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Seroepidemiological Evidence of Avian Influenza A Virus Transmission to Pigs in Southern China

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Recently, three novel avian-origin swine influenza viruses (SIVs) were first isolated from pigs in Guangdong Province, southern China, yet little is known about the seroprevalence of avian influenza among pigs in southern China. Here, we report for the first time the seroprevalence of avian H3, H4, and H6 influenza viruses in swine populations and the lack of seroepidemiological evidence of avian H5 influenza transmission to pigs in China.

***Keywords*: Seroepidemiological; avian; swine; influneza virus; sporadic**

Influenza A viruses are known to infect a wide variety of animals, including humans, pigs, horses, birds, and sea mammals. The primary reservoir of the influenza A virus is aquatic waterfowl, and birds are the source of all influenza viruses in other species (8). Pigs are susceptible to both human and avian influenza viruses and have been proposed to be intermediate hosts, or mixing vessels, for the generation of pandemic influenza viruses through reassortment or adaptation to the mammalian host (7). Experimentally, the pig is able to be infected by all of the avian H1–H13 subtypes studied (3). In nature, however, interspecies transmission of avian influenza viruses to pigs is not often documented (4). Recently, three novel avian-origin swine influenza viruses (SIVs) were first isolated from pigs in Guangdong Province, southern China (6, 5, 9). The appearance of avian influenza viruses (AIVs) among pigs poses concerns for both veterinary and human health, yet little is known about the seroprevalence of avian influenza among pigs in southern China.

To investigate the prevalence of AIVs infections in pigs in southern China, a total of 1,080 blood samples were collected from April 2010 to June 2012 from 20 swine farms distributed throughout Guangdong, Guangxi, Fujian, and Jiangxi Provinces, southern China(Figure 1). The 1,080 serum samples from 21-to-25-week-old growing-finishing pigs were chosen using a stratified random sampling method for farms and for pigs within farms. In addition, 550 serum samples were retrospectively analyzed from apparently healthy pigs in Guangdong Province in 2001. All animal researches were conducted under the guidance of CDC's Institutional Animal Care and Use Committee and in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited facility. Our animal research in this study has been approved by Guangdong Province Animal Disease Control Center. These serums were separated by centrifugation at 3,000 rpm for 15 min. The serum samples were transferred to new Eppendorf tubes and stored at −20°C until tested for antibodies against influenza A virus. All serum samples were treated with a receptor-destroying enzyme and absorbed with erythrocytes to remove nonspecific inhibitors before the assays. All samples were tested by hemagglutination inhibition (HI) and virus neutralization (VN) assays according to standard protocols (2). According to previous reports, the HI assay does not reliably detect antibodies to AIVs in mammalian sera because nonspecific hemagglutination inhibitors in the mammalian sera, even inactivated, can cause false-positives for AIVs (1). Therefore, in this study ,the VN test is carried out in parallel with the HI test by four viruses, A/Swine/Guangdong/L21/2011(H3N2), A/Swine/Guangdong/K4/2011(H4N8),

A/Swine/Guangdong/K6/2010 (H6N6) , and A/Chicken/Guangdong/178/04(H5N1). The following antigens were also used for HI testing: H1N1 influenza virus [A/Swine/Guangdong/L6/2009(H1N1)] for classical H1N1 SIV, H3N2 influenza virus [A/Swine/Guangdong/01/2005(H3N2)] for human-like H3N2 SIV. These influenza viruses were provided by the College of Veterinary Medicine, South China Agricultural University. HI titer ≥ 40 or VN titer ≥ 40 are considered seropositive and indicate previous infection.

A total of 1,630 serum (include 550 serum samples were collected in 2001) were examined by VN and HI for AIVs antibodies . Results of the VN and HI tests are reported in Table 1. The serum samples collected in 2001 had no HI or VN antibodies against any of these AIVs. In addition, 35.2% (380/1,080) of the serum samples were HI positive for A/Swine/Guangdong/L6/2009(H1N1), and 19.7% (213/1,080) of the serum samples were HI positive for A/Swine/Guangdong/01/2005(H3N2).

