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Live poultry market workers are susceptible to both avian and swine influenza viruses, Guangdong Province, China

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ABSTRACT

Guangdong Province is recognized for dense populations of humans, pigs, poultry and pets. In order to evaluate the threat of viral infection faced by those working with animals, a cross-sectional, sero-epidemiological study was conducted in Guangdong between December 2013 and January 2014. Individuals working with swine, at poultry farms, or live poultry markets (LPM), and veterinarians, and controls not exposed to animals were enrolled in this study and 11 (4 human, 3 swine, 3 avian, and 1 canine) influenza A viruses were used in hemagglutination inhibition (HI) assays (7 strains) and the cross-reactivity test (9 strains) in which 5 strains were used in both tests. Univariate analysis was performed to identify which variables were significantly associated with seropositivity. Odds ratios (OR) revealed that swine workers had a significantly higher risk of elevated antibodies against A/swine/Guangdong/L6/2009(H1N1), a classical swine virus, and A/swine/Guangdong/SS1/2012 (H1N1), a Eurasian avian-like swine virus than non-exposed controls. Poultry farm workers were at a higher risk of infection with avian influenza H7N9 and H9N2. LPM workers were at a higher risk of infection with 3 subtypes of avian influenza, H5N1, H7N9, and H9N2. Interestingly, the OR also indicated that LPM workers were at risk of H1N1 swine influenza virus infection, perhaps due to the presence of pigs in the LPM. While partial confounding by cross-reactive antibodies against human viruses or vaccines cannot be ruled out, our data suggests that animal exposed people as are more likely to have antibodies against animal influenza viruses.

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1. Introduction

As an international commercial hub, Guangdong Province is home to some of the world's largest populations of humans, pigs, and poultry. Located in southern China, this province has been the site of a number of outbreaks of novel human infections, including Severe Acute Respiratory Syndrome (SARS) and highly pathogenic avian H5N1 influenza A virus, leading some scientists to refer to this region as an epicenter of pandemic influenza viruses (Shortridge et al., 2003). In recent years, a variety of novel avian and swine influenza viruses have been detected in poultry and pigs in Guangdong (Kong et al., 2011a; Su et al., 2012b,c). An avian-like H7N9 influenza strain, first detected in March 2013 (Gao et al., 2013), has silently spread among poultry flocks in at least ten of China's provinces and causes rapidly progressive lower respiratory tract infections in humans. As of January 19, 2015 this novel reassortant avian influenza A virus H7N9 had affected 500 patients with a case-fatality rate of over 30% (as reported by the National Health and Family Planning Commission of the People's Republic of China).

While the general population is exposed to novel zoonoses, individuals with occupational exposure to animals are at a higher

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risk of infection from zoonotic influenza viruses. Swine influenza H1N1 has been reported to infect pig farm workers, and Avian influenza H5N1 and H7N9 which have caused fatal infections (Center for Infectious Disease Research and Policy, University of Minnesota, 2005; Gray et al., 2007; Myers et al., 2006; Yu et al., 2013). This cross-sectional study was designed to examine the risk of zoonotic influenza virus infection in animal workers in Guangdong Province.

2. Materials and methods

2.1. Study population

This study's protocol was reviewed and approved by the Institutional Review Board at the Guangdong Center for Disease Control and Prevention. Between December 2013 and January 2014, a total of 546 animal-exposed participants were enrolled, including four animal-exposed groups: 171 swine workers, 150 poultry farm workers, 105 live poultry market (LPM) workers, and 120 veterinarians of companion animal clinics. Contact information was provided by the Bureau of Animal Husbandry and Veterinary of Guangdong. Initial contact was made by telephone; if contacts were interested in participating, a study director met with them to explain the project's objectives and procedures, and answer any questions. Participants completed a standard questionnaire that captured data regarding general health status and history of occupational exposure to animals. Occupational exposure was defined as working at more than 5 h per day in close proximity (less than 1 m) to pigs, poultry, or dogs. Only individuals with animal exposure for more than 3 months (90 days) were included in the exposure groups. 264 volunteers with no occupational animal exposure, no house pets were selected from healthy individuals visiting the Third Affiliated Hospital of Sun Yat-Sen University in Guangdong for a physical examination during the same time period in which exposed individuals were enrolled were chosen as control group. All participants never got flu vaccine and provided written informed consent.

