Limited Susceptibility of Chickens, Turkeys, and Mice to Pandemic (H1N1) 2009 Virus

Donata Kalthoff, Christian Grund,
Timm C. Harder, Elke Lange,
Thomas W. Vahlenkamp, Thomas C. Mettenleiter,
and Martin Beer

To determine susceptibility of chickens, turkeys, and mice to pandemic (H1N1) 2009 virus, we conducted contact exposure and inoculation experiments. We demonstrated that chickens were refractory to infection. However, oculo-oronasally inoculated turkeys and intranasally inoculated mice seroconverted without clinical signs of infection.

The current outbreak of pandemic (H1N1) 2009 continues to expand in humans, with occasional spillover into domestic pigs. Pandemic (H1N1) 2009 virus causes only mild disease compared with pandemic influenza viruses from the 20th century. However, this characteristic might change if pandemic (H1N1) 2009 viruses acquire virulence markers by reassorting with influenza viruses that cause severe disease in humans, such as highly pathogenic avian influenza viruses (HPAIVs) of the H5N1 subtype. Such reassortment might occur in humans but appears more likely in so-called mixing vessels. Pigs, which had been described as potential mixing vessels (1), are highly susceptible to infection with pandemic (H1N1) 2009 virus (2,3). In addition, pigs have been infected subclinically by HPAIV (H5N1) in countries to which HPAIV (H5N1) is endemic (4). However, whether poultry can be infected with pandemic (H1N1) 2009 virus is not well understood. Therefore, we determined the susceptibility of chickens, turkeys, and mice to pandemic (H1N1) 2009 virus.

The Study

All animal experiments were reviewed and approved by the responsible state ethics committee (LALLF M-V/TSD/7221.3–2.1.-014/09). Five chickens (12 weeks of age, specific pathogen–free [spf]) were inoculated oculo-oronasally by dripping a 10^6 50% tissue culture infectious dose

($TCID_{50}$) of virus A/Regensburg/D6/09/H1N1 on the cornea, nares, and oropharynx. Ten chickens (3 months of age, spf) were inoculated intravenously with 10^4 $TCID_{50}$. Five chickens (15 weeks of age, spf) were housed (permanent contact behind bars and daily direct contact for 30 min) with pigs infected with pandemic (H1N1) 2009 virus (3).

A second transmission experiment was performed with the same contact exposure experimental setup, which included 8 infected pigs and 5 turkeys (4 weeks of age). In addition, 28 chickens (1 week of age) and 28 turkeys (1 week of age) were inoculated with 200 µL of virus suspension (10^6 $TCID_{50}$) directly into the left air sac. Six fattening turkeys (16 weeks of age) from a local flock were inoculated oculo-oronasally with 10^6 $TCID_{50}$ of A/Regensburg/D6/09 (H1N1) virus. Six fattening turkeys were inoculated oculo-oronasally with 10^8 $TCID_{50}$ of the more recently isolated pandemic (H1N1) 2009 A/Bayern/74/2009 virus. Cloacal and oropharyngeal swab samples from poultry infected oculo-oronasally, through the air sac, or by contact with pigs were collected daily. Samples were tested by using a real-time reverse transcription–PCR with subtype H1N1–specific primers (http://offlu.net) (Table).

Additional studies were conducted to determine the 50% lethal dose of strain A/Regensburg/D6/2009 pandemic (H1N1) 2009 virus for mice. Sixteen BALB/c mice (6 weeks of age) were inoculated intranasally with 10^2–10^5 $TCID_{50}$/animal (30 µL), and 4 mice were inoculated intraperitoneally with 10^5 $TCID_{50}$/animal (30 µL).

None of the inoculated animals became ill after infection by any tested route; the intravenous pathogenicity index of the virus for chickens was 0. All swab samples from poultry after contact with infected pigs or from poultry inoculated through the air sac were negative for virus (Table). Virus excretion by pigs was detected (3); real-time reverse transcription–PCR cycle threshold values ≥26 (contact experiment with chickens) or ≥17 (contact experiment with turkeys). Virus RNA was detected (cycle threshold values 27–39) in swab samples obtained 1–6 days postinoculation from the oropharynx of turkeys inoculated with 10^8 $TCID_{50}$ of the A/Bayern/74/2009 (H1N1) strain. Small amounts of virus RNA also were detected in the lung and left air sac from a few chicks and poults early after infection through the air sac (Table), which most likely represent residual inoculum. Although contact exposure pigs were infected (3), seroconversion was not detected in any of the tested contact exposure poultry species. Poultry inoculated intravenously or through the air sac and chickens inoculated oculo-oronasally were negative for antibodies against influenza A virus nucleoprotein.

Fattening turkeys seroconverted after oculo-oronasal inoculation with 10^6 $TCID_{50}$ or 10^8 $TCID_{50}$ (Table). In addition, 7 of 8 mice inoculated with 10^4 $TCID_{50}$ or 10^5 $TCID_{50}$ by the oculo-oronasal route seroconverted; 4 mice inocu-

lated with 10^5 TCID$_{50}$ by the intraperitoneal route showed no detectable antibody response to the virus (Table).

