Published in final edited form as:

Vaccine. 2008 September 12; 26(Suppl 4): D54–D58. doi:10.1016/j.vaccine.2008.07.072.

Pathogenicity of highly pathogenic avian influenza virus in mammals

Emmie de Wit^a, Yoshihiro Kawaoka^b, Menno D. de Jong^c, and Ron A. M. Fouchier^{a,*}

^aDepartment of Virology and National Influenza Center, Erasmus Medical Center, Rotterdam, The Netherlands ^bDepartment of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706, USA and Division of Virology, Department of Microbiology and Immunology, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan ^cOxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Abstract

In recent years, there has been an increase in outbreaks of highly pathogenic avian influenza (HPAI) in poultry. Occasionally, these outbreaks have resulted in transmission of influenza viruses to humans and other mammals, with symptoms ranging from conjunctivitis to pneumonia and death. Here, the current knowledge of the determinants of pathogenicity of HPAI viruses in mammals is summarized. It is becoming apparent that common mechanisms exist across influenza A virus strains and subtypes, through which influenza viruses adapt to mammals and gain or lose pathogenicity.

Keywords

influenza A virus; pathogenicity; HPAI

1. Introduction

Although influenza A virus is commonly known as the cause of annual influenza epidemics in humans, wild aquatic birds represent the natural reservoir of this virus. Influenza A viruses are divided into subtypes based on antigenicity of their hemagglutinin (HA) and neuraminidase (NA) proteins. During circulation in poultry, viruses of subtypes H5 and H7 may acquire multiple basic amino acids at the HA cleavage site, rendering these viruses highly pathogenic with a mortality of up to 100% in poultry.

Occasionally, avian influenza A viruses cross the species barrier into humans. A pandemic may arise if such viruses are able to spread efficiently from human to human, causing worldwide morbidity and mortality. The introduction of a virus of the H1N1 subtype in the human population in 1918 is probably the best-known example of a pandemic. An estimated 50 million people died as a result of this so-called 'Spanish influenza' pandemic [1].

© 2008 Elsevier Ltd. All rights reserved.

*Corresponding author: Dept. of Virology, Erasmus MC, P.O. Box 2040, 3000CA Rotterdam, The Netherlands. Tel.: +31107044066; fax: +31107044060. E-mail address: r.fouchier@erasmusmc.nl (R.Fouchier).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

In recent years, the increase in outbreaks of highly pathogenic avian influenza (HPAI) in poultry and occasional transmission of these viruses to humans has caused great concern for the emergence of a new influenza A virus pandemic. Several outbreaks of low pathogenic avian influenza (LPAI) and HPAI H7 viruses in poultry have resulted in transmission of these viruses to humans (Table 1). Most of these human infections were in one or a few persons and resulted in conjunctivitis and, occasionally, mild respiratory symptoms [2–7]. However, an outbreak of HPAI H7N7 virus in the Netherlands in 2003 resulted in infection of 89 humans. Although most individuals developed conjunctivitis or mild respiratory symptoms, one patient died as a result of severe pneumonia and related complications [8,9].

The first recorded direct transmissions of avian influenza viruses to humans leading to deaths occurred in 1997, when 18 people were infected during an outbreak of HPAI H5N1 in poultry markets in Hong Kong, six of whom died [10]. Since 1997, HPAI H5N1 viruses have continued to circulate in Asia and reemerged in Hong Kong in 2003. Subsequently, the HPAI H5N1 viruses have surfaced in poultry and wild birds across the Eastern hemisphere, also causing disease in several terrestrial carnivores [11–15] and humans [16,17]. In humans, ~ 400 cases of HPAI H5N1 infection were detected to date. Patients infected with HPAI H5N1 often suffer from severe pneumonia, progressing to acute respiratory distress syndrome, with a fatal outcome in ~60% of infections [17,18]. In patients infected with HPAI H5N1, virus cannot only be detected in the respiratory tract, but also in blood, cerebrospinal fluid and viscera [17,19,20]. Below, known determinants of pathogenicity of HPAI viruses in mammals are summarized.