Sera were collected by medical professionals from the Center for Disease Control and Prevention of Guangdong Province or from the Third Affiliated Hospital of Sun Yat-Sen University during December 2013 through January 2014.

2.2. Influenza A strains

A total of eleven influenza A strains originating from poultry, pigs, humans and dogs were used in the hemagglutination inhibition serological assays and the cross-reactivity test (Table 1). Among the avian viruses, A/duck/Anhui/1/2006(H5N1) (AV/H5N1) belongs to the 2.3.4 clade which circulated in mainland of China in recent years (Jiang et al., 2010; Li et al., 2010; Smith et al., 2009b). A/Chicken/Shanghai/10/2001(H9N2) (AV/H9N2), A/Chicken/Beijing/1/1994-like strain is one of the most prevalent influenza viruses circulating among poultry in southern China today (Choi et al., 2004; Chu et al., 2011; Xu et al., 2007; Zhou et al., 2015). The genome of A/pigeon/Shanghai/S1421/2013(H7N9) (AV/H7N9) is similar to the virus which caused the fatal human infection in eastern China at the beginning of 2013, and into 2014 (Zhang et al., 2013). Among the swine viruses, A/swine/Guangdong/L6/2009 (H1N1) (CS/H1N1) is a wholly classic swine H1N1 virus, representative of the overall phylogenetic lineage of viruses that have circulated among pigs in south China at least 10 years (Chen et al., 2013; Kong et al., 2011b). A/swine/Guangdong/SS1/2012(H1N1) (EA/H1N1) is a Eurasian avian-like swine influenza A virus H1N1 swine virus representative of the viruses that have recently appeared among swine in south China (Chen et al., 2013). A/swine/Guangxi/13/2006(H1N2)(CS/H1N2) is a H1N2 swine influenza virus isolated from lung tissue of a pig in Guangxi province, China (Chen et al., 2008). Genes from A/Guangdong/1057/2010(H1N1) (Pdm09/H1N1) are very similar to the influenza A (H1N1)/pdm09 virus lineage which is representative of A/California/7/2009(H1N1) (data unpublished). A/New Caledonia/20/99(H1N1), an antigenic variant of Beijing/262/95, was first reported in New Caledonia in the Southern Pacific (Daum et al., 2002). A/Brisbane/10/2007(H3N2), another seasonal influenza H3N2 virus was first found in Brisbane. The canine virus, A/canine/Guangdong/2/2011 (H3N2) (CAN/H3N2) is a virus which is genetically similar to the avian influenza virus subtype H3N2 found recently circulating in dogs and cats in Guangdong province (Su et al., 2012a). All the avian influenza virus reference strains were inactivated and kindly supplied by the Harbin Veterinary Research Institute (Chinese Academy of Agricultural Sciences, Harbin, China) (as indicated in Table 1). The swine influenza virus reference strains (CS/H1N1, EA/H1N1), human (Pdm09/H1N1) influenza virus and canine (CAN/H3N2) influenza virus were isolated and kept by the College of Veterinary Medicine, South China Agricultural University (as indicated in Table 1).

Table 1
Viruses used in the hemagglutination inhibition serological assays and the cross-reactivity test.

