ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.2010

International Journal of Pharma and Bio Sciences

H5N1 VIRUS: A PANDEMIC THREAT

A. R. MALLICK[1], ABHERI DAS SARMA[2] AND A. K. GHOSH[1]*

[1]Department of Pharmacology, Gupta College of Technological Sciences,Asansol, Burdwan, West Bengal, Pin - 713301, India
[2]Department of Pharmaceutics, Gupta College of Technological Sciences,Asansol, Burdwan, West Bengal, Pin - 713301, India

Correspondence Author akg_mail@yahoo.com

ABSTRACT

H5N1 is a subtype of the Influenza A virus. Not all H5N1 subtypes are the same. Some may be highly pathogenic while others cause mild disease of none at all in birds and so are called low pathogenic strains of the avian influenza virus. Viruses, by nature, change all the time and even a low pathogenic strain can turn into a highly pathogenic strain and vice versa. The highly pathogenic avian influenza virus subtype presently of global concern. Highly pathogenic bird flu (HPAI) is highly contagious disease affecting wild bird and poultry. There are three types of flu virus – A, B & C. Only types A & B Cause significant human disease. Flu A is further sub typed according to the type of protein (H) and (N) protein on its outer layer. There are 16 different H types (H1, H2 etc) and 9 different N types (N1, N2 etc). H5N1 viruses are spherically or longitudinally shaped enveloped particles with an up to eight-fold segmented, single-stranded RNA genome of negative polarity. H5N1 is a specific form that had not previously infected humans and is spreading rapidly amongst wild-bird populations. To avoid H5N1 infection one should strictly follow the precaution and preventive measures. Researchers across the world are trying to develop vaccines to check the pandemic threat that is rising in a tsunamic fashion.

KEY WORDS

H5N1 Virus, avian influenza, pandemic, Bird flu

INTRODUCTION

Avian influenza virus (H5N1) emerged in Hong Kong in 1997. In recent years, several outbreaks have been reported in different parts of Asia, Europe and Africa, raising concerns of dissemination of a new and highly lethal influenza pandemic. Although H5N1 has not been capable of sustaining human-to-human transmission, the ability of the virus to undergo variation due to mutations poses the possibility of viral adaptation to the human species. To avoid influenza pandemic a great deal of awareness is necessary to stop initial outbreaks, through the use of case recognition, sensitive and rapid diagnostic methods, appropriate therapeutic and preventive measures. Influenza pandemic

awareness involves coordinated pharmacologic and vaccinal strategies, and controlled travel. In recent years, notably since 1997, increased circulation of highly pathogenic avian influenza has been detected consistently in poultry and wild birds in several countries, mostly in Asia. In addition, the World Health Organization (WHO) as of this writing [1] has officially reported 271 human cases of avian influenza (59.1% of them lethal). The occurrence of human infection outbreaks of avian influenza, can lead to highly lethal influenza pandemic.

According to the FAO Avian Influenza Disease Emergency Situation Update, H5N1 pathogenicity is gradually rising in wild birds in endemic areas whereas the avian influenza disease in farmed birds has been checked by vaccination. Eleven outbreaks of H5N1 were reported worldwide in June 2008 in five countries (China, Egypt, Indonesia, Pakistan and Vietnam) compared to 65 outbreaks in June 2006 and 55 in June 2007. The "global HPAI situation can be said to have improved markedly in the first half of 2008, cases of HPAI are still underestimated and underreported in many countries because of limitations in country disease surveillance systems".[2]

BIOLOGY OF H5N1

H5 stands for the fifth of several known types of the protein hemagglutinin a glycosylated and acylated protein consisting of 562 - 566 amino acids. N1 stands for the first of several known types of the protein neuraminidase. Influenza viruses are RNA viruses in the family Orthomyxoviridae, which includes four genera: Influenza A, B and C viruses and Thogotovirus, as recently proposed by the International Committee on Taxonomy of Viruses (ICTV) [3, 4]. They are enveloped negative-stranded RNA viruses with nucleocapsid (N) and matrix (M) proteins. [5]

Influenza A viruses are roughly spherical (120 nM) with glycoprotein spikes on the surface and genome consisting of eight RNA fragments that encode 10 proteins. The haemagglutinin (HA), neuraminidase (NA) and matrix (M2) proteins are embedded in the envelope lipid bilayer derived from the host cell. The M1 protein underlying the envelope is the major determinant of virion morphology. [6] The nucleoprotein (NP) associates with each RNA segment to form the ribonucleoprotein (RNP) complex, which also contains small amounts of the three polymerase subunits. The nonstructural proteins NS1 and NS2 are found in infected cells.

