Journal of Virology, Dec. 2009, p. 13046–13047 Vol. 83, No. 24
0022-538X/09/$12.00 doi:10.1128/JVI.01645-09
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

Letter to the Editor

Experimental Infection of Turkeys with Pandemic (H1N1) 2009 Influenza Virus (A/H1N1/09v)▽

Interspecies influenza virus transmission between turkeys and pigs is well documented (8). Reverse zoonosis of pandemic (H1N1) 2009 influenza virus (A/H1N1/09v) to pigs has been reported to have occurred naturally (4, 5). As a consequence, there is a potential for spread of A/H1N1/09v to turkeys, which would pose a significant threat to the poultry industry; therefore, in this study, the susceptibility of turkeys to experimental infection with A/H1N1/09v was investigated.

The study used a total of 45 3-week-old turkeys: each bird in three groups of 10 was challenged with 0.1 ml of virus containing either $10^{3.6}$, $10^{4.4}$, or 10^{6} 50% egg infective doses of A/California/07/09v, administered equally between the intranasal and intraocular routes. One day postinfection (dpi), four in-contact birds were introduced to each group. All birds were observed twice daily for clinical signs. Buccal and cloacal swabs were collected daily up to 10 dpi for the detection of viral RNA using a modified matrix gene real-time reverse transcription-PCR (RRT-PCR). Total RNA, including a positive extraction control that was prepared to yield a predetermined threshold cycle value, was extracted as previously described (1). The modified matrix gene RRT-PCR utilized the forward primer and probe described originally by Spackman et al. (7); the reverse primer consisted of an equimolar mixture of the original reverse primer and a reverse primer modified to provide a perfect sequence match with A/H1N1/09v, typified by A/California/07/09v (GenBank accession no. FJ969537). The four altered nucleotides in the modified reverse primer are underlined: 5′-TGCAAA$\underline{\text{G}}$ACA$\underline{\text{CT}}$TTC$\underline{\text{C}}$AGTCTCTG-3′. RNA extracted from a titrated allantoic fluid preparation of A/California/07/09v was used to construct a 10-fold dilution series; this served to calibrate the threshold cycle values derived from testing extracted clinical specimens by the RRT-PCR.

At 6 dpi, two birds from each group were selected randomly and killed humanely for necropsy, tissues samples were collected for RRT-PCR analysis and immunohistopathology. At 20 dpi, the experiment was concluded, buccal and cloacal swabs were collected for RRT-PCR, and clotted blood was examined for the detection of antibodies to A/H1N1/09v using the hemagglutination inhibition test. All turkeys remained clinically normal throughout the study. In totality, RRT-PCR analysis revealed only two buccal swabs to be weakly positive: both were obtained at 2 dpi from turkeys infected with the highest viral dose, and virus isolation was negative for both birds. RRT-PCR and immunohistopathology of tissue samples collected at 6 dpi were all negative. Hemagglutination inhibition tests were negative for all birds.

The hemagglutinin (HA) gene was PCR amplified, sequenced, and compared to the sequence of the inoculum virus for the two RRT-PCR-positive birds. The HA sequence for the inoculum was identical to the published sequence of egg-adapted A/California/07/09v (GenBank accession no. FJ969540) containing a mixture of wild-type virus (225D/226Q) and two mutants (225G/226R). Challenge of turkeys resulted in the selection of the 225G/226Q variant, with no other changes compared to the inoculum virus sequence. The mutation Q226R has been previously observed in egg-adapted, seasonal human H1N1 viruses; in that case, the 226R variant grew well in eggs and cell culture but was suspected to be strongly impaired in vivo (3). Data presented here support this hypothesis since the 226R variant was absent from challenged turkeys. The receptor binding specificity of the HA depends on the virus host species: swine and human viruses preferentially bind α2,6-linked sialyl receptors, whereas avian viruses bind α2,3-linked receptors (2). An aspartic acid at codon 190 of H1N1 human and swine influenza A viruses is essential for their binding to α2,6-linked receptors, whereas glycine at codon 225 allows them additionally to bind to α2,3-linked receptors (2), possibly explaining why the virus variant with 225G was capable of limited replication in turkeys, although a suboptimal human virus-like 190D variant prevented the establishment of efficient infection.