Species	Virus names	Lineage of HA segment
Human viruses	A/Guangdong/1057/2010(H1N1)[c,d]	Influenza A(H1N1) pdm09 virus
	A/California/04/2009(H1N1)[a,e]	Influenza A(H1N1) pdm09 virus
	A/New Caledonia/20/99(H1N1)[a,e]	Seasonal influenza H1N1 virus
	A/Brisbane/10/2007(H3N2)[a,e]	Seasonal influenza H1N1 virus
Swine viruses	A/swine/Guangdong/L6/2009(H1N1)[c,d]	Classic swine influenza H1N1
	A/swine/Guangdong/SS1/2012(H1N1)[c,d,e]	Eurasian avian-like swine influenza H1N1
	A/swine/Guangxi/13/2006(H1N2)[a,e]	Classic swine influenza H1N1
Avian viruses	A/duck/Anhui/1/2006(H5N1)[b,d,e]	Highly pathogenic avian influenza H5N1 2.3.4 clade
	A/chicken/Shanghai/10/2001(H9N2)[b,d,e]	A/Chicken/Beijing/1/1994-like lineage
	A/pigeon/Shanghai/S1421/2013(H7N9)[b,d,e]	
Canine virus	A/canine/Guangdong/02/2011(H3N2)[c,d,e]	Avian-like canine H3N2 virus

[a] Human cell-derived influenza A virus hemagglutination proteins extracellular domain, supplied by Sino Biological Inc., China.
[b] Inactivated avian influenza virus reference strains, supplied by the Harbin Veterinary Research Institute.
[c] Virus strains, isolated and kept by the College of Veterinary Medicine, South China Agricultural University.
[d] Used as a referrence strains in the HI assay.
[e] Used to immunize the rabbits in order to generate the reference serums for the cross-reactivity test.

Human cell-derived influenza A virus hemagglutination proteins extracellular domain of three human influenza viruses A/California/04/2009(H1N1), A/New Caledonia/20/99(H1N1), A/Brisbane/10/2007(H3N2) and swine influenza virus A/swine/Guangxi/13/2006(H1N2) were supplied by Sino Biological Inc., China (as indicated in Table 1).

2.3. Hemagglutination inhibition (HI) assay

Sera were tested for the presence of antibodies using a hemagglutination inhibition (HI) assay as previously described (Hassantoufighi et al., 2010). Horse red blood cells (supplied by Guangzhou Ruite Bio-Tec Co., Ltd.) were used to test the antibodies against avian influenza virus as described (Kayali et al., 2008). Seven full viruses, included A/Guangdong/1057/2010(H1N1), A/swine/Guangdong/L6/2009(H1N1), A/swine/Guangdong/SS1/2012 (H1N1), A/duck/Anhui/1/2006(H5N1), A/chicken/Shanghai/10/2001(H9N2), A/pigeon/Shanghai/S1421/2013(H7N9) and A/canine/Guangdong/2/2011(H3N2) (all three avian influenza viruses were inactived) mentioned in above sections were enrolled as the reference strains for HI assay. Collected sera were treated with a 1:5 (vol:vol) solution of receptor destroying enzyme (RDE) at 37 °C for 18 h, followed by incubation at 56 °C for 30 min to remove non-specific inhibitors. Serum samples were titrated in 2-fold dilutions in phosphate buffered saline (PBS) (PH 7.4), and tested at an initial dilution of 1:10. In this study, HI titers ≥1:40 were considered positive.

2.4. Cross-reactivity test

To examine potential confounding through cross-reactivity, HI titers from control antisera were determined against the reference virus strains. The tests were performed with a panel of rabbit antisera directed against 3 human (A/New Caledonia/20/99 (H1N1), A/Califonia/04/2009(H1N1), A/Brisbane/10/2007(H3N2)), 2 swine (A/swine/Guangxi/13/2006(H1N2), A/swine/Guangdong/SS1/2012 (H1N1)) 3 avian (A/chicken/Shanghai/10/2001(H9N2), A/duck/Anhui/1/2006(H5N1), A/pigeon/Shanghai/S1421/2013 (H7N9)) and 1 canine (A/canine/Guangdong/02/2011(H3N2)) influenza viruses (as indicated in Table 1).