2.1 Polymerase complex

The influenza A virus ribonucleoprotein complex contains 4 proteins necessary for virus replication: PB2, PB1, PA and NP. Several substitutions in the polymerase complex proteins have been described, mainly in PB2, that are important for adaptation of avian viruses to replication in mammalian hosts. A glutamic acid (E) to lysine (K) substitution at position 627 of PB2 was first described as a determinant of host range in vitro [21]; 627E is typically found in avian viruses, while human viruses generally have 627K. In mouse models for infection with HPAI H5N1 and HPAI H7N7 viruses, this substitution was shown to be the main determinant of pathogenicity. The PB2 E627K substitution was the main reason why a HPAI H5N1 virus isolated from a patient with a fatal infection in 1997 caused a lethal systemic infection in mice, whereas a virus isolated from a patient with relatively mild disease caused a non-lethal respiratory infection [22]. Due to this same E627K substitution, the virus isolated from a fatal case of infection with HPAI H7N7 virus in the Netherlands in 2003 was intrinsically more pathogenic in mice than a virus isolated from a patient with conjunctivitis [23]. Other indications of the importance of the E627K substitution in PB2 are that adaptation of A/equine/London/1416/73 (H7N7) to mice resulted, amongst others, in a E627K substitution in PB2, leading to a 1000x increased virulence of this virus [24]; that a HPAI H7N1 virus with 627K in PB2, isolated in Italy during the 1999–2000 outbreak, was more pathogenic in mice than viruses lacking this substitution [25]; that a E627K substitution in PB2 resulted in increased replication of viruses in the upper respiratory tract of mice [26] and that avian viruses without the E627K substitution acquire it spontaneously upon a single passage in mice [26–28]. There was no direct correlation between the presence of 627K in PB2 and the outcome of disease in HPAI H5N1 virus infected humans, but in this cohort almost all viruses which did not contain the E627K substitution harbored another substitution (D701N) in the polymerase proteins that has been implicated in adaptation to replication in mammals [19].

The pathogenicity of a variant of A/seal/Massachusetts/1/80 (H7N7), SC35M that is highly pathogenic to mice, was determined by a combination of two substitutions in PB2, D701N and S714R. These substitutions resulted in an increased polymerase activity in human cells [29].

A possible explanation for the increased replication efficiency may be that the D701N substitution in PB2 and a N319K substitution in NP resulted in a more efficient binding of these proteins to importin α 1 and an increased nuclear localization of PB2, NP and importin α 1 in human, but not avian cells [30]. The D701N substitution in PB2 was also found in HPAI H5N1 viruses isolated from healthy ducks in southern China from 1999 onwards. The viruses with 701N in PB2 were more pathogenic in mice than those with 701D in PB2 [27].

Although the effect was not mapped to individual substitutions, PB2 and PB1 contributed to the pathogenicity of A/Vietnam/1203/04 as compared to A/Chicken/Vietnam/C58/04 in mouse and ferret models. In both models, the chicken isolate was non-lethal whereas the human isolate caused considerable mortality [31]. In human cells, the polymerase activity of A/Vietnam/1203/04 was significantly higher than that of the chicken virus polymerase complex, again indicating that increased virus replication is related to pathogenicity [31].

Sequence comparison of avian and human influenza A viruses have identified 52 species-associated positions in the influenza A virus genome. Of these 52 positions, 35 were in the polymerase complex [32]. In similar genome comparisons, 32 amino acid positions were described that distinguish human and avian influenza viruses, of which 26 were in PB2, PA and NP [33]. Thus, substitutions in the polymerase complex proteins may facilitate influenza virus adaptation to mammals, and contribute to pathogenicity.

2.2 PB1-F2

The recently discovered PB1-F2 protein, transcribed from an alternative reading frame in the PB1 gene, induces apoptosis in infected cells. In mice, PB1-F2 contributed to the pathogenicity of HPAI H5N1 viruses isolated in 1997. All viruses that were highly pathogenic to mice had 66S in PB1-F2, whereas less virulent viruses had 66N. The N66S substitution resulted in more severe infection, higher virus titers and higher production of the cytokines IFN- γ and TNF- α in the lungs of infected mice [34]. Interestingly, 66S was also present in PB1-F2 of the 1918 Spanish influenza virus.