The surface glycoproteins HA and NA are critical for the biology of influenza virus. HA is responsible for the virus attachment to the cell surface, binding to sialic acid residues in cell membrane glycoproteins, thus triggering viral fusion and entry. [7] The proteolytic cleavage of HA by serine proteases present in the infected tissue exposes hydrophobic fusion domains that mediate membrane fusion. This is an important molecular determinant of host range and tissue pathology. [8, 9] Some HA types can be cleaved by different serine proteases, which enables the virus to spread more efficiently in vivo. [10, 11] The RNA-dependent RNA polymerase and the NS1 proteins of influenza virus are also determinants of viral pathogenicity and host range. [12, 13] NA cleaves terminal sialic acid from glycoconjugates present on respiratory mucins, cells, and progeny virions. [5] This action is of key importance for the release of the virus from infected cells and, consequently, for the spreading of infection throughout the respiratory tract. The antigenic diversity of HA and NA provides for influenza A virus sub typing. Sixteen HA and nine NA subtypes are currently recognized, with amino acid sequences differing by 30% or more. [14] Six HA (H1, H2, H3, H5, H7 and H9) and three NA (N1, N2 and N7) subtypes have been identified in strains of influenza virus causing human infection, but only viruses of three HA (H1, H2 and H3) and two NA (N1 and N2) subtypes have remained in sustained circulation in the human population after causing pandemics. [15] After the binding of HA to sialic acid residues present in glycoproteins or glycolipids on the cell surface, influenza virus enters susceptible cells by

receptor mediated endocytosis This internalization mediated by clathrin0 coated vesicles is followed by the release of the genome into the host cell cytoplasm, a phenomenon dependent on endosomal acidification. [5] Transcription of the negative-stranded RNA into either messenger RNA (mRNA), which directs viral protein synthesis, or complementary RNA (cRNA), which serves as template for the viral RNA (vRNA) genome synthesis, is mediated by a viral RNA polymerase complex active in the nucleus of host cell. [16] Assembly and packaging of vRNAs into infectious virions involve several cellular compartments. Nuclear export is promoted by the binding of M1 viral protein to RNPs, followed by the assembly of nucleocapsids in association with the cytoplasmic membrane and budding through the cell surface. Only viruses with a full complement of genome segments are infectious. [17-20]

PROPERTIES OF H5N1

Infectivity: H5N1 is easily transmissible between birds. H5N1 as an avian virus preferentially binds to a type of galactose receptors that populate the avian respiratory tract from the nose to the lungs and are virtually absent in humans, occurring only in and around the alveoli. Therefore, the virus is not easily expelled by coughing and sneezing, the usual route of transmission. [21-25] Low pathogenic avian influenza H5N1 (LPAI H5N1) also called "North American" H5N1 occurs in wild birds. In most cases, it causes minor sickness or no noticeable signs of disease in birds.

Virulence: Through antigenic drift, H5N1 has mutated into dozens of highly pathogenic varieties divided into genetic clades which are known from specific isolates, but all currently belonging to genotype Z of avian influenza virus H5N1, now the dominant genotype. [26-29]

Transmission and host range: H5N1 is transmitted through saliva, nasal secretions, feces and blood of infected birds. Other animals get infected through direct contact or surface contamination with these bodily fluids [30]. The H5N1 can pass through placenta to infect the fetus. the gastrointestinal tract, the brain, liver, and blood cells.

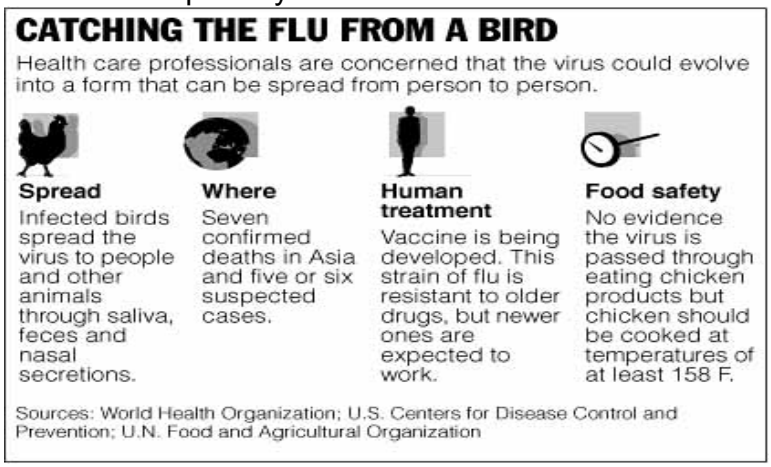

Fig.2

Spreading of flu in humans.

High mutation rate: Influenza viruses have high mutation rate. [26, 27] Genetic mutations in the hemagglutinin gene that cause single amino acid substitutions can significantly alter the ability of viral hemagglutinin proteins to bind to receptors on the surface of host cells. Mutation can cause them capable to infect human beings. [31]

SURVIVAL CAPABILITY OF H5N1 [32]

Avian flu virus can last indefinitely at a temperature dozens of degrees below freezing, as is found in the northern most areas that migratory birds frequent.