2.5. Statistical methods

Univariate analysis was performed to identify which variables were significantly associated with seropositivity. The magnitude of association between the variables and seropositivity is expressed as an odds ratio (OR) with 95% confidence intervals (95% CI). Statistical significance was set at $P < 0.01$. Analyses were performed using SPSS software version 17.0 (IBM, USA).

3. Results

3.1. Study participant characteristics

Between December 2013 and January 2014, a total of 810 participants were enrolled in the study. Exposed participants included 171 swine workers from three production farms, 150 poultry farm workers from 4 poultry farms, 120 veterinarians from 20 companion animal clinics, and 105 LPM workers were enrolled from two LPMs. Each LPM contains hundreds of individual stores, which each sell more than 100 birds (chicken, ducks, and geese) and/or 20 pigs per day. We selected stores which sold both poultry and pigs. A total of 264 non-animal workers (controls) were enrolled during physical examination at the Third Affiliated Hospital of Sun Yat-Sen University in Guangdong.

Participants were grouped based on age (≤39 years and >39 years), gender, and occupation. On average, swine farm workers, LPM workers, and veterinarians were older than control subjects, while poultry farm workers were younger ($P < 0.01$). Swine workers and poultry workers contained more males than females ($P < 0.01$) (Table 2). All of the participants denied previously receiving influenza vaccines.

3.2. Age and animal exposure were associated with elevated antibody titers against some influenza A strains

Of all participants (animal exposed and non-animal exposed controls) >39 years of age, 17% had positive antibody titer against CS/H1, 8.4% against EA/H1, 5.7% against AV/H7, and 8.9% against AV/H9. These rates of positive antibody titer were significantly higher than the rates in individuals ≤39 years of age (Table 3). 17% of males and 8.9% of females had positive antibody titers against CS/H1; while only 7.4% of males and 2.8% of females had positive antibody titers against EA/H1 (Table 3). Age, gender, and animal contact were not associated with elevated antibody titers against pdm09/H1 or CAN/H3.

Participants >39 years of age had higher odds of antibody titer against CS/H1 (OR = 1.69, 95% CI = 1.1–2.5), EA/H1 (OR = 3.0, 95% CI = 1.5–5.9), AV/H7 (OR = 2.7, 95% CI = 1.2–5.8), and AV/H9 (OR = 2.0, 95% CI = 1.1–3.5) compared to those age 39 and under (Table 4). Males had significant higher odds of antibody titer against influenza A CS/H1 and EA/H1 strains (OR = 2.1, 95% CI = 1.3–3.3; OR = 2.77, 95% CI = 1.3–5.9) compared to females (Table 4). Participants with any animal exposure had significantly higher odds of antibody titer against 5 influenza A strains: CS/H1 (OR = 4.7, 95% CI = 2.5–8.7), EA/H1 (OR = 5.5, 95% CI = 1.9–15.5), AV/H5 (OR = 20.1, 95% CI = 1.2–333.7), AV/H7 (OR = 4.9, 95% CI = 1.5–16.2) and AV/H9 (OR = 5.2, 95% CI = 2.1–13.3) when compared to those with no animal contact (Table 4).

Table 2
Characteristics of exposed and non-exposed subjects upon enrollment, Guangdong, 2013.

Exposure variables	Total (%) $n = 810$	Swine workers[a] (%) $n = 171$	Poultry workers (%) $n = 150$	LPM workers (%) $n = 105$	Veterinarians[b] (%) $n = 120$	Non-animal workers, controls (%) $n = 264$
Age (years)						
≤39	406 (50.1)	51 (29.8)	120 (80.0)	21 (20.0)	40 (33.3)	174 (65.9)
>39	404 (49.9)	120 (70.1)	30 (20.0)	84 (80)	80 (66.7)	90 (34.1)
Mean age (years)	38.4	**43.2**	**32.4**	**44.9**	**40.6**	36.7[c]
Gender						
Female	308 (38.0)	57 (33.3)[c]	39 (26.0)[c]	48 (45.7)	50 (41.7)	114 (43.2)
Male	502 (62.0)	**114 (66.7)**	**111 (74.0)**	57 (54.3)	70 (58.3)	150 (56.8)

