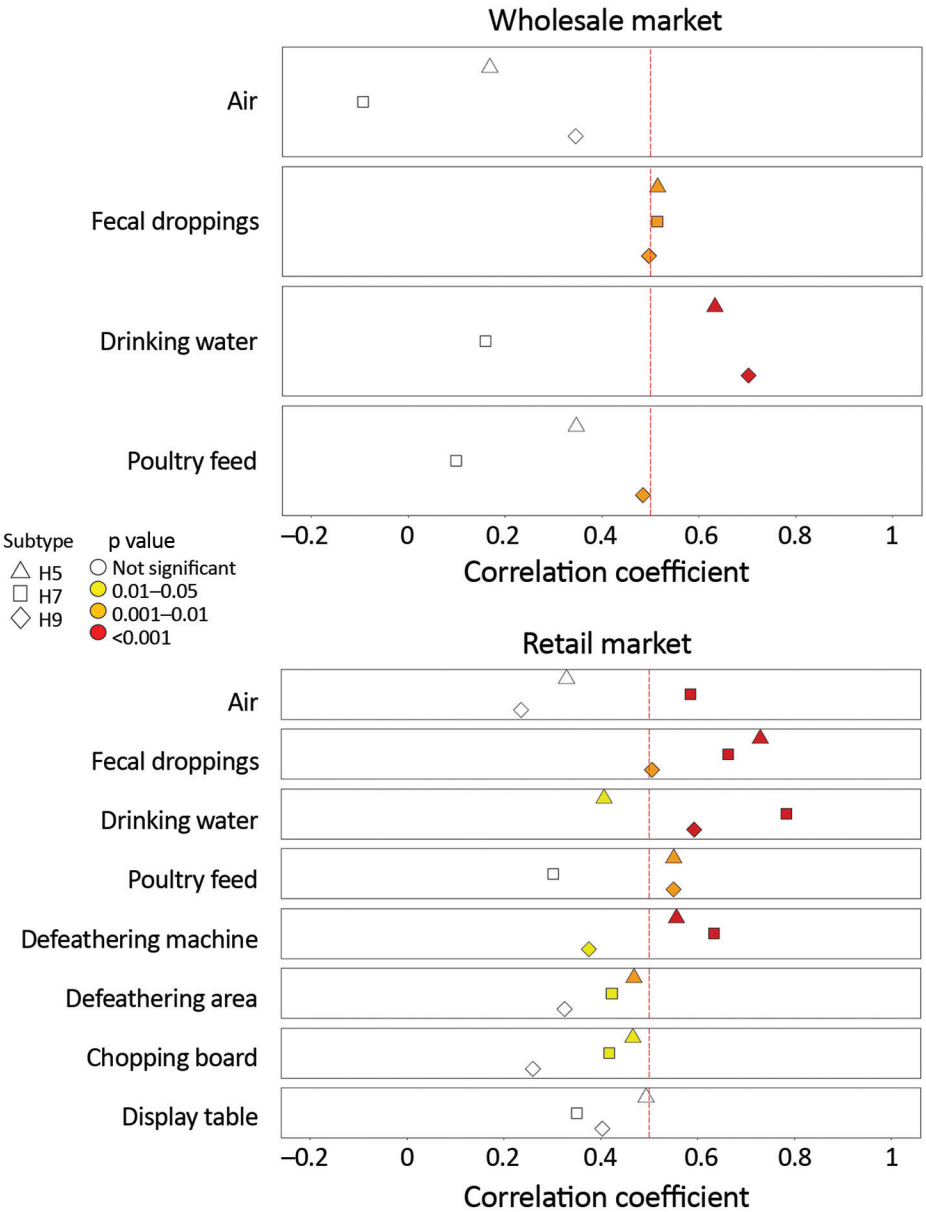

Figure 2. Correlation between AIV detection rates in poultry and environmental samples at live poultry markets (LPMs), Guangdong, China, December 2015–July 2018. Monthly AIV, H5, H7, and H9 detection rates in chicken and environmental samples were analyzed using Spearman's rank correlation. The vertical red dashed line indicates correlation coefficient (r_s) at 0.5. Subtypes and significance levels are indicated. AIV, avian influenza virus.