2.3 Hemagglutinin

One obvious determinant in HA of HPAI viruses is the multi basic cleavage site that renders these viruses highly pathogenic in chickens by facilitating systemic replication [35]. A HPAI H5N1 strain isolated in Hong Kong in 1997 that was pathogenic in mice, was highly attenuated upon replacement of the multi basic cleavage site with that of a low pathogenic virus [22]. Thus, cleavage of HA is not only a determinant of pathogenicity in poultry, but also in mammals.

HA also plays a role in viral adaptation through its' function as the receptor binding protein. Whereas human influenza A viruses preferentially use α -2,6-linked sialic acids (SA) as a receptor, avian viruses prefer α -2,3-linked SA [36]. In virus attachment studies, human H3N2 viruses attach abundantly to the human trachea, in contrast to HPAI H5N1 virus. In the lower respiratory tract of humans, HPAI H5N1 virus attached predominantly to type II pneumocytes, alveolar macrophages and nonciliated cuboidal epithelial cells in terminal bronchioles [37]. The attachment pattern of H5N1 virus to these cells was in concordance with the diffuse alveolar damage observed in human cases of HPAI H5N1 virus infection [37]. In agreement with this attachment pattern of HPAI H5N1 virus, it was shown that α -2,3-linked SA are mainly present on nonciliated cuboidal bronchiolar cells and type II pneumocytes and are virtually absent in the human upper respiratory tract [38].

Immunohistochemical studies on lung samples from a patient infected with HPAI H5N1 virus showed that type II pneumocytes were predominantly infected with this virus [39]. However,

replication of HPAI H5N1 virus was shown in human ex vivo tissues originating from the upper respiratory tract [40].

Mutations in HA that affect pathogenicity have been described, although the effect of these substitutions on receptor binding was not always shown. An I227S substitution in HA of a HPAI H5N1 virus isolated from a fatal case in Hong Kong in 1997 increased the virulence of this virus in mice [22]. The removal of a potential glycosylation site at position 154 of HA of the 1997 HPAI H5N1 viruses increased the virulence of these viruses [41]. HA of the HPAI H7N7 virus isolated from the fatal case in the Netherlands in 2003 contained 3 substitutions compared to a virus isolated from a patient with conjunctivitis, including a substitution introducing a potential glycosylation site near the receptor binding site. This HA caused increased virus titers in the lungs and distribution of virus to different organs in infected mice. In virus attachment studies, it was shown that there are subtle differences in attachment to the human lower respiratory tract of the virus isolated from the fatal case and a virus from a conjunctivitis case, that may explain differences in pathogenicity [23].

Adaptation of HA to enable efficient binding to α -2,6-linked SA is currently considered one of the most important prerequisites for efficient human-to-human transmission and thus for the emergence of a new pandemic. Although several HPAI H5N1 viruses with mutations that increase the affinity of HA for α -2,6-linked SA have been isolated, a HA with sole preference for human receptors has not yet been identified [42–46].

2.4 NS1

Influenza A virus replication induces an innate immune response, that can be modulated by NS1. Virus pathogenicity may in part be determined by NS1. In contrast to earlier HPAI H5 strains, the HPAI H5N1 strains isolated in Hong Kong in 1997 did not lead to increased production of the proinflammatory cytokine TGF- β in serum of infected mice [47]. A glutamic acid at position 92 of NS1 rendered 1997 HPAI H5N1 viruses insensitive to IFN- α , IFN- γ and TNF- α in vitro and was a determinant of pathogenicity in infected pigs [48]. HPAI H5N1 viruses that are pathogenic to mice induced much higher levels of inflammatory cytokines in mouse lungs [49]. Higher levels of proinflammatory cytokines were produced in human monocyte-derived macrophages infected with 1997 HPAI H5N1 virus, than in those infected with a human H1N1 or H3N2 virus [50]. In human primary bronchial and alveolar epithelial cells, higher levels of proinflammatory cytokines were induced by HPAI H5N1 viruses from 1997 and 2004 than by a human H1N1 virus [51]. NS1 of 1997 HPAI H5N1 viruses did not bind efficiently to CPSF30, resulting in higher levels of IFN- β mRNA and inhibition of viral replication. HPAI H5N1 viruses isolated after 1997 do bind to CPSF30, resulting in more efficient virus replication in vitro, due to a L103F and I106M substitution in NS1 [52]. This lack of NS1 binding to CPSF30 could be partially overcome by the internal genes of this virus [52]. In HPAI H5N1 viruses isolated from ducks in 1997, a P42S substitution in NS1 led to a 10000x more virulent virus in mice and lower IFN α / β levels in vitro [53]. Finally, NS of A/Vietnam/1203/04 was shown to be an important determinant of pathogenicity in ferrets [31].