Exposed participants indicated they worked in close contact with pigs, poultries or dogs for more than 8 h per day, 5 days a week.
Boldfaced values differ from reference group values in a statistically significant manner ($P < 0.01$).
[a] Participants indicated they lived and worked at the selected pig farms (4 days off each month).
[b] Participants indicated they belong to the pet clinics (we selected half of the staff), who contact with at least 5 dogs per day.
[c] Reference group.

Table 3
Antibodies positive rate of serum samples against reference influenza virus strains, Guangdong, 2013.

Groups		Postive no. (%, 95% CI)							
		Pdm09/H1N1	CS/H1N1	EA/H1N1	CAN/H3N2	AV/H5N1	AV/ H7N9	AV/ H9N2
Age	≤39 (406)[a]	226 (55.7%, 50.7–60.6%)	44 (10.8%, 8.0–14.3%)	12 (3.0%, 1.5–5.1%)	5 (1.2%, 0.4–2.9%)	4 (1.0%, 0.3–2.5%)	9 (2.2%, 1.0–4.2%)	19 (4.7%, 2.8–7.2%)
	>39 (404)	198 (49.0%, 44.0–54.0%)	**69 (17.1%, 13.5–21.1%)**	**34 (8.4%, 5.9–11.6%)**	10 (2.5%, 1.2–4.5%)	7 (1.7%, 0.7–3.5%)	**23 (5.7%, 3.6–8.4%)**	**36 (8.9%, 6.3–12.1%)**
Gender	Female (308)[a]	171 (55.4%, 49.7–61.0%)	27 (8.9%, 6.0–12.7%)	9 (2.8%, 1.3–5.3%)	8 (2.6%, 1.1–5.1%)	4 (1.3%, 0.4–3.3%)	12 (3.9%, 2.0–6.7%)	25 (8.2%, 5.4–11.8%)
	Male (502)	253 (50.4%, 45.9–54.9%)	**86 (17.1%, 13.9–20.7%)**	**37 (7.4%, 5.3–10.0%)**	7 (1.4%, 0.6–2.8%)	7 (1.4%, 0.6–2.8%)	20 (3.98%, 2.4–6.1%)	30 (5.9%, 4.0–8.3%)
Occupation	Swine farm (171)	93 (54.4%, 46.6–62%)	**57 (33.3%, 26.3–40.9%)**	**26 (15.2%, 10.2–21.5%)**	2 (1.17%, 0.2–4.5%)	1 (0.6%, 0.0–3.2%)	2 (1.2%, 0.1–4.2%)	6 (3.5%, 1.3–7.5%)
	Poultry farm (150)	78 (52.0%, 43.7–60.2%)	13 (8.5%, 4.6–14.2%)	4 (2.7%, 0.7–6.7%)	3 (2%, 0.3–5.4%)	3 (2%, 0.4–5.7%)	**7 (4.7%, 1.9–9.4%)**	**17 (11.3%, 6.7–17.5%)**
	LPM workers (105)	51 (48.6%, 38.7–58.6%)	**21 (19.8%, 12.7–28.7%)**	**8 (7.6%, 3.3–14.4%)**	3 (2.9%, 0.5–7.7%)	5 (4.8%, 1.6–10.8%)	**18 (17.1%, 10.5–25.7%)**	**24 (22.9%, 15.2–32.1%)**
	Veterinarian (120)	68 (56.7%, 47.3–65.7%)	10 (8.3%, 4.0–14.8%)	4 (3.3%, 0.9–8.3%)	4 (3.3%, 0.9–8.3%)	2 (1.7%, 0.2–5.9%)	2 (1.7%, 0.2–5.9%)	3 (2.5%, 0.5–7.1%)
	Non-animal (264)[a]	134 (50.8%, 44.6–57.0%)	12 (4.6%, 2.4–7.9%)	4 (1.5%, 0.4–3.8%)	3 (1.1%, 0.3–3.4%)	0 (0%, 0.0–1.4%)	3 (1.1%, 0.2–3.3%)	5 (1.9%, 0.6–4.4%)