Table 2. AIV detection rates from chicken and environmental samples collected at a retail LPM, Guangdong, China, December 2015–July 2018*

Type of samples	No. samples	Median monthly positive rate, % (95% CI)		
		H5	H7	H9
Poultry samples†	1,239	0.0 (0.0–2.5)	5.8 (0.0–15.0)	64.6 (55.0–67.5)
Oropharyngeal	1,239	0.0 (0.0–2.5)	5.8 (0.0–13.3)	60.0 (52.5–67.5)
Cloacal	1,239	0.0 (0.0–2.5)	0.0 (0.0–2.5)	20.0 (13.2–26.5)
Environmental samples	1,734	6.2 (0.0–11.8)	4.2 (1.6–22.0)	73.8 (60.0–79.4)
Poultry holding zone‡	965	2.9 (0.0–6.3)	3.0 (0–20.0)	68.2 (56.0–75.9)
Fecal droppings	424	0.0 (0.0–7.7)	0.0 (0.0–11.1)	58.3 (50.0–66.7)
Drinking water	364	0.0 (0.0–8.3)	3.3 (0.0–15.4)	83.3 (66.7–100.0)
Poultry feed	177	0.0 (0.0–11.1)	0.0 (0.0–9.1)	50.0 (33.3–70.0)
Poultry slaughtering zone	457	2.2 (0.0–25.0)	6.1 (0.0–22.2)	78.6 (59.5–87.5)
Defeathering machine	250	0.0 (0.0–20.0)	0.0 (0.0–20.0)	86.2 (70.0–100.0)
Defeathering area	207	0.0 (0.0–25.0)	0.0 (0.0–12.5)	73.2 (50.0–87.5)
Poultry selling zone	194	27.9 (0.0–50.0)	0.0 (0.0–14.3)	91.2 (60.0–100.0)
Chopping board	141	33.0 (0.0–50.0)	0.0 (0.0–25.0)	100.0 (71.4–100.0)
Display table	53	0.0 (0.0–66.7)	0.0 (0.0–14.3)	92.9 (25.0–100.0)
Air§	118	0.0 (0.0–25.0)	0.0 (0.0–16.7)	75.0 (50.0–100.0)

*AIV, avian influenza virus; LPM, live poultry market; qRT-PCR, quantitative reverse transcription PCR.
†A positive poultry sample may detect AIV in the oropharyngeal samples, cloacal samples, or both by qRT-PCR.
‡Environmental swab specimens were collected within the same poultry stall at LPMs but may not be from the same cage where the chickens were sampled. Fecal droppings were collected from the ground or cages, drinking water was collected from the water troughs, and poultry feed was sampled from the surface of the bowls or feeders.
§A positive air sample may be positive for AIV by qRT-PCR in any of the 3 size fractions collected by a NIOSH sampler (*11*). Two to 6 NIOSH samplers were applied monthly to sample air at the retail markets.

p = 0.003) in fecal droppings correlated moderately well with viral prevalence in chickens, whereas drinking water provided the best correlation for H5 (r_s = 0.633, p<0.001) and H9 (r_s = 0.702, p<0.001) (Figure 2) and was more sensitive for H9 (Appendix Figure 3). At the rLPM, H9 detection rates in drinking water (r_s = 0.593, p<0.001), poultry feed (r_s = 0.550, p = 0.002), and fecal droppings (r_s = 0.506, p = 0.003) best correlated with H9 prevalence in chickens; drinking water was most sensitive (Appendix Figure 3). H7 detection rates in drinking water (r_s = 0.784, p<0.001), fecal droppings (r_s = 0.663, p<0.001), defeathering machines (r_s = 0.634, p<0.001), and air (r_s = 0.585, p<0.001) best correlated with H7 prevalence in chickens. The H5 detection rates in fecal droppings (r_s = 0.729, p<0.001), defeathering machines (r_s = 0.556, p<0.001), and poultry feed (r_s = 0.550, p = 0.02) best correlated with H5 prevalence in chickens. Collectively, these results suggest that fecal droppings may provide a good estimation for H5, H7, and H9 prevalence in chickens at LPMs and that drinking water can be more sensitive in some settings and useful for determining virus contamination in LPMs. For viruses present at low prevalence (e.g., H5), low sensitivity is expected.