At the time of writing, an occurrence of A/H1N1/09v infection in turkeys was reported in Chile (6). Analysis of the HA sequence of this isolate (GenBank: GQ866225) did not reveal any alteration which may be expected to significantly affect receptor binding. Therefore, it appears the Chilean turkeys were infected with a virus containing "human"-like HA. The significance of this is unclear; however, it does raise the question as to why more disease occurrence in avian species has not occurred if the currently circulating A/H1N1/09v strains are able to infect this species. Host range and tropism are polygenic traits; therefore, further investigation of the Chilean isolate is required.

Here, we have shown the failure of A/H1N1/09v to initiate clinical infection and establish efficient virus replication and transmission; however, viral RNA was transiently detected in two turkeys. As the H1N1 pandemic progresses, variants with better ability to infect avian species may be generated; therefore, further investigations into A/H1N1/2009v host genetic adaptations necessary to support avian infection, potential for reassortment with avian influenza strains, and transmission are required.

ACKNOWLEDGMENTS

This work was supported by the EU research project FLUTEST and Defra UK.

REFERENCES

1. **Löndt, B. Z., A. Nunez, J. Banks, H. Nili, L. K. Johnson, and D. J. Alexander.** 2008. Pathogenesis of highly pathogenic avian influenza A/turkey/Turkey/1/2005 H5N1 in Pekin ducks (Anas platyrhynchos) infected experimentally. Avian Pathol. **37:**619–627.
2. **Matrosovich, M., A. Tuzikov, N. Bovin, A. Gambaryan, A. Klimov, M. R. Castrucci, I. Donatelli, and Y. Kawaoka.** 2000. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. J. Virol. **74:**8502–8512.
3. **Matrosovich, M. N., T. Y. Matrosovich, T. Gray, N. A. Roberts, and H. D. Klenk.** 2004. Human and avian influenza viruses target different cell types in cultures of human airway epithelium. Proc. Natl. Acad. Sci. USA **101:**4620–4624.
4. **OIE.** 2009. OIE disease notification in pigs Canada (early May). http://www.oie.int/wahis/public.php?page=single_report&pop=1&reportid=8065.
5. **OIE.** 2009. OIE disease notification in pigs Argentina (early June). http://www.oie.int/wahis/public.php?page=single_report&pop=1&reportid=8227.
6. **ProMed.** 2009. Chile finds H1N1 swine flu in turkeys. http://apex.oracle.com/pls/otn/f?p=2400:1001:1642184153826356::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1004,78889.
7. **Spackman, E., D. A. Senne, T. J. Myers, L. L. Bulaga, L. P. Garber, M. L.**

Perdue, K. Lohman, L. T. Daum, and D. L. Suarez. 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. J. Clin. Microbiol. **40:**3256–3260.

8. **Wright, S. M., Y. Kawaoka, G. B. Sharp, D. A. Senne, and R. G. Webster.** 1992. Interspecies transmission and reassortment of influenza A viruses in pigs and turkeys in the United States. Am. J. Epidemiol. **136:**488–497.

Christine Russell
Amanda Hanna
Laura Barrass
Department of Virology
Veterinary Laboratories Agency
Weybridge KT15 3NB, United Kingdom

Mikhail Matrosovich
Institute of Virology
Philipps University
D-35043 Marburg, Germany

Alejandro Núñez
Ian H. Brown
Bhudipa Choudhury
Jill Banks*
Department of Virology
Veterinary Laboratories Agency
Weybridge KT15 3NB, United Kingdom

*Phone: 44 01932 357307
Fax: 44 01932 347046
E-mail: j.banks@vla.defra.gsi.gov.uk

▿ Published ahead of print on 30 September 2009.