Boldfaced values differ from control group values in a statistically significant manner ($P < 0.01$). Pdm09/H1N1, CS/H1N1, EA/H1N1, CAN/H3N2, AV/H9N2AV/H5N1 and AV/H7N9 stands for A/Guangdong/1057/2010(H1N1), A/swine/Guangdong/SS1/2012(H1N1), A/swine/Guangdong/L6/2009(H1N1), A/canine/Guangdong/2/2011(H3N2), A/chicken/Shanghai/10/2001(H9N2), A/duck/Anhui/1/2006(H5N1) and A/pigeon/Shanghai/S1421/2013(H7N9) respectively.
[a] Control group.

Occupational exposure was a statistically significant risk factor for elevated antibody titers against some influenza A strains. Swine workers had significantly higher odds of antibody titer against CS/H1 (OR = 10.5, 95% CI = 5.4–20.3) and EA/H1 (OR = 11.7, 95% CI = 4.0–34.1). Poultry farm workers had significantly higher odds of antibody titer against AV/H7 (OR = 4.3, 95% CI = 1.1–16.7) and AV/H9 (OR = 6.6, 95% CI = 2.4–28.3). LPM workers had significantly higher odds of antibody titer against CS/H1 (OR = 5.3, 95% CI = 2.5–11.1), EA/H1 (OR = 5.4, 95% CI = 1.6–18.2), AV/H5 (OR = 20.9, 95% CI = 1.1–399.1), AV/H7 (OR = 18.0, 95% CI = 5.2–62.6) and AV/H9 (OR = 15.4, 95% CI = 5.7–41.5). Veterinarians had no increased odds of elevated antibody titers against zoonotic influenza viruses. No exposed groups had significant odds for elevated antibody titer against pdm09/H1 or CAN/H3, when compared to controls.

Only swine workers had significantly higher antibodies against CS/H1 and EA/H1 (OR = 10.5, 95% CI = 5.4–20.3; OR = 11.7, 95% CI = 4.0–34.1, respectively) compared to non-animal workers (Table 3).

Poultry farm and LPM workers had higher antibodies positive rate against AV/H7 and AV/H9 (OR = 4.3, 95% CI = 1.1–16.7; OR = 6.6, 95% CI = 2.4–28.3, respectively) compared to non-animal workers. Though, the number of the positive samples against AV/H5 in poultry farm and LPM workers were slightly higher than other groups, no significant differences were observed. Interestingly, compared to the control group, LPM workers showed significantly higher antibody titer against three avian influenza virus strains: AV/H7 (OR = 18.0; 95% CI = 5.2–62.6), AV/H9 (OR = 15.4; 95% CI = 5.7–41.3), and AV/H5 (OR = 20.9; 95% CI = 1.1–399.1), and CS/H1 and EA/H1 swine influenza viruses (OR = 5.3, 95% CI = 2.5–11.1; OR = 5.4, 95% CI = 1.6–18.2, respectively). These results indicate that seroreactivity may be associated with daily contact with both poultry and pigs at the live markets.

3.3. Serologic cross-reactivity between human, swine, and avian viral strains

Serologic cross-reactivity between human, swine, and avian viral strains was assessed through cross-testing of reference antisera. None of the avian influenza strains showed cross-reactivity against the human, pigs and dogs strains, including H1 and H3 subtype influenza viruses. Human seasonal H1N1 antisera showed no cross-reactivity against pdm09/H1N1 and swine H1N1 viruses. Pdm09/H1N1 antisera showed cross-reactivity against Classical swine influenza H1N1 and Eurasian avian-like swine influenza virus, both with HI titers of 1:80. Human H3N2 antisera demonstrated little cross-reactivity to canine H3N2 virus, with HI titers of less than 1:10 (data unpublished).