We summarized H5, H7, and H9 detection rates in various environmental samples at the rLPM (Table 2). H5 virus was most frequently detected from poultry selling zones (median monthly positive rate 27.9%, 95% CI 0%–50%), especially from chopping boards (33%, 95% CI 0%–50%), whereas H7 virus was most frequently detected from poultry slaughtering zones (6.1%, 95% CI 0%–22.2%), especially from defeathering machines. H9 virus was frequently detected from all sampling sites. However, we found no clear difference in environmental sites for detecting H5, H7, or H9 (Appendix Tables 1,2).

Conclusions

AIV detection rates in environmental samples correlated moderately with AIV activity in chickens at LPMs. Environmental sampling at rLPMs provides greater sensitivity in detecting H5, H7, and H9 AIV in poultry than that at the wLPMs and should be included as routine surveillance to monitor AIV activity. At the rLPM, H5 and H7 viruses were most frequently detected from poultry selling and poultry slaughtering areas, whereas the highly prevalent H9 viruses were detected frequently at poultry holding, slaughtering, and selling areas. Environmental samples with the highest detection rate for H5, H7, and H9 viruses may not provide the best indication of virus activity in poultry, however. Some market stalls containing viruses with low prevalence would be misclassified if only environmental or bird samples were collected. To detect viruses expected to be present at low prevalence, environmental samples should be collected from multiple sites in each market stall, including samples from holding, processing, and selling areas.

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Appendix

Appendix Table 1. Monthly AIV detection rates from chicken and environmental samples collected at the wholesale market during December 2015–July 2018 in Guangzhou, Guangdong, China

Year	Month	Monthly positive rate (%)					
		Oropharyngeal	Cloacal	Fecal droppings	Drinking water	Poultry feed	Air
				H5 virus			
2015	Dec	2	3	24	10	50	29
2016	Jan	5	2	40	43	0	0
	Feb	0	0	0	0	0	0
	Mar	0	2	0	0	0	0
	Apr	0	3	4	8	0	0
	May	2	2	0	0	0	0
	Jun	2	0	4	0	0	0
	Jul	0	0	10	0	0	25
	Aug	2	0	0	0	0	0
	Sep	0	0	0	0	0	0
	Oct	0	0	0	0	0	0
	Nov	0	0	0	0	0	0
	Dec	0	0	0	0	0	0
2017	Jan	0	0	0	0	0	0
	Feb	0	0	0	0	0	0
	Mar	0	0	0	0	0	0
	Apr	0	0	0	0	0	0
	May	2	0	0	0	0	0
	Jun	0	0	0	0	0	0
	Jul	0	0	0	0	0	0
	Aug	0	0	0	0	0	0
	Sep	0	0	0	0	0	0
	Oct	2	0	0	0	0	0
	Nov	0	0	0	0	0	0
	Dec	2	2	0	0	0	0
2018	Jan	0	0	0	0	0	0
	Feb	0	0	0	0	0	0
	Mar	0	0	0	0	0	0
	Apr	0	0	0	0	0	0
	May	0	0	0	0	0	0
	Jun	2	0	0	7	0	0
	Jul	0	0	0	0	0	0
				H7 virus			
2015	Dec	0	0	0	0	0	0
2016	Jan	5	3	3	0	0	0
	Feb	3	0	0	0	0	29
	Mar	2	2	0	0	0	0
	Apr	2	0	7	25	100	0
	May	2	0	0	0	0	0
	Jun	0	0	0	0	0	0
	Jul	3	0	0	5	0	0
	Aug	7	0	0	6	0	0
	Sep	0	0	0	0	0	0
	Oct	2	0	0	7	0	25
	Nov	2	0	8	11	17	25
	Dec	0	0	0	0	0	25
2017	Jan	0	0	7	9	0	50