In patients infected with HPAI H5N1 in Vietnam in 2004 and 2005, cytokine levels in peripheral blood were elevated, especially in patients with a fatal outcome of disease. Whether this was related to NS1 or merely due to enhanced virus replication is not known [19]. Likewise, it is unclear at present whether and to what extent the increased cytokine levels contribute to disease pathogenesis.

3. Conclusions

Adaptation of the polymerase complex proteins, NP, PB1-F2, HA and NS1 are all important features in the pathogenicity of avian influenza viruses in humans. Although the summary

above focused on HPAI H5 and H7 viruses, remarkable similarities were observed in pathogenicity studies using the 1918 Spanish influenza virus. It was shown that the 1918 virus polymerase complex (including PB2 E627K) [54,55], PB1-F2 (with 66N) [34], HA [55–57], NA [55,57] and possibly NS1 [58] contributed to the pathogenicity of this virus. From these data, two important conclusions can be drawn. First, multiple genes determine the pathogenicity of influenza A viruses (Fig.1). Second, common mechanisms exist across influenza A virus strains and subtypes, through which influenza viruses adapt to mammals and gain or loose pathogenicity. While the determinants of avian influenza virus pathogenicity in mammals are thus becoming clear, the determinants for transmission between mammals remain largely unknown, requiring more attention in the future.

Acknowledgments

E.W and R.A.M.F. are funded in part by EU FP6 program RiviGene (No. SSPE-CT-2005-022639) and contract NIAD-NIH HHNSN266200700010C.

Y.K is funded in part by U.S. National Institute of Allergy and Infectious Diseases Public Health Service research grants, by U.S., by grants-in-aid from the Ministries of Education, Culture, Sports, Science, and Technology, of Health, Labor, and Welfare of Japan, by CREST (Japan Science and Technology Agency), and by a contract research fund from the Ministry of Education, Culture, Sports, Science and Technology, Japan, for the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases.

References

1. Patterson KD, Pyle GF. The geography and mortality of the 1918 influenza pandemic. *Bull Hist Med* 1991 Spring;65(1):4–21. [PubMed: 2021692]
2. Tweed SA, Skowronski DM, David ST, Larder A, Petric M, Lees W, et al. Human illness from avian influenza H7N3, British Columbia. *Emerg Infect Dis* 2004 Dec;10(12):2196–2199. [PubMed: 15663860]
3. Webster RG, Geraci J, Petursson G, Skirnisson K. Conjunctivitis in human beings caused by influenza A virus of seals. *N Engl J Med* 1981 Apr 9;304(15):911. [PubMed: 7207529]
4. Kurtz J, Manvell RJ, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. *Lancet* 1996 Sep 28;348(9031):901–902. [PubMed: 8826845]
5. Nguyen-Van-Tam JS, Nair P, Acheson P, Baker A, Barker M, Bracebridge S, et al. Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. *Euro Surveill* 2006 May;11(5):E060504 2. [PubMed: 16816456]
6. ProMed. Avian influenza H7N2, human - United Kingdom (WALES) (08). 2007. cited; Available from: <http://www.promedmail.org> [archive no. 20070606.1830]
7. Prevention CfDCa. Update: influenza activity--United States and worldwide, 2003–04 season, and composition of the 2004–05 influenza vaccine. *MMWR Morb Mortal Wkly Rep* 2004 Jul 2;53(25):547–552. [PubMed: 15229411]
8. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 2004 Feb 21;363(9409):587–593. [PubMed: 14987882]
9. Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, Munster V, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci U S A* 2004 Feb 3;101(5):1356–1361. [PubMed: 14745020]
10. de Jong JC, Claas EC, Osterhaus AD, Webster RG, Lim WL. A pandemic warning? *Nature* 1997 Oct 9;389(6651):554. [PubMed: 9335492]
11. Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RA, Amonsin A, Payungporn S, et al. Avian influenza H5N1 in tigers and leopards. *Emerg Infect Dis* 2004 Dec;10(12):2189–2191. [PubMed: 15663858]
12. Butler D. Thai dogs carry bird-flu virus, but will they spread it? *Nature* 2006 Feb 16;439(7078):773. [PubMed: 16482119]

