立法會CB(2)1365/16-17(04)號文件
LC Paper No. CB(2)1365/16-17(04)

我係李乾新，人稱白鴿佬
就住呢份「香港活家禽業未來路向的顧問研究」所做出嘅建議，本人有以下嘅睇法。

我由 1984 年就已經喺洪水橋開鴿場，喺上世紀嘅九十年代已經將鴿場搬咗去中山石岐，家陣嗰鴿場喺珠海嘅高新區，佔地超過六十亩，喺主要供港注冊鴿場。由最初嘅每日一車到最近嘅隔日一車由珠海將啲毛鴿運到長沙灣家禽批發市場，直至 2017 年 2 月 16 日，在毫無預警下國家質檢總局突然發出暫停活鴿供港，致使我嘅供港注冊鴿場至今都無法輸入活鴿到香港。令到我在香港嘅所有經濟活動完全停止。

本人經營活鴿生意已三十幾年，基本上睇住呢個行業嘅高低起跌，從來都未試過咁嚴峻嘅時候。每個月伙記人工、供車、中港車嘅管理費加加埋埋嘅支出大概六萬蚊左右，我已經頂咗三個月，唔知仲可以捱到幾時。悽涼！

我每日自我反省查找不足，喺咪喺大陸防疫做得唔夠好呢？原來唔喺。

打鐵還需自身硬！喺呢廿幾三十年嚟，我哋喺内地嘅注冊養鴿場從未發生過禽流感疫情同埋亦從未驗到過違規嘅殘留物。由此證明，我哋喺内地所經營嘅注冊供港養殖場所出產嘅白鴿係安全、可靠、質優嘅食物。有咁嘅成績，全懶我哋嘅白鴿繁殖場用科學嘅養殖方法，喺珠海動物檢驗檢疫局專業、負責任嘅態度，嚴密嘅監督同埋呢方面嘅專家指導之下，我嘅白鴿繁殖場先有咁嘅成績。所以我非常讚同顧問報告所言：並無證據顯示從內地供港註冊農場進口活家禽的禽流感風險較本地活家禽為高。

高永文局長多次喺公開場合話過：白鴿嘅禽流感風險喺低嘅！我提供一份國外嘅微生學學家所做嘅研究報告，得出嘅結論都證明到白鴿嘅禽流感風險喺極低嘅。而且顧問報告亦建議無需禁止内地活家禽入口。

既然白鴿嘅含禽流感風險極低，國家質檢總局怎解突然暫停活鴿供港呢？好可惜，呢份顧問報告冇做研究。

本人在與業界代表赴廣州拜會廣東省動檢局，會議嘅結果喺冇結果，只因香港食衛局嘅官員與省局嘅官員沟通嘅唔融洽。我哋都有同珠海動檢局嗰邊商討過，所得出嘅結論都係一樣，等國家質檢總局通知。我認為問題喺係香港食衛局，因為食衛局啲官員對内地嘅制度存在極大嘅偏見，採取極不信任嘅態度，也不信任我哋呢班喺内地經營家禽養殖業嘅從業員。

由此可見，令到我啲白鴿唔能夠繼續由內地輸入香港嘅原因，喺唔關禽流感嘅事，完全喺因爲兩地政府嘅唔融洽，未能通力合作所致，累到我哋半生不死。

就算呢份顧問報告寫得幾天花龍鳳，遠景如何咁美好，香港人對新鮮家禽如何咁喜愛，對本人嚟講都毫無意義。因為香港本地已經冇白鴿繁殖場，我內地嘅所出產嘅活鴿又唔能够輸入本港，喺香港我想繼續經營新鮮白鴿，都係死路一條。

出現咁嘅問題已經唔係我哋呢啲平民百姓可以解決得到，已經上到政府同政府嘅層面，係政府行為。解鈴還須繫鈴人，本人强烈要求高永文局長協助我哋，盡快請求中央政府俾我哋啲活鴿繼續輸入香港，已解本人之困。

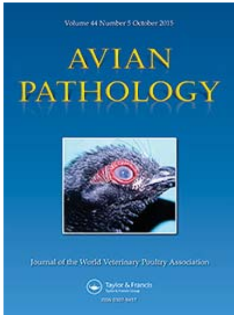
就以上嘅問題，本人提出以下嘅建議：

1. 香港政府和內地政府盡快達成科學、有效、可行的方案，使我們在內地的注冊養殖場可持續發展。
2. 如未有有效的方案，香港政府出家禽養殖牌，本人愿意將內地的養鴿業搬回香港。
3. 一刀切方案，禁止所有活家禽在市面上出售，政府提出恩恤辦法。
4. 全面開放，百花齊放。