4. Discussion

In this study, we aimed to demonstrate that the animal workers in Guangdong province were facing an increased risk of zoonotic influenza viruses infection compared to the general population. The overall seropositive rate and univariate analysis indicate that all participants >39 years had higher odds of elevated antibodies against two swine H1N1 influenza strains and two avian influenza virus subtypes AV/H7 and AV/H9.

Univariate analysis compared seroprevalence rates among occupations; and as expected, individuals with occupational exposures to animals had higher odds of antibody titers against respective species' influenza virus subtypes. However, antibody titers against CAN/H3N2 were found to not be significantly elevated among veterinarians with frequent dog contact. This could be due to the poor transmissibility of CAN/H3N2, in which the HA gene is thought to be derived from A/aquatic bird/Korea/JN-

Table 4
Odds ratios (ORs) for elevated sera antibody titers of four exposed groups against reference strains, determined by univariate analysis.

Group	OR (95% CI)[a]						
	Pdm09/H1N1	CS/H1N1	EA/H1N1	CAN/H3N2	AV/H5N1	AV/H7N9	AV/H9N2
Age							
>39 (404)	0.8 (0.6–1.0)	**1.7 (1.1–2.5)**	**3.0 (1.5–5.9)**	2.0 (0.7–6.0)	1.8 (0.5–6.1)	**2.7 (1.2–5.8)**	**2.0 (1.1–3.5)**
≤39 (406)	Ref						
Gender							
Male (508)	0.8 (0.6–1.1)	**2.1 (1.3–3.3)**	**2.8 (1.3–5.9)**	0.5 (0.2–1.5)	0.9 (0.3–3.2)	1.0 (0.5–2.0)	0.7 (0.4–1.2)
Female (302)	Ref						
Occupation risk							
Swine workers (171)	1.2 (0.8–1.7)	**10.5 (5.4–20.3)**	**11.7(4.0–34.1)**	1.0 (0.2–6.2)	3.1 (0.1–93.1)	1.0 (0.2–6.2)	1.9 (0.6–6.3)
Poultry farm workers (150)	1.1 (0.7–1.6)	2.0 (0.9–4.5)	1.8 (0.4–7.2)	1.8 (0.4–8.9)	10.8 (0.5–216.6)	**4.3 (1.1–16.7)**	**6.6 (2.4–28.3)**
LPM workers (105)	0.9 (0.6–1.4)	**5.3 (2.5–11.1)**	**5.4 (1.6–18.2)**	2.6 (0.5–12.9)	**20.9 (1.1–399.1)**	**18.0 (5.2–62.6)**	**15.4 (5.7–41.5)**
Vet (120)	1.3 (0.8–2.0)	1.9 (0.8–4.6)	2.2 (0.6–9.1)	3.0 (0.7–13.6)	9.0 (0.4–200.0)	1.5 (0.2–8.9)	1.3 (0.3–5.7)
General people (264)	Ref						
Animal exposure							
Any (546)	1.1 (0.8–1.5)	**4.7 (2.5–8.7)**	**5.5 (1.9–15.5)**	2.0 (0.6–7.4)	**20.1 (1.2–333.7)**	**4.9 (1.5–16.2)**	**5.2 (2.1–13.3)**
None (264)	Ref						
Pigs (171)	1.1 (0.8–1.6)	**5.2 (3.4–7.9)**	**5.6 (3.0–10.2)**	0.6 (0.1–2.6)	0.4 (0.1–3.1)	0.2 (0.1–1.0)	0.4 (0.2–1.0)
No pigs (639)	Ref						
Avian (255)	0.9 (0.7–1.2)	0.9 (0.6–1.4)	0.8 (0.4–1.5)	1.5 (0.5–4.2)	**4.5 (1.2–16.2)**	**8.5 (3.6–20.0)**	**7.4 (4.0–13.9)**
No avian espoused (555)	Ref						
House pets espoused (120)	1.2 (0.8–1.8)	0.5 (0.3–1.0)	0.5 (0.2–1.5)	2.1 (0.7–6.8)	1.4 (0.3–6.4)	0.4 (0.1–1.6)	0.3 (0.1–1.0)
No house pets (690)	Ref						