13. Klopffleisch R, Wolf PU, Wolf C, Harder T, Starick E, Niebuhr M, et al. Encephalitis in a stone marten (*Martes foina*) after natural infection with highly pathogenic avian influenza virus subtype H5N1. *J Comp Pathol* 2007 Aug–Oct;137(2–3):155–159. [PubMed: 17689552]
14. Kuiken T, Rimmelzwaan G, van Riel D, van Amerongen G, Baars M, Fouchier R, et al. Avian H5N1 influenza in cats. *Science* 2004 Oct 8;306(5694):241. [PubMed: 15345779]
15. Songserm T, Amonsin A, Jam-on R, Sae-Heng N, Pariyothorn N, Payungporn S, et al. Fatal avian influenza A H5N1 in a dog. *Emerg Infect Dis* 2006 Nov;12(11):1744–1747. [PubMed: 17283627]
16. Beigel JH, Farrar J, Han AM, Hayden FG, Hyer R, de Jong MD, et al. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 2005 Sep 29;353(13):1374–1385. [PubMed: 16192482]
17. Abdel-Ghaffar, AN.; Chotpitayasunondh, T.; Gao, Z.; Hayden, FG.; Nguyen, DH., et al. Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza AV. Update on avian influenza A (H5N1) virus infection in humans; *N Engl J Med*. 2008 Jan 17. p. 261-273.
18. WHO. Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO. 2008. cited; Available from: http://www.who.int/csr/disease/avian_influenza/country/cases_table_2008_04_30/en/index.html
19. de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006 Oct;12(10):1203–1207. [PubMed: 16964257]
20. Wang H, Feng Z, Shu Y, Yu H, Zhou L, Zu R, et al. Probable limited person-to-person transmission of highly pathogenic avian influenza A (H5N1) virus in China. *Lancet* 2008 Apr 26;371(9622):1427–1434. [PubMed: 18400288]
21. Subbarao EK, London W, Murphy BR. A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. *J Virol* 1993 Apr;67(4):1761–1764. [PubMed: 8445709]
22. Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science* 2001;293(5536):1840–1842. [PubMed: 11546875]
23. Munster VJ, de Wit E, van Riel D, Beyer WE, Rimmelzwaan GF, Osterhaus AD, et al. The Molecular Basis of the Pathogenicity of the Dutch Highly Pathogenic Human Influenza A H7N7 Viruses. *J Infect Dis* 2007 Jul 15;196(2):258–265. [PubMed: 17570113]
24. Shinya K, Watanabe S, Ito T, Kasai N, Kawaoka Y. Adaptation of an H7N7 equine influenza A virus in mice. *J Gen Virol* 2007 Feb;88(Pt 2):547–553. [PubMed: 17251573]
25. Rigoni M, Shinya K, Toffan A, Milani A, Bettini F, Kawaoka Y, et al. Pneumo- and neurotropism of avian origin Italian highly pathogenic avian influenza H7N1 isolates in experimentally infected mice. *Virology* 2007 Jul 20;364(1):28–35. [PubMed: 17408714]
26. Hatta M, Hatta Y, Kim JH, Watanabe S, Shinya K, Nguyen T, et al. Growth of H5N1 Influenza A Viruses in the Upper Respiratory Tracts of Mice. *PLoS Pathog* 2007 Oct 5;3(10):e133.
27. Li Z, Chen H, Jiao P, Deng G, Tian G, Li Y, et al. Molecular basis of replication of duck H5N1 influenza viruses in a mammalian mouse model. *J Virol* 2005 Sep;79(18):12058–12064. [PubMed: 16140781]
28. Mase M, Tanimura N, Imada T, Okamatsu M, Tsukamoto K, Yamaguchi S. Recent H5N1 avian influenza A virus increases rapidly in virulence to mice after a single passage in mice. *J Gen Virol* 2006 Dec;87(Pt 12):3655–3659. [PubMed: 17098982]
29. Gabriel G, Dauber B, Wolff T, Planz O, Klenk HD, Stech J. The viral polymerase mediates adaptation of an avian influenza virus to a mammalian host. *Proc Natl Acad Sci U S A* 2005 Dec 20;102(51):18590–18595. [PubMed: 16339318]
30. Gabriel G, Herwig A, Klenk HD. Interaction of Polymerase Subunit PB2 and NP with Importin alpha1 Is a Determinant of Host Range of Influenza A Virus. *PLoS Pathog* 2008 Feb 1;4(2):e11. [PubMed: 18248089]
31. Salomon R, Franks J, Govorkova EA, Ilyushina NA, Yen HL, Hulse-Post DJ, et al. The polymerase complex genes contribute to the high virulence of the human H5N1 influenza virus isolate A/Vietnam/1203/04. *J Exp Med* 2006 Mar 20;203(3):689–697. [PubMed: 16533883]
32. Chen G-W, Chang S-C, Mok C-K, Lo Y-L, Kung Y-N, Huang J-H, Shih Y-H, Wang J-Y, Chiang C, Chen C-J, Shih S-R. Genomic signatures of human versus avian influenza A viruses. *Emerg Infect Dis* 2006;12:1353–1360. [PubMed: 17073083]