In the present study, antibodies against H3, H4, and H6 AIVs were detected in the serum samples collected from pigs in southern China. None of the samples tested showed seropositivity against the avian H5 virus according to the HI and VN tests, suggesting that they have been sporadically infected with H3, H4, and H6 AIVs. H3N2, H4N8, and H6N6 viruses were recently isolated from pigs in China and the viral genes were derived from wholly AIVs of the Asia lineage (6, 5, 9). Therefore, we report for the first time the seroprevalence of avian H3, H4, and H6 influenza viruses in swine populations and the lack of seroepidemiological evidence of avian H5 influenza transmission to pigs in China. All the four provinces of southern China

participating in the investigation have now been confirmed with seropositivity against avian-origin SIVs in pigs, indicating that the avian-origin SIVs have already been prevailing among the swine population in southern China, not only in Guangdong Province. The number of commercial pig farms is certainly increasing in southern China, and most of these are large-scale swine farms. Thus, humans and pigs are in close proximity in farming villages, providing the opportunity for the interspecies transmission of influenza viruses. Given the evidence that pigs can sustain reassortment of human and avian influenza viruses, it is important to be cautious and to enhance surveillance for atypical influenza viruses in pigs as part of our overall pandemic preparedness plans, and that we consider the potential for avian-like SIVs, or the novel reassortant viruses, to enter the human population.

Conflict of Interest

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Table 1. Seroprevalence of avian-origin H3N2 SIV ，avian-origin H4N8 SIV， avian-origin H6N6 SIV and H5N1 AIV in swine farms in different provinces, southern China.

Province	H3N2			H4N8			H5N1			H6N6		
	No. examined	Seroprevalence (%)		No. examined	Seroprevalence (%)		No. examined	Seroprevalence (%)		No. examined	Seroprevalence (%)	
		VN[a]	HI[a]		VN[a]	HI[a]		VN[a]	HI[a]		VN[a]	HI[a]
Guangdong	300	2%(6/300)	1.0%(3/300)	300	2.3%(7/300)	0.7%(2/300)	300	0%(0/300)	0%(0/300)	300	2.7%(8/300)	0%(0/300)
Guangxi	280	0%(0/280)	0%(0/280)[c]	280	2.9%(8/280)	0.4%(1/280)	280	0%(0/280)	0%(0/280)	280	1.4%(4/280)	0%(0/280)
Fujian	200	2%(4/200)	0%(0/200)	200	0%(0/200)	0%(0/200)	200	0%(0/200)	0%(0/200)	200	2.5%(5/200)	0%(0/200)
Jiangxi	300	0%(0/300)	0%(0/300)	300	0.7%(2/300)	0.7%(2/300)	300	0%(0/300)	0%(0/300)	300	0.7%(2/300)	0%(0/300)
GD2001[b]	550	0%(0/550)	0%(0/550)	550	0%(0/550)	0%(0/550)	550	0%(0/550)	0%(0/550)	550	0%(0/550)	0%(0/550)
Total[c]	1080	0.93% (10/1080)	0.27% (3/1080)	1080	1.6% (17/1080)	0.5% (5/1080)	1080	0% (0/1080)	0% (0/1080)	1080	1.8% (19/1080)	0% (0/1080)

a HI titer ≥ 40 or VN titer ≥ 40 are considered seropositve and indicate previous infection.

b 550 serum samples were collected from apparently healthy pigs in Guangdong Province in 2001.

c The total does not include the 550 serum samples were collected in 2001

Fig. 1. Survey sites in southern China where the sero-epidemiological study was conducted.GX, GD, FJ, JX are short for the provinces Guangxi, Guangdong, Fujian, Jiangxi respectively.