Conclusions

Our results demonstrate lack of susceptibility of chickens and minor susceptibility of turkeys for infection by pandemic (H1N1) 2009 virus. Transmission of swine influenza viruses to poultry, particularly to turkeys, has been demonstrated (5), and experimental infection of chickens with virus subtype H3N2 resulted in a low replication rate, primarily in the alimentary tract (6). In contrast, our data indicate that pandemic (H1N1) 2009 virus cannot productively infect chickens at the ages of 1 week (air sac inoculation), 12 weeks (contact exposure, intravenous inoculation), or 15 weeks (oculo-oronasal inoculation), or turkeys at the ages of 1 week (air-sac inoculation) and 4 weeks (contact exposure) because neither virus excretion nor seroconversion was observed during the 10-day observation period. Fattening turkeys from a local flock seroconverted after oculo-oronasal inoculation, but virus RNA was detected in respiratory samples only in turkeys inoculated with a high dose of the A/Bayern/74/2009 (H1N1) virus (Table).

On the basis of our experiments, risk for transmission of pandemic (H1N1) 2009 virus strain to chicken and turkeys and subsequent possible reassortment with other avian influenza viruses should be low. However, we observed a slightly higher susceptibility of older turkeys to high doses of pandemic (H1N1) 2009 virus, which is consistent with recent reports of infected layer turkey flocks in Chile and Canada (7,8). Analysis of specific virus strains involved

in these outbreaks would be useful for confirming these observations and analyzing different strains. Host factors (age, physiologic state, stress levels, and concurrent infections) influencing susceptibility of turkeys should be investigated. Our data, which demonstrate absence of illness in poultry after inoculation with pandemic (H1N1) 2009 virus, are consistent with those of recent experimental infections of poultry, including turkeys (9–11). Seroconversion was detected only in turkeys by using a hemagglutination-inhibition test in 1 study (9), which might indicate higher sensitivity of competitive ELISAs used in our study.

As in other studies (12,13), characterization of pandemic (H1N1) 2009 virus strains in BALB/c mice showed differences in lethal dose and clinical signs dependent on the virus strain. None of the infected BALB/c mice in our study showed clinical signs. These findings may have resulted from the fact that mice were infected intranasally without anesthesia and with a dose <10^6 TCID$_{50}$/animal or because of a different phenotype of the virus strain used. However, replication competence of pandemic (H1N1) 2009 virus in mice without prior adaptation was indicated by seroconversion, at least for the higher infectious doses (Table). Intraperitoneal inoculation did not cause development of influenza virus–specific antibodies. This finding cannot be explained by receptor specificity of pandemic (H1N1) 2009 virus because Childs et al. (14) showed that representative pandemic (H1N1) 2009 viruses bound not only to most α2–6-linked sialyl moieties, irrespective of the backbone chain length and type, but also to a considerable range of α2–3-linked sialyl residues. Although virus rep-

Table. Susceptibility of chicken, turkeys, and mice to pandemic (H1N1) 2009 virus*

Animal and dose (virus strain)	No. animals	Route of inoculation	Swab samples	Tissue samples, no. positive/no. tested†	Seroconversion in NP-ELISA (no. positive/no. tested)‡
Chicken	5	Oculo-oronasal	–	NA	–
Chick	28	Intra–air sac	–	Day 2: 2/3; Day 4: 2/3; Day 8: 1/3	–
Chicken	10	Intravenous	NA	NA	–
Chicken	5	Contact with infected pigs	–	NA	–
Turkey, 10^6 TCID$_{50}$ (A/Regensburg/D6/2009)	6	Oculo-oronasal	–	NA	+ (3/6)
Turkey, 10^8 TCID$_{50}$ (A/Bayern/74/2009)	6	Oculo-oronasal	+ (respiratory), – (cloacal)	NA	+ (4/6)
Poult	28	Intra–air sac	–	Day 2: 3/3; Day 4: 1/3; Day 6: 1/3	–
Turkey	5	Contact with infected pigs	–	NA	–
Mouse, 10^2–10^3 TCID$_{50}$	8	Oculo-oronasal	NA	NA	–
Mouse, 10^4 TCID$_{50}$	4	Oculo-oronasal	NA	NA	+ (3/4)
Mouse, 10^5 TCID$_{50}$	4	Oculo-oronasal	NA	NA	+ (4/4)
Mouse, 10^5 TCID$_{50}$	4	Intraperitoneal	NA	NA	–

*NP, nucleoprotein; –, negative; NA, not applicable; +, positive; TCID$_{50}$, 50% tissue culture infectious dose.
†Three juvenile chickens and turkeys inoculated through the air sac were killed on days 0, 2, 4, 6, 8, and 14 posinoculation, and left and right lung lobes were removed for analysis by real-time reverse transcription–PCR. Tissues from the left (inoculation site) lung lobe of 5 chicks and 4 poults killed at the indicated days postinfection were positive for virus RNA with high cycle threshold values (>35), and 1 additional poult was positive for virus RNA with a cycle threshold value of 27 on day 2 postinfection.
‡Seroconversion was verified by testing for antibodies against virus NP by commercially available ELISAs (ELISA Avian Influenza Type A; Pourquier, Montpellier, France, and FlockChek AI MultiS-Screen; IDEXX, Ludwigsburg, Germany).

lication may be reduced after intraperitoneal inoculation, development of neutralizing antibodies after inoculation by this route has been reported (*15*).