香港人對新鮮美食的嚮往，就是我的奮鬥目標！

李乾新

二零一七年五月六日



Avian Pathology

ISSN: 0307-9457 (Print) 1465-3338 (Online) Journal homepage: http://www.tandfonline.com/loi/cavp20

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To cite this article: Yuehuan Liu, Zhiyuan Yang, Xiuqing Wang, Jiming Chen, Jiezhang Yao, Yanjun Song, Jian Lin, Chunhua Han, Huijuan Duan, Jicheng Zhao, Jie Pan & Jia Xie (2015) Pigeons are resistant to experimental infection with H7N9 avian influenza virus, Avian Pathology, 44:5, 342-346, DOI: 10.1080/03079457.2015.1055235

To link to this article: http://dx.doi.org/10.1080/03079457.2015.1055235

Published online: 06 Oct 2015.

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Avian Pathology, 2015
Vol. 44, No. 5, 342–346, http://dx.doi.org/10.1080/03079457.2015.1055235

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Pigeons are resistant to experimental infection with H7N9 avian influenza virus

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To determine the susceptibility of pigeons to the newly emerged avian influenza virus subtype H7N9, we experimentally infected three different types of pigeons (meat, town, and racing) with two different doses (2×10^4 or 2×10^5 EID_{50}) of H7N9 avian influenza virus A/Chicken/China/2013 by either intranasal and intraocular inoculation (IN + IO) or intravenous injection (IV). In addition, the potential transmission of H7N9 to pigeons by direct close contact with experimentally infected pigeons and chickens was assessed. Results showed that none of the experimentally infected pigeons exhibited any clinical signs regardless of the infection route and dose. Of the 12 racing pigeons that were randomly selected and necropsied, none of them had any gross lesions. In agreement with this finding, virus was not isolated from all pigeons. No detectable H7-specific antibodies were found in any pigeon. In contrast, 11 of 31 chickens that were either directly infected with H7N9 by IN + IO inoculation or by contact with IN + IO-infected chickens had conjunctivitis. Virus was isolated from all 31 chickens and H7-specific antibodies were detected in these chickens. However, none of the IV-infected chickens or chickens in direct contact with IV-infected chickens had any clinical signs. No virus was isolated from these chickens and no H7-specific antibody was detected. Overall, we conclude that pigeons are less or not susceptible to the H7N9 virus at the doses used and are not likely to serve as a reservoir for the virus. However, the virus does cause conjunctivitis in chickens and can transmit to susceptible hosts by direct contact.

Introduction

A novel H7N9 avian influenza virus A that is capable of causing a high fatality rate in humans was first isolated in China in 2013 (Shi *et al*., 2013). Further genetic analysis indicates that the novel H7N9 virus contains the haemagglutinin (HA) and neuraminidase (NA) gene segments of H7N9 avian influenza virus, while the remaining six gene segments came from H9N2 subtype avian influenza virus (Gao *et al*., 2013). Human infections with both low-pathogenic and high-pathogenic H7 subtypes of avian influenza viruses are generally limited to mild respiratory illness and conjunctivitis (Kalthoff *et al*., 2010). The isolation of the novel H7N9 subtype virus from pigeons suggests that pigeons may be either susceptible to infection or may carry and spread the virus (Shi *et al*., 2013). We have previously reported that the respiratory tract of pigeons mainly expresses the receptor for human influenza virus, sialic acids linked to galactose by an a-2,6 linkage (SAa2,6Gal), but not the avian influenza virus receptor, an a-2,3 linkage (SAα2,3 Gal) (Liu *et al*., 2009). Other studies have shown that pigeons are generally resistant to H7N7 and H7N1 subtype infection (Panigrahy *et al*., 1996). Two more recent studies have reported that pigeons are resistant to H7N9 infection, but virus was detected from oropharyngeal swabs at early time points after intranasal inoculation (Kalthoff *et al*., 2014; Pantin-Jackwood *et al*., 2014), suggesting low level of virus replication in pigeons. Abolnik reviewed many studies regarding the susceptibility of pigeons to avian influenza virus and concluded that minimal virus replication occurs in pigeons, but is not sufficient to transmit the virus to other susceptible hosts (Abolnik, 2014). Here, we used three different types of pigeon, two different doses of virus and two inoculation methods to further determine the susceptibility of pigeons and chickens to this novel H7N9 avian influenza virus.