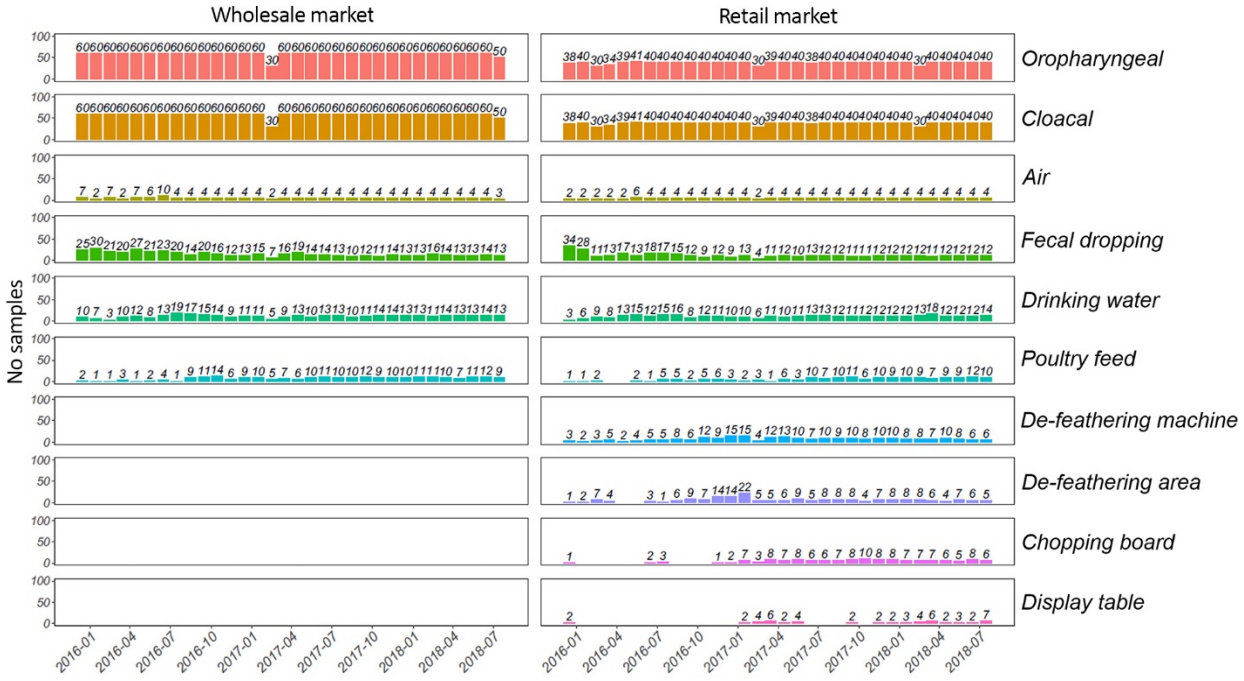
Year	Month	Monthly positive rate (%)					
		Oropharyngeal	Cloacal	Fecal droppings	Drinking water	Poultry feed	Air
	Feb	0	0	0	0	0	0
	Mar	0	0	0	0	0	25
	Apr	3	2	5	0	0	0
	May	15	3	7	10	0	50
	Jun	3	3	7	8	0	0
	Jul	0	0	0	8	10	75
	Aug	0	0	0	0	0	0
	Sep	5	3	8	0	17	0
	Oct	2	0	0	0	0	0
	Nov	2	0	0	0	0	0
	Dec	0	0	0	0	0	0
2018	Jan	2	3	0	0	0	0
	Feb	0	0	0	0	0	0
	Mar	0	3	0	0	0	0
	Apr	3	0	0	0	14	25
	May	13	8	8	0	0	0
	Jun	0	0	0	0	0	0
	Jul	0	0	0	0	0	0
		H9 virus					
2015	Dec	45	22	20	50	100	57
2016	Jan	38	17	37	57	0	100
	Feb	67	28	43	100	100	71
	Mar	7	3	0	50	33	0
	Apr	30	17	33	83	100	14
	May	0	0	0	0	0	33
	Jun	40	7	44	77	50	0
	Jul	25	0	10	68	0	100
	Aug	68	20	100	100	89	75
	Sep	50	3	5	67	46	0
	Oct	40	13	6	50	50	75
	Nov	30	2	8	44	17	50
	Dec	30	15	31	55	33	75
2017	Jan	3	0	7	46	30	50
	Feb	0	0	0	0	0	50
	Mar	30	3	13	44	29	50
	Apr	38	10	21	39	17	50
	May	38	2	7	80	40	100
	Jun	28	7	36	39	55	75
	Jul	2	2	23	31	10	50
	Aug	5	0	10	20	20	25
	Sep	32	2	17	82	42	75
	Oct	60	15	36	64	0	100
	Nov	7	2	0	21	10	50
	Dec	40	7	8	39	20	50
2018	Jan	40	2	15	92	46	75
	Feb	5	2	0	18	9	75
	Mar	32	2	7	29	0	50
	Apr	37	3	0	39	71	50
	May	52	25	31	54	36	75
	Jun	47	10	7	93	75	50
	Jul	68	2	15	77	22	100