33. Finkelstein DB, Mukatira S, Mehta PK, Obenauer JC, Su X, Webster RG, et al. Persistent host markers in pandemic and H5N1 influenza viruses. *J Virol* 2007 Oct;81(19):10292–10299. [PubMed: 17652405]
34. Conenello GM, Zamarin D, Perrone LA, Tumpey T, Palese P. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. *PLoS Pathog* 2007 Oct 5;3(10):1414–1421. [PubMed: 17922571]
35. Kawaoka Y, Webster RG. Sequence requirements for cleavage activation of influenza virus hemagglutinin expressed in mammalian cells. *Proc Natl Acad Sci U S A* 1988 Jan;85(2):324–328. [PubMed: 2829180]
36. Rogers GN, Paulson JC. Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. *Virology* 1983 Jun;127(2):361–373. [PubMed: 6868370]
37. van Riel D, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, et al. H5N1 Virus Attachment to Lower Respiratory Tract. *Science* 2006 Apr 21;312(5772):399. [PubMed: 16556800]
38. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: influenza virus receptors in the human airway. *Nature* 2006 Mar 23;440(7083):435–436. [PubMed: 16554799]
39. Liem NT, Nakajima N, Phat le P, Sato Y, Thach HN, Hung PV, et al. H5N1-infected cells in lung with diffuse alveolar damage in exudative phase from a fatal case in Vietnam. *Jpn J Infect Dis* 2008 Mar;61(2):157–160. [PubMed: 18362412]
40. Nicholls JM, Chan MC, Chan WY, Wong HK, Cheung CY, Kwong DL, et al. Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. *Nat Med* 2007 Feb;13(2):147–149. [PubMed: 17206149]
41. Chen H, Bright RA, Subbarao K, Smith C, Cox NJ, Katz JM, et al. Polygenic virulence factors involved in pathogenesis of 1997 Hong Kong H5N1 influenza viruses in mice. *Virus Res* 2007 Sep; 128(1–2):159–163. [PubMed: 17521765]
42. Auewarakul P, Suptawiwat O, Kongchanagul A, Sangma C, Suzuki Y, Ungchusak K, et al. An avian influenza H5N1 virus that binds to a human-type receptor. *J Virol* 2007 Sep;81(18):9950–9955. [PubMed: 17626098]
43. Gambaryan A, Tuzikov A, Pazynina G, Bovin N, Balish A, Klimov A. Evolution of the receptor binding phenotype of influenza A (H5) viruses. *Virology* 2006 Jan 20;344(2):432–438. [PubMed: 16226289]
44. Stevens J, Blixt O, Tumpey TM, Taubenberger JK, Paulson JC, Wilson IA. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science* 2006 Apr 21;312(5772): 404–410. [PubMed: 16543414]
45. Yamada S, Suzuki Y, Suzuki T, Le MQ, Nidom CA, Sakai-Tagawa Y, et al. Haemagglutinin mutations responsible for the binding of H5N1 influenza A viruses to human-type receptors. *Nature* 2006 Nov 16;444(7117):378–382. [PubMed: 17108965]
46. Yang ZY, Wei CJ, Kong WP, Wu L, Xu L, Smith DF, et al. Immunization by avian H5 influenza hemagglutinin mutants with altered receptor binding specificity. *Science* 2007 Aug 10;317(5839): 825–828. [PubMed: 17690300]
47. Dybing JK, Schultz-Cherry S, Swayne DE, Suarez DL, Perdue ML. Distinct pathogenesis of hong kong-origin H5N1 viruses in mice compared to that of other highly pathogenic H5 avian influenza viruses. *J Virol* 2000 Feb;74(3):1443–1450. [PubMed: 10627555]
48. Seo SH, Hoffmann E, Webster RG. Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nat Med* 2002 Sep;8(9):950–954. [PubMed: 12195436]
49. Lipatov AS, Andreansky S, Webby RJ, Hulse DJ, Rehg JE, Krauss S, et al. Pathogenesis of Hong Kong H5N1 influenza virus NS gene reassortants in mice: the role of cytokines and B- and T-cell responses. *J Gen Virol* 2005 Apr;86(Pt 4):1121–1130. [PubMed: 15784906]
50. Cheung CY, Poon LL, Lau AS, Luk W, Lau YL, Shortridge KF, et al. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 2002 Dec 7;360(9348):1831–1837. [PubMed: 12480361]
51. Chan MC, Cheung CY, Chui WH, Tsao SW, Nicholls JM, Chan YO, et al. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Respir Res* 2005;6:135. [PubMed: 16283933]