Values shown in boldface are statistically significant ($p<0.01$). Pdm09/H1N1, CS/H1N1, EA/H1N1, CAN/H3N2, AV/H5N1, AV/H7N9 and AV/H9N2, abbreviated for A/Guangdong/1057/2010(H1N1), A/canine/Guangdong/1/2012(H1N1), A/swine/Guangdong/L6/2009(H1N1), A/swine/Guangdong/L5/2010(H3N2), A/duck/Anhui/1/2006 (H5N1), A/pigeon/Shanghai/S1421/2013(H7N9) and A/chicken/Shanghai/10/2001(H9N2), respectively.

[a] Odds ratio and 95% confidence interval compared to the control group.

2 (Lin et al., 2012; Su et al., 2012a). Further research suggests that canine influenza viruses of avian origin are poorly transmission from dogs to humans (Song et al., 2008).

More participants were seropositive for H9 than the avian influenza virus H5 and H7, suggesting that H9N2 viruses infect humans more frequently than other avian influenza virus subtypes. Avian influenza subtype H7N9 is composed of six gene segments (PB1, PB2, PA, NP, M, and NS) similar to the H9N2 subtype, which is currently circulating in waterfowl in China (Gao et al., 2013). Given this information, the results of this study recommend continuing surveillance of avian influenza subtype H9N2, which has the potential to pose a serious public health threat, especially in the event of further reasstortment.

LPM workers commonly exposed to both poultry and pigs daily, with no use of personal protective equipment or annual influenza vaccines likely face a higher risk of infection with both avian and swine influenza viruses. The rate of seropositivity for CS/H1, EA/H1, AV/H7 and AV/H9 were all significantly higher than controls. Pigs have been known to serve as a "mixing vessel" of influenza viruses from multiple species. Reassortment of viruses from different species tends to occur in pigs, which go on to infect humans (Smith et al., 2009a); LPMs provide an optimal opportunity for reassortment and are suspected to have contributed to the emergence of avian influenza virus H7N9 (Pepin et al., 2013; Suarez et al., 1999; Wang et al., 2006).

This study had some limitations, such as the cross-sectional study design which did not allow monitoring of changes in antibody titer over time, or following influenza-like illness events. Secondly, all participants reported no receipt of human influenza vaccination. Including some vaccinated individuals would allow for additional statistical analyses. Additional serological tests would also strengthen the detection of antibodies to specific viruses. As we assay mainly detected antibodies against the hemagglutinin receptor, without assessing reactivity to neuraminidase, we cannot exclude the possibility that participants were seroreactive to other viruses with identical hemagglutinins. Also, wider inclusion of reference virus strains would expand our serological search for zoonotic influenza virus subtypes.

Our results support existing literature which identifies a need for improvement of LPM management. Previous studies have recommended the development of quarantine guidelines for Chinese poultry, and separation of species within the crowded conditions of LPMs (Martin et al., 2011; Tam, 2002). Frequent use of disinfectants within LPM and as delivery trucks enter and exit the facilities could reduce transmission of influenza viruses. Our results further underline the risk persons with occupational animal exposure are at, and we thus recommend expanding utilization of personal protective equipment and annual influenza vaccination in this population. To effectively reduce the threat zoonotic influenza viruses pose to humans, through either occupational or recreational exposure, continuous epidemiological monitoring of zoonotic influenza viruses in the population must be conducted.

Conflict of interest

None declared.

Ethical approval

This study's protocol was reviewed and approved by the Institutional Review Board at the Guangdong Center for Disease Control and Prevention.

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