Appendix Table 2. Monthly AIV detection rates from chicken and environmental samples collected at retail markets during December 2015–July 2018 in Guangzhou, Guangdong, China

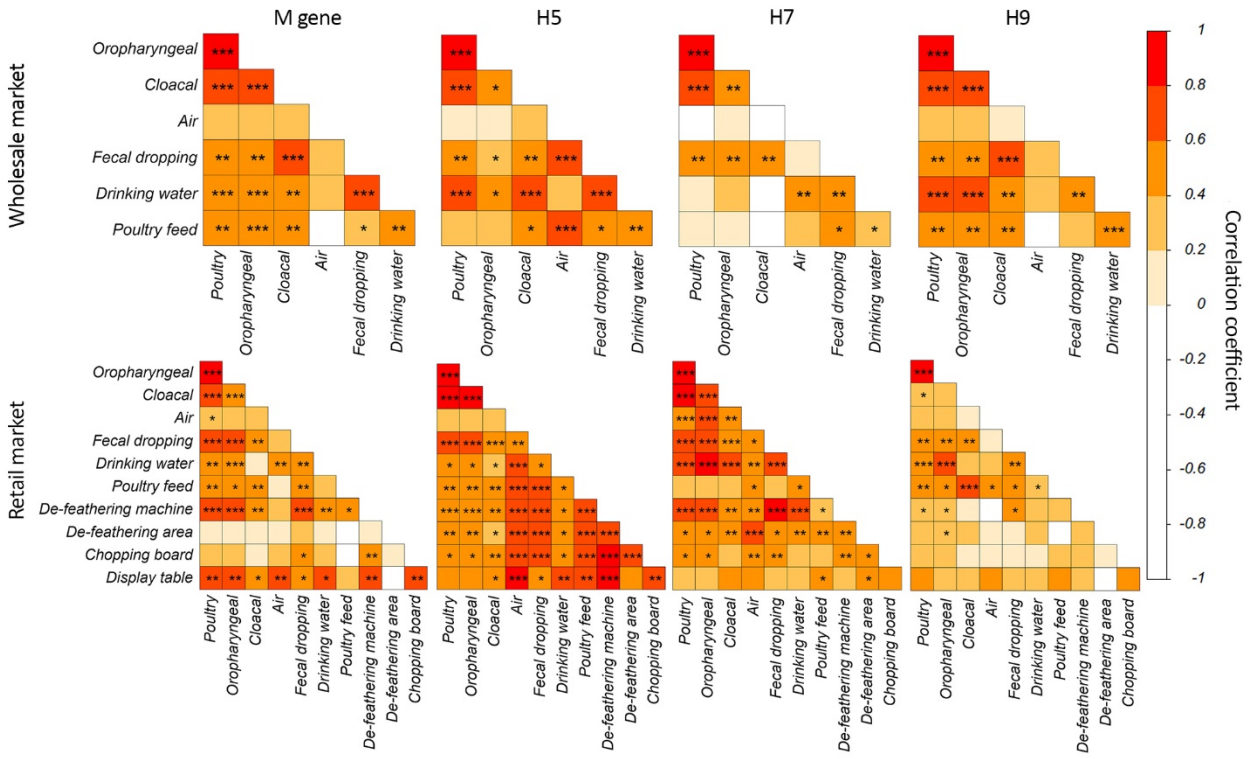
		Monthly positive rate (%)									
Year	Month	Oropharyngeal	Cloacal	Fecal droppings	Drinking water	Poultry feed	Defeathering machine	Defeathering area	Chopping board	Display table	Air
						H5 virus					
2015	Dec	32	42	32	0	100	33	0	100	50	0
2016	Jan	50	33	54	83	100	100	50	*	*	50
	Feb	0	0	0	0	0	0	0	*	*	0
	Mar	0	0	8	0	*	40	50	*	*	0
	Apr	3	3	0	31	*	0	*	*	*	0
	May	5	5	8	0	0	50	*	*	*	0
	Jun	3	0	0	0	0	0	0	0	*	0
	Jul	0	0	0	0	0	0	0	0	*	0
	Aug	0	0	0	0	0	0	0	*	*	0
	Sep	0	0	0	0	0	0	22	*	*	0
	Oct	0	0	0	0	0	0	0	*	*	0
	Nov	0	0	8	9	0	11	0	100	*	25
	Dec	0	0	0	0	33	13	7	50	*	25
2017	Jan	0	0	0	10	0	20	14	43	100	50
	Feb	0	0	0	0	0	0	0	0	0	0
	Mar	0	0	0	0	0	0	0	0	0	0
	Apr	0	0	0	0	0	0	0	0	0	0
	May	0	0	0	0	0	0	0	0	0	0
	Jun	0	0	0	0	0	0	0	0	*	0
	Jul	0	0	0	15	0	0	0	0	*	0
	Aug	0	0	0	0	0	0	0	29	*	0
	Sep	0	0	0	0	0	0	0	0	0	0
	Oct	3	0	9	0	0	25	25	20	*	0
	Nov	0	0	0	0	0	0	0	38	0	0
	Dec	0	0	0	0	0	10	50	38	0	0
2018	Jan	3	3	8	0	0	0	25	43	0	0
	Feb	0	0	0	0	11	13	25	43	0	0
	Mar	0	0	0	17	0	0	0	0	0	0
	Apr	5	0	25	8	22	30	100	67	0	25
	May	0	0	8	8	44	63	100	100	67	50
	Jun	23	3	8	33	42	83	100	88	100	50
	Jul	15	3	17	43	30	83	80	100	100	75
						H7 virus					
2015	Dec	0	0	0	0	0	0	0	0	0	0
2016	Jan	18	20	25	17	0	50	0	*	*	0
	Feb	13	7	9	44	0	33	43	*	*	50
	Mar	32	15	46	50	*	40	0	*	*	0
	Apr	15	10	24	15	*	0	*	*	*	0
	May	17	12	15	33	50	25	*	*	*	17
	Jun	0	0	0	0	0	0	0	0	*	0
	Jul	8	0	0	7	0	0	0	0	*	0
	Aug	3	0	0	13	0	0	0	*	*	0
	Sep	8	0	0	0	0	0	0	*	*	0
	Oct	10	0	11	0	0	17	0	*	*	25
	Nov	0	0	0	0	0	0	0	0	*	0
	Dec	18	0	11	40	33	53	36	50	*	25
2017	Jan	23	10	23	50	0	20	36	29	50	75
	Feb	17	20	0	33	33	0	20	100	100	100
	Mar	0	0	0	0	0	0	0	25	0	0
	Apr	0	0	17	0	33	39	33	29	50	0
	May	20	3	40	46	0	70	56	50	0	50
	Jun	11	5	0	8	10	14	40	0	*	50
	Jul	15	8	17	46	14	30	50	0	*	25
	Aug	3	0	0	8	0	0	13	0	*	0
	Sep	0	0	0	9	9	0	0	0	0	0
	Oct	0	0	0	0	0	0	0	0	*	0
	Nov	10	3	0	8	0	20	0	13	0	0
	Dec	3	0	0	0	0	0	0	0	0	0
2018	Jan	0	0	0	0	0	0	13	29	0	0
	Feb	7	0	0	0	0	0	0	0	0	0
	Mar	0	0	0	0	0	0	0	0	0	0
	Apr	5	0	8	0	0	10	0	17	0	50
	May	0	3	0	0	0	0	14	0	0	0
	Jun	0	0	0	0	0	0	0	0	0	0