Our results demonstrate lack of susceptibility and absence of serologic responses of chickens and turkeys to contact infection with an early human-origin isolate of pandemic (H1N1) 2009 virus. However, direct inoculation of fattening turkeys resulted in seroconversion and detection of virus RNA in oropharyngeal swab samples. Generation of new variants and reassortants caused by double infections with other influenza A viruses and pandemic (H1N1) 2009 virus in poultry other than turkeys appears unlikely. However, further adaptation of pandemic (H1N1) virus strains in turkeys cannot be excluded.

Acknowledgments

Dr Kalthoff is a veterinarian at the Friedrich-Loeffler-Institut in Greifswald-Insel Riems, Germany. Her research interests are virus pathogenesis and vaccine development.

References

1. Scholtissek C, Burger H, Kistner O, Shortridge KF. The nucleoprotein as a possible major factor in determining host specificity of influenza H3N2 viruses. Virology. 1985;147:287–94. DOI: 10.1016/0042-6822(85)90131-X
2. Brookes SM, Irvine RM, Nunez A, Clifford D, Essen S, Brown IH, et al. Influenza A (H1N1) infection in pigs. Vet Rec. 2009;164:760–1.
3. Lange E, Kalthoff D, Blohm U, Teifke JPT, Breithaupt A, Maresch C, et al. Pathogenesis and transmission of the novel swine origin influenza virus A/H1N1 after experimental infection of pigs. J Gen Virol. 2009;90:2119–23.
4. Takano R, Nidom CA, Kiso M, Muramoto Y, Yamada S, Shinya K, et al. A comparison of the pathogenicity of avian and swine H5N1 influenza viruses in Indonesia. Arch Virol. 2009;154:677–81. DOI: 10.1007/s00705-009-0353-5
5. Choi YK, Lee JH, Erickson G, Goyal SM, Joo HS, Webster RG, et al. H3N2 influenza virus transmission from swine to turkeys, United States. Emerg Infect Dis. 2004;10:2156–60.
6. Thomas C, Manin TB, Andriyasov AV, Swayne DE. Limited susceptibility and lack of systemic infection by an H3N2 swine influenza virus in intranasally inoculated chickens. Avian Dis. 2008;52:498–501. DOI: 10.1637/8210-011408-RESNOTE.1
7. PRO/AH/EDR. Influenza pandemic (H1N1) 2009, animal health (03): Chile, avian, RFI [cited 2010 Jan 14]. http://www.promedmail.org, archive no. 20090821.2961.
8. PRO/AH/EDR. Influenza pandemic (H1N1) 2009, animal health (14): Canada (ON), avian [cited 2010 Jan 14]. http://www.promedmail.org, archive no. 20091020.3602.
9. Terregino C, De Nardi R, Nisi R, Cilloni F, Salviato A, Fasolato M, et al. Resistance of turkeys to experimental infection with an early 2009 Italian human influenza A(H1N1)v virus isolate. Euro Surveill. 2009;14:19360.
10. Swayne DE, Pantin-Jackwood M, Kapczynski D, Spackman E, Suarez DL. Susceptibility of poultry to pandemic (H1N1) 2009 virus. Emerg Infect Dis. 2009;15:2061–3. DOI: 10.3201/eid1512.091060
11. Russell C, Hanna A, Barrass L, Matrosovich M, Núñez A, Brown IH, et al. Experimental infection of turkeys with pandemic (H1N1) 2009 influenza virus (A/H1N1/09v). J Virol. 2009;83:13046–7. DOI: 10.1128/JVI.01645-09
12. Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature. 2009;460:1021–5.
13. Maines TR, Jayaraman A, Belser JA, Wadford DA, Pappas C, Zeng H, et al. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. Science. 2009;325:484–7.
14. Childs RA, Palma AS, Wharton S, Matrosovich T, Liu Y, Chai W et al. Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. Nat Biotechnol. 2009;27:797–9. DOI: 10.1038/nbt0909-797
15. Bertram EM, Lau P, Watts TH. Temporal segregation of 4–1BB versus CD28-mediated costimulation: 4–1BB ligand influences T cell numbers late in the primary response and regulates the size of the T cell memory response following influenza infection. J Immunol. 2002;168:3777–85.

Address for correspondence: Martin Beer, Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany; email: martin.beer@fli.bund.de

International Conference on Emerging Infectious Diseases
ICEID
Atlanta, Georgia, USA
July 11-14 2010

Which infectious diseases are emerging?
Who are they affecting?
Why are they emerging?
What can be done to control them?