- Over 30 days at 0°C (32°F) (over one month at freezing temperature)
- 6 days at 37°C (98.6°F) (one week at human body temperature)
- decades in permanently frozen lakes
- on hard non-porous surface such as plastic or stainless steel for 24-48 hours
- on clothes, paper and tissues for 8-12 hours. [33]
- While cooking poultry to 70°C (158°F) kills the H5N1 virus, it is recommended to cook meat to 165°F to kill all foodborne pathogens. [34]

DISINFECTION OF H5N1

- 30 minutes 60°C (140.0°F) (half hour at a temperature that causes first and second degree burns in humans in ten seconds). [35]
- Acidic pH conditions
- Presence of oxidizing agents such as sodium dodecyl sulfate, lipid solvents, and B-propiolactone
- Exposure to disinfectants: formalin, iodine compounds. [36]
- Ordinary levels of chlorine in tap water kill H5N1 in public water systems. [37]

To kill avian flu viruses, the "World Health Organization recommends that environmental surfaces be cleaned by the following:

- Disinfectants such as sodium hypochloride, 1% in-use dilution, 5% solution to be diluted 1:5 in clean water, for materials contaminated with blood and body fluids
- Bleaching powder seven grams per liter with 70% available chlorine for toilets and bathrooms
- 70% alcohol for smooth surfaces, tabletops, and other surfaces where bleach cannot be used. [38]
- Influenza viruses can be shed by adults up to 7 days after resolution of fever and up to 21 days in children after onset of illness. [39]
- Inactivation by 56°C/3 hours; 60°C/30 min. [40]

AVIAN INFLUENZA IN BIRDS AND HUMANS

Avian influenza viruses are carried by wild birds in their intestine and causes infection. Contamination occurs through direct contact with infected waterfowl or other infected poultry, or through contact with surfaces (such as dirt or cages) or materials (such as water or feed) that have been contaminated with the virus.

Two form of infection occur, the “low pathogenic” form may go undetected and usually causes only mild symptoms (such as ruffled feathers and a drop in egg production). However, the highly pathogenic form spreads more rapidly through flocks of poultry. This form may cause disease that affects multiple internal organs and has a mortality rate that can reach 90-100% often within 48 hours.

The risk from avian influenza is generally low to most people, because the viruses do not usually infect humans. The period when an infected person is contagious depends on the age of the person. Adults may be contagious from one day before they become sick and for three to seven days after they first develop symptoms. Some children may be contagious for longer than a week.

POULTRY FARMING PRACTICES

There have been a number of farming practices that have changed in response to outbreaks of the H5N1 virus, including:

- vaccinating poultry against bird flu
- vaccinating poultry workers against human flu
- limiting travel in areas where H5N1 is found
- increasing farm hygiene
- reducing contact between livestock and wild birds
- reducing open-air wet markets

- limiting workers contact with cock fighting
- reducing purchases of live fowl
- Improving veterinary vaccine availability and cost. [41, 42]

VACCINATION

H5N1 vaccines for chickens exist and are sometimes used, although there are many difficulties, and it's difficult to decide whether it helps more or hurts more. H5N1 pre-pandemic vaccines exist in quantities sufficient to inoculate a few million people [15] and might be useful for priming to "boost the immune response to a different H5N1 vaccine tailor-made years later to thwart an emerging pandemic". [16]

ROUTES OF CONTAMINATIONS [43]

- Direct Contact with Infected Birds
- Ingestion, Inhalation and Contact with Bird Faeces
- Killing, Cleaning, Plucking Feathers or Disposing of Dead Birds
- Contaminated Water
- Feathers
- Consumption of Improperly Cooked Infected Poultry Products
- In most of the cases of wild birds found with bird flu, it is thought that the wild birds contracted bird flu from neighboring poultry operations

CLINICAL PRESENTATION

Fever (>38:C), Headache, Myalgia Diarrhea, Abdominal pain, Vomiting, Cough, Sputum, Sore Throat, Rhinorrhea, Shortness of Breath, Pulmonary infiltrates, Lymphopenia, Thrombocytopenia, Increased aminotransferase levels, Development of respiratory failure (usually with Acute Respiratory Distress Syndrome), High levels of inflammatory mediators may contribute to ARDS and multiorgan failure. H5N1 has also been found to infect the blood (viremia) and other parts of the body not normally attacked by influenza. [44]

SIGNS AND TESTS

In February 2006, the U.S. Food & Drug Administration approved a new, faster test for diagnosing strains of bird flu in people suspected of having the virus. The test is called the Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set. The test gives preliminary results within 4 hours. Older tests required 2 to 3 days.