Year	Month	Monthly positive rate (%)									
		Oropharyngeal	Cloacal	Fecal droppings	Drinking water	Poultry feed	Defeathering machine	Defeathering area	Chopping board	Display table	Air
	Jul	3	0	0	0	0	0	0	0	14	0
						H9 virus					
2015	Dec	55	45	65	33	100	67	0	100	100	100
2016	Jan	58	43	68	100	100	50	50	*	*	100
	Feb	20	13	27	33	100	67	14	*	*	100
	Mar	59	27	77	100	*	80	75	*	*	100
	Apr	46	41	77	85	*	100	*	*	*	50
	May	73	22	62	93	50	50	*	*	*	33
	Jun	93	35	50	100	100	100	100	100	*	100
	Jul	83	23	53	100	80	60	100	33	*	100
	Aug	73	23	80	100	100	100	50	*	*	50
	Sep	70	18	50	63	50	100	22	*	*	25
	Oct	53	15	33	92	60	92	86	*	*	100
	Nov	65	23	92	73	50	78	64	0	*	100
	Dec	65	10	67	100	33	100	71	100	*	50
2017	Jan	43	8	39	90	0	87	41	29	100	50
	Feb	50	3	0	67	33	50	20	100	100	100
	Mar	31	8	27	36	0	58	60	38	0	75
	Apr	63	30	67	70	83	92	100	100	100	50
	May	60	3	60	73	33	100	56	100	50	75
	Jun	90	13	92	100	70	86	80	100	*	100
	Jul	43	13	17	62	14	60	50	100	*	50
	Aug	68	8	50	83	40	89	88	100	*	100
	Sep	63	15	55	100	91	100	25	88	100	75
	Oct	88	15	82	92	67	100	100	90	*	100
	Nov	63	20	33	75	40	70	100	75	0	50
	Dec	45	5	33	58	33	70	88	100	0	50
2018	Jan	60	25	75	100	70	100	75	71	33	50
	Feb	40	30	50	46	33	75	100	57	25	50
	Mar	50	0	46	56	29	43	33	57	50	25
	Apr	60	38	58	75	56	100	25	100	100	75
	May	53	20	75	67	33	100	100	100	100	75
	Jun	80	33	58	83	83	100	100	100	100	75
	Jul	88	38	58	100	50	83	80	100	86	100