Materials and Methods

Virus. Avian influenza virus A/Chicken/China/2013 (H7N9) was obtained from the China Animal Health and Epidemiology. Virus was propagated in 10-day-old specific-pathogen-free (SPF) chicken embryos and stored in −70°C. SPF chicken embryos were purchased from Beijing Experimental Animal Technology Inc. (Beijing, China). The median egg infectious dose (EID_{50}) of the virus was determined by using the Reed & Muench method (Reed & Muench, 1938).

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(*Received 14 October 2014; accepted 3 April 2015*)
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Birds. Forty-eight six-month-old pigeons (*Columba livia*) were provided by a Beijing racing pigeon farm. Twenty-one 6–24 month-old town pigeons were obtained from the Beijing Conference. Twenty-five six-month-old meat pigeons were provided by the YanQin Meat Pigeon farm. Eighty 4–5 weeks old SPF chickens were purchased from Beijing Experimental Animal Technology Inc. (Beijing, China). All birds were tested negative for H7- and H9-specific antibodies by the haemagglutination inhibition (HI) test and an ELISA specific for all subtypes of AIV (ID Screen® Influenza A Antibody Competition Multi-Species, IDVET) prior to infection.

Birds were randomly divided into different groups and used in the experiments shown in Table 1. Birds were housed in standard negative pressure isolators. Two different doses of H7N9 were inoculated into pigeons and chickens by intranasal and intraocular route (IN+IO) or by intravenous injection (IV). In some experiments, pigeons or chickens were housed together with either IN + IO or IV-infected chickens and pigeons at three days post infection to determine the transmission of the virus by close contact. Clinical signs were monitored and recorded daily. Oropharyngeal swabs were collected from all animals at three, four, and five days after inoculation for virus isolation. Cloacal swabs were taken at day four after infection and used for virus isolation. Twenty-four randomly selected chickens and pigeons from experiment 1 (Table 1) were necropsied at day four and five after infection and gross lesions were examined. Lungs and trachea tissues were harvested and used for virus isolation.

Animal protocols in this study were approved by the Beijing Animal Welfare Committee (SYXK, Beijing, 2012–0005).

Virus isolation. Nasal and oropharyngeal and cloacal swabs were placed in 1.3 ml of tryptose phosphate broth (TPB) buffer supplemented with penicillin (10,000 units/ml) and streptomycin (10,000 µg/ml). After centrifugation for 5 min at 4500g (Beckman Coulter Avanti J-26XP), the supernatant was collected and used for inoculation into SPF chicken embryos. Embryos were observed daily for viability. Allantoic fluid was collected from dead embryos and haemagglutination assays were performed to determine the HA titres as described previously (Yamada *et al*., 1985). HA titre equal or greater than 16 were considered as positive. HA-negative allantoic fluid samples were passed one more time in embryos to confirm the results. Influenza A virus M gene-specific reverse transcription polymerase chain reaction (RT-PCR) was used to further confirm the HA-positive virus isolation results.

RT-PCR. RT-PCR was performed as described previously (Starick *et al*., 2000). Briefly, total RNA was extracted from the HA-positive allantoic fluids. Primers specific for the membrane protein gene (M) were from the ShengGong Biotechnology Inc. (Shanghai, China). Forward and reverse primers are 5′ AGC GTA GAC GCT TTG TC 3′ and 5′ GAC GAT CAA GAA TCC AC 3′, respectively. RT-PCR was performed by using the TaKaRa RNA PCR kit (AMV) Ver 3.0. The PCR cycling condition was 35 cycles of 94°C 1 min, 53°C 1 min and 72°C 1 min, and 72°C 10 min for final extension after the initial denaturing at 94°C for 3 min. PCR products were detected by gel electrophoresis.

Serology. Sera collected from birds prior to virus infection and 21 days post infection were tested for the presence of H7-specific haemagglutination inhibition (HI) antibody response as described previously (Klopfleisch *et al*., 2006). Avian influenza H7 and H9 antigen, Newcastle disease virus antigen and their respective positive sera were provided by the Institute of Animal and Husbandry Medicine of Beijing Academy of Agriculture and Forestry Sciences. Four HA units of H7 antigen was used in the assay. A HI titre 1:16 was considered as positive. The same serum samples were also tested for the presence of antibody response using a commercially available H7 subtype-specific ELISA kit (ID Screen® Influenza H7 Antibody Competition, FLUAC H7, IDVET, France) by following the manufacturer's instructions.