52. Twu KY, Kuo RL, Marklund J, Krug RM. The H5N1 influenza virus NS genes selected after 1998 enhance virus replication in mammalian cells. *J Virol* 2007 Aug;81(15):8112–8121. [PubMed: 17522219]
53. Jiao P, Tian G, Li Y, Deng G, Jiang Y, Liu C, et al. A single-amino-acid substitution in the NS1 protein changes the pathogenicity of H5N1 avian influenza viruses in mice. *J Virol* 2008 Feb;82(3): 1146–1154. [PubMed: 18032512]
54. Taubenberger JK, Reid AH, Lourens RM, Wang R, Jin G, Fanning TG. Characterization of the 1918 influenza virus polymerase genes. *Nature* 2005 Oct 6;437(7060):889–893. [PubMed: 16208372]
55. Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solorzano A, Swayne DE, et al. Characterization of the reconstructed 1918 spanish influenza pandemic virus. *Science* 2005 Oct 7;310(5745):77–80. [PubMed: 16210530]
56. Kobasa D, Takada A, Shinya K, Hatta M, Halfmann P, Theriault S, et al. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. *Nature* 2004 Oct 7;431 (7009):703–707. [PubMed: 15470432]
57. Tumpey TM, Garcia-Sastre A, Taubenberger JK, Palese P, Swayne DE, Pantin-Jackwood MJ, et al. Pathogenicity of Influenza Viruses with Genes from the 1918 Pandemic Virus: Functional Roles of Alveolar Macrophages and Neutrophils in Limiting Virus Replication and Mortality in Mice. *J Virol* 2005 Dec;79(23):14933–14944. [PubMed: 16282492]
58. Geiss GK, Salvatore M, Tumpey TM, Carter VS, Wang X, Basler CF, et al. Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: the role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. *Proc Natl Acad Sci U S A* 2002;99(16):10736–10741. [PubMed: 12149435]
59. Peiris M, Yuen KY, Leung CW, Chan KH, Ip PL, Lai RW, et al. Human infection with influenza H9N2. *Lancet* 1999 Sep 11;354(9182):916–917. [PubMed: 10489954]
60. ProMed. Avian influenza A (H1N7), humans, avians - Egypt: correction. 2004

Figure 1. Determinants of adaptation and pathogenicity of avian influenza viruses in mammals. The functions of eight viral proteins, that are discussed in the main text as important determinants of pathogenicity, are indicated.