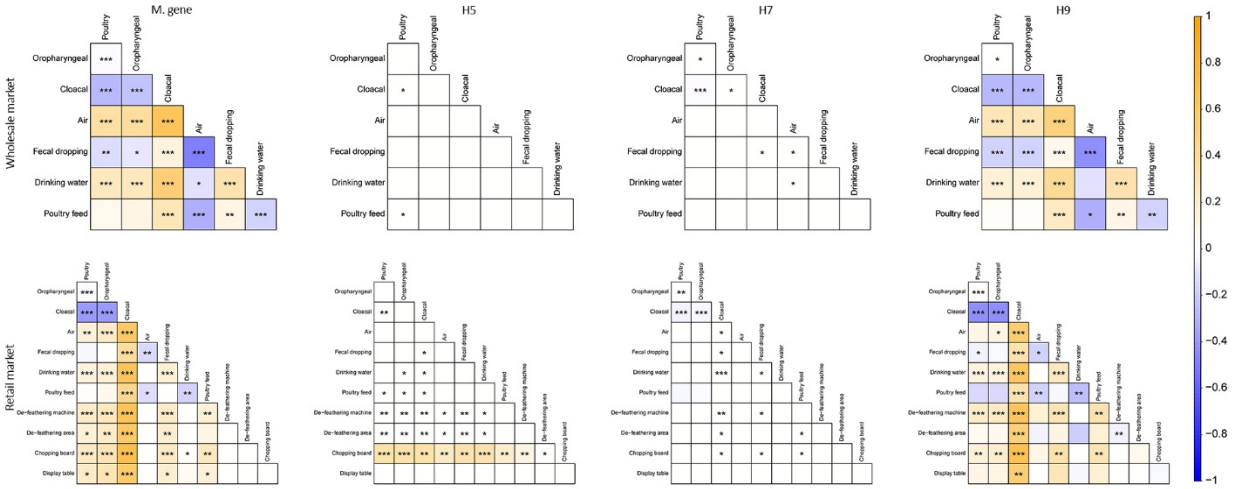
*No samples were collected.



Appendix Figure 1. Number of poultry swabs, environmental swabs, and air samples collected monthly at the wholesale and retail live poultry markets, Guangzhou, China, December 2015–July 2018.



Appendix Figure 2. Correlation matrices for monthly AIV detection frequencies across different sample types. Gradient of shades from light orange to red indicate increasing positive correlation coefficient (r_s) by Spearman's rank correlation. The significance level is indicated as follows:



Appendix Figure 3. Differences monthly AIV detection rates across different sample types. Gradient of shades from light orange to red indicate increasing median of monthly difference (detection rates of the sample type shown by the row labels minus that shown by the column labels). The significance level is indicated as follows: *0.01≤p<0.05; **0.001≤p<0.01; ***p<0.001.