Results

Clinical signs and gross lesions. None of the pigeons exhibited any clinical signs after either direct virus inoculation or housing together with experimentally infected SPF chickens. Of the 12 pigeons necropsied, none showed any gross lesions.

In contrast, five out of 10 SPF chickens showed transient conjunctivitis after close contact with SPF chickens that were inoculated with H7N9 by IO + IN route (experiment 1). Three out of the 10 SPF chickens that were infected with H7N9 by the IN + IO route had conjunctivitis (experiment 2). Three of the six SPF chickens developed conjunctivitis after close contact with SPF chickens that were experimentally infected with H7N9 by IN + IO route (experiment 2). None of the IV-inoculated SPF chickens or SPF chickens in close contact with IV-infected chickens showed any clinical signs.

Virus isolation. Regardless of inoculation route, all experimentally infected racing pigeons showed negative virus isolation results from oropharyngeal and cloacal swabs (Table 2). Virus was not isolated from lungs and tracheas of 12 pigeons that were necropsied. Similarly, no virus was isolated from town and meat pigeons regardless of the inoculation route (Table 2).

In contrast, virus was isolated from all SPF chickens that were infected with H7N9 by the IN + IO route (Table 2). In addition, all three SPF chickens from experiment 1 necropsied at 4 days after infection showed positive virus isolation from lungs and trachea. Two of the three SPF chickens necropsied at day 5 after infection showed positive virus isolation from lungs and trachea (Table 2).

For the SPF chickens that were infected with H7N9 by the IV route, only one of the 15 (experiment 1) and one of the eight (experiment 2) showed positive virus isolation (Table 2). Of the three chickens necropsied at days 4 and 5 after virus infection, no virus was isolated from the three birds necropsied at day 4 while one of the three chickens necropsied at day 5 showed positive virus isolation (Table 2).

All pigeons that were housed together with SPF chickens that were infected with H7N9 by the IN + IO route showed negative virus isolation results. While all the SPF chickens that were housed together with IN+IO-infected chickens showed positive virus isolation results (experiments 1 and 2, Table 2). None of the pigeons or SPF chickens that were housed together with infected pigeons had positive virus isolation results (experiments 1 and 2, Table 2).

Antibody response. All pigeons showed negative H7-specific antibody response at 21 days after infection as detected by both ELISA and HI regardless of the route of virus inoculation (Table 2). All the SPF chickens that were infected by the IN + IO route showed positive H7-specific antibody response as detected by both ELISA and/or HI assays. However, a variable antibody response was observed for SPF chickens that were infected by the IV route. In experiment 1, all nine chickens were negative for H7 antibody by ELISA and HI, but in experiment 2, two of the eight chickens had positive HI antibody response.

All pigeons or chickens that were housed together with infected pigeons showed negative antibody responses in experiment 1. However, all five chickens that were housed together with IN + IO-infected chickens showed positive HI antibody responses. Similarly, in experiment 2, all pigeons that were housed together with IN + IO-infected chickens showed negative HI antibody responses. All chickens that were housed together with IN + IO-infected chickens showed positive HI antibody responses. All SPF chickens that were housed with IN + IO-infected pigeons showed negative HI antibody responses (Table 2).

Table 1. Summary of bird experiments.

Experiment no.	Birds	Route of infection	Infection dose	Purpose of experiment
1	14 racing pigeons	IN+IO	0.2 ml (2×10^4 EID_{50})	Susceptibility of pigeons
	15 SPF chickens			Positive control
	14 racing pigeons	IV	0.2 ml (2×10^4 EID_{50})	Susceptibility of pigeons
	15 SPF chickens			Positive control
	10 racing pigeons[a]	Housed together with inoculated pigeons		Transmission by close contact
	10 SPF chickens			
	10 racing pigeons[a]	Housed together with infected chickens		Transmission by close contact
	10 SPF chickens			
2	8 town pigeons	IN + IO	0.2 ml (2×10^5 EID_{50})	Susceptibility of pigeons
	10 meat pigeons			Susceptibility of pigeons
	10 SPF chickens			Positive control
	8 town pigeons	IV	0.2 ml (2×10^5 EID_{50})	Susceptibility of pigeons
	10 meat pigeons			Susceptibility of pigeons
	8 SPF chickens			Positive control
	5 town pigeons	Housed together with IN + IO infected SPF chickens		Transmission by close contact
	5 meat pigeons			Transmission by close contact
	6 SPF chickens			Positive control
	6 SPF chickens	Housed together with IN + IO infected pigeons		Transmission by close contact

[a]Five racing pigeons were housed together with IN + IO infected chickens. Five racing pigeons were housed together with IV infected chickens. Five SPF chickens were housed together with IN+IO infected pigeons. Five SPF chickens were housed together with IN+IO infected chickens.

Discussion

Numerous previous studies have examined the susceptibility of pigeons to different subtypes of avian influenza virus and the role of pigeons in the transmission of avian influenza virus (Panigrahy *et al*., 1996; Abolnik, 2014). Although several reports described the isolation of avian influenza virus from pigeons or the detection of different subtypes of influenza virus by RT-PCR (Gronesova *et al*., 2009; Mansour *et al*., 2014), the majority of the studies showed that pigeons are generally resistant to avian influenza virus replication and are not likely to be a major reservoir and transmission host for avian influenza virus (Liu *et al*., 2007; Kohls *et al*., 2011; Smietanka *et al*., 2011; Yamamoto *et al*., 2012; Teske *et al*., 2013; Kalthoff *et al*., 2014; Pantin-Jackwood *et al*., 2014). Here, we investigated the susceptibility of pigeons to avian H7N9 subtype. Two different doses of H7N9, 2×10^4 EID_{50} and 2×10^5 EID_{50} were used to infect three different types of pigeons (racing, meat and town) by either the IN + IO route or by IV route. The IN + IO route mimics the natural infection in the field situation. Results showed that none of the pigeons exhibited any clinical signs. No gross lesions were observed in pigeons necropsied at days 4 and 5 after virus infection. Virus was not isolated from any of 64 experimentally infected pigeons or 30 close contact pigeons. No H7 subtype-specific antibody was detected in racing pigeons by using both ELISA and HI assays. HI antibody was negative for all town and meat pigeons. SPF chickens that were in close contact with infected pigeons showed no H7-specific antibody response and negative virus isolation results, suggesting that H7N9 did not replicate in pigeons. Our results are in agreement with the finding of an earlier study which showed that pigeons are resistant to experimental infection with highly pathogenic H7N7 influenza virus by IN + IO and IV routes (Panigrahy *et al*., 1996). Similarly, pigeons are also resistant to experimental infection with low-pathogenic H7N1 infection by IO + IN and IV routes (Panigrahy *et al*., 1996). Two recent studies have showed low levels of virus replication in pigeons after intranasal inoculation (Kalthoff *et al*., 2014; Pantin-Jackwood *et al*., 2014). In our study, we were not able to isolate virus from pigeons. We observed that three of 10 chickens infected with the higher dose of H7N9 (2×10^5 EID_{50}) by the IN + IO route showed conjunctivitis (experiment 2, Table 2), but none of 15 chickens in the lower dose groups (2×10^4 EID_{50}) showed any clinical signs (experiment 1, Table 2). Interestingly, chickens in close contact with IN + IO-infected chickens also exhibited conjunctivitis. No clinical signs in H7N9-infected chickens were reported in two recent studies (Kalthoff *et al*., 2014; Pantin-Jackwood *et al*., 2014). The discrepancy may be due to multiple factors such as the different virulence of the virus strains used, different inoculation methods, different age, genetics, and immune status of pigeons used in these studies. Nevertheless, all studies demonstrated that chickens, but not pigeons, can shed virus and serve as a reservoir for the virus. None of the IV-infected chickens or chickens in close contact with IV-infected chickens had any clinical signs. This indicates that chickens are susceptible to H7N9 during natural infection in a dose dependent manner. This is further confirmed by the positive virus isolation and H7-specific antibody response in these diseased chickens. No H9-specific or Newcastle disease virus-specific antibody responses were detected. There is a concern whether the use of IV infection to determine the virulence of low-pathogenic avian influenza virus H7N9 adopted by the World Organisation for Animal Health reflects the true pathogenicity of the virus. An outbreak of H7 subtype avian influenza virus occurred in Holland in 2003, which resulted in conjunctivitis in people handling chickens (Belser *et al*., 2009). In 2004, two human cases of H7N3 avian influenza virus infection exhibited conjunctivitis and mild respiratory illness (Tweed *et al*., 2004). It remains to be determined whether the virus has a tropism for eye epithelial cells, which may have the receptor for virus entry.

We compared the susceptibility of chickens to H7N9 using two different doses of infection via either IN + IO or IV inoculation route. Interestingly, 23 chickens receiving IV infection of either 2×10^4 EID_{50} or 2×10^5 EID_{50} H7N9 showed no clinical signs. Only two of the 23 chickens

Table 2. Virus isolation and antibody response in SPF chickens and pigeons infected with H7N9 avian influenza virus.

			Number of birds with positive virus isolation/total number of birds					Number of birds with H7 subtype specific antibody response /total number of birds			
								Pre-infection		21 days post infection	
Experiment no.	Birds	Route of infection	3d op swab	4d op swab	4d cloacal swab	5d op swab	total	HI	ELISA	HI	ELISA
1	14 racing pigeons	IN + IO	/	0/14	0/14	0/11	0/14	0/14	0/14	0/8	0/8
	15 SPF chickens		/	15/15	10/15	11/12	15/15	0/15	0/15	9/9	9/9
	14 racing pigeons	IV	/	0/14	0/14	0/11	0/14	0/14	0/14	0/8	0/8
	15 SPF chickens		/	1/15	1/15	0/12	1/15	0/15	0/15	0/9	0/9
	10 racing pigeons	housed together with IN+IO or IV infected racing pigeons	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	10 SPF chickens		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	10 racing pigeons	Housed together with infected SPF chickens	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	10 SPF chickens		5/10[a]	5/10[a]	5/10[a]	5/10[a]	5/10[a]	0/10	0/10	5/10[a]	5/10[a]
2	8 town pigeons	IN+IO	0/8	0/8	/	0/8	0/8	0/8	/	0/8	/
	10 meat pigeons		0/10	0/10	/	0/10	0/10	0/10	/	0/10	/
	10 SPF chickens		10/10	10/10	/	10/10	10/10	0/10	/	10/10	/
	8 town pigeons	IV	0/8	0/8	/	0/8	0/8	0/8	/	0/8	/
	10 meat pigeons		0/10	0/10	/	0/10	0/10	0/10	/	0/10	/
	8 SPF chickens		0/8	0/8	/	1/8	1/8	0/8	/	2/8	/
	5 town pigeons	Housed together with IN+IO infected chickens	0/5	0/5	0/5	/	0/5	0/5	/	0/5	/
	5 meat pigeons		0/5	0/5	0/5	/	0/5	0/5	/	0/5	/
	6 SPF chicken		6/6	6/6	6/6	/	6/6	6/6	/	6/6	/
	6 SPF chicken	Housed together with IN+IO infected meat and town pigeons	0/6	0/6	0/6	/	0/6	0/6	/	0/6	/

op, oropharyngeal.
[a]Five chickens positive for virus isolation and H7 specific antibody were housed together with IN+IO infected chickens. Five chickens negative for virus isolation and H7 antibody were housed together with IV infected chickens. Pigeons were housed with either IN+IO or IV-infected chickens. / indicates not tested.

had positive virus isolation. While all 25 chickens receiving IN+IO infection of either 2×10^4 EID_{50} or 2×10^5 EID_{50} H7N9 had positive virus isolation results. All IN+IO-infected chickens showed H7-specific HI antibody response. In contrast, no HI antibody was detected in IV low-dose group, but two of eight high-dose IV chickens developed H7-specific antibody response. Overall, the results suggest that the IN + IO route of infection is more efficient than IV infection in terms of virus isolation and antibody response.

The isolation of virus from the lungs and trachea tissues at 4 and 5 days after virus infection suggests that virus replication occurred in the respiratory tract of chickens. This result is consistent with the positive virus isolation from oropharyngeal swabs. In experiment 1, virus isolation from oropharyngeal and cloacal swabs was 100% and 66.7%, respectively, at day 4 after infection. In experiment 2, all chickens in close contact with IN+IO-infected chickens showed 100% virus isolation from both oropharyngeal and cloacal swabs at day 4 after infection. Overall, the results suggest that chickens are the reservoirs for the virus and can efficiently transmit H7N9 avian influenza virus by close contact, possibly through both aerosol and contaminated materials.

We conclude that pigeons used are less or not susceptible to the H7N9 virus at the doses used regardless of the route of infection. Furthermore, pigeons do not serve as a transmission host and reservoir for H7N9. The molecular mechanism by which pigeons are resistant to H7N9 will be investigated in future studies.

Acknowledgements

This work was supported by Beijing Municipal Bureau of Agriculture and Beijing Academy of Agriculture and Forestry Sciences cooperation project [Grant No. 2013006].

Disclosure statement

No potential conflict of interest was reported by the authors.

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