Doctor might also perform the following tests:

- Chest x-ray
- Nasopharyngeal culture
- Blood differential
- Auscultation (to detect abnormal breath sounds)

Other tests may be done to look at the functions of heart, kidneys, and liver.

MANAGEMENT

Studies done in laboratories suggest that some strains of H5N1 are still susceptible to the older drugs, which are inexpensive and widely available. However, influenza viruses can become resistant to these drugs, so these medications may not always work [45-47]. The medications that can be undertaken are given in table1.

Table .1

Medications for H5N1 avian influenza

	Amantadine	Oseltamivir	Rimantadine
Brand Name	Symmetrel	Tamiflu	Flumadine

Mechanism of Action	Amantadine inhibits the replication of viruses in cells. The drug should be taken before exposure to the virus.	Oseltamivir blocks the action of neuraminidase (it is a neuraminidase inhibitor) thereby reducing the spread of influenza. By preventing the spread of virus from cell to cell, the symptoms and duration of influenza infection are reduced.	Rimantadine prevent viruses in cells from multiplying. To prevent a viral infection, the drug should be started before exposure to the virus.
Preparations	Amantadine is available as 100mg soft gelatin capsules and as a syrup containing 50mg per each teaspoon.	75 mg tablets	Tablets: 100mg oval (peach-colored). Syrup: 50mg per teaspoonful.
Drug Interaction	Amantadine amplifies the actions of dopamine in the brain Amantadine adds to the sedating effects alcohol and other sedating drugs such as the benzodiazepine class of anti-anxiety drugs	There are no known interactions between oseltamivir and other drugs. Oseltamivir does not interact with the flu vaccine.	There are no known, clinically important drug interactions with Rimantadine.
Dosing	Amantadine is taken once or twice daily with or without food. If it causes an upset stomach, it can be taken with food.	Oseltamivir is administered orally. The recommended dose is one tablet twice daily for five days.	Once or twice daily with or without food. It should be continued for 5 to 7 days or for 24 to 48 hours after the disappearance of symptoms.
Contra-indication	No well-controlled studies have been done in pregnant women to evaluate amantadine's safety.	Oseltamivir has not been adequately evaluated in pregnant women.	Oseltamivir has not been adequately evaluated in pregnant women.
Side effects	dizziness, loss of coordination, inability to sleep, and nervousness, nausea, and vomiting	nausea, vomiting, diarrhea, bronchitis, abdominal pain, headache and dizziness	loss of appetite

CLINICAL TRIALS FOR H5N1 VACCINES

Current Status: Candidate vaccines were developed in the United States and the United Kingdom during 2003 for protection against the strain that was isolated from humans in Hong Kong in February 2003 but the 2003 strain died out in 2004 making the vaccine of little use. In April 2004, WHO made an H5N1 prototype seed strain available to manufacturers. The National Institute of Allergy and Infectious Diseases (NIAID) awarded H5N1 vaccine contracts to Aventis Pasteur (now Sanofi Pasteur) of Swiftwater, Pennsylvania, and to Chiron Corporation of Emeryville, California [48, 49]. As of July 2007, phase I clinical trials on humans are underway in which a vaccine that focuses on the M2 viral protein "is being administered to a small group of healthy people in order to verify the safety of the product and to provide an initial insight into the vaccine's effect on the human immune system." [50]. In June 2006, the National Institutes of Health (NIH) began enrolling participants in a Phase 1 H5N1 study of an intranasal influenza vaccine candidate based on MedImmune's live, attenuated vaccine technology. [51]

APPROVED HUMAN H5N1 VACCINES

On April 17, 2007 the US FDA approved "Influenza Virus Vaccine, H5N1" by manufacturer Sanofi Pasteur Inc for manufacture at its Swiftwater, PA facility. [52-54]

PRECAUTION AND CONTROL

Three international organizations coordinate the global H5N1 influenza surveillance effort: The World Health Organization (WHO) monitors human cases, while the World Organization of Animal Health (OIE) and the Food and Agriculture Organization (FAO) collect information of H5N1 in birds and other animals. Rapid detection method of H5N1 will play a critical role in the control of a rapidly spreading flu pandemic.

- Poultry and eggs should be properly handled and cooked until whites and yolks are firm as recommended by the U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA).
- Hands must be washed with soap and warm water for at least 20 seconds before and after handling raw poultry and eggs.
- Cutting boards and other utensils must be washed with soap and hot water to keep raw poultry from contaminating other foods.
- Food thermometer can be used to make sure while cooking at least 165 degrees Fahrenheit temperature has reached.
- If one intends to travel to visit countries with cases of bird flu one should adopt - good hygiene to minimize risk of contamination.
- Crowded areas must be avoided and one should stay in places with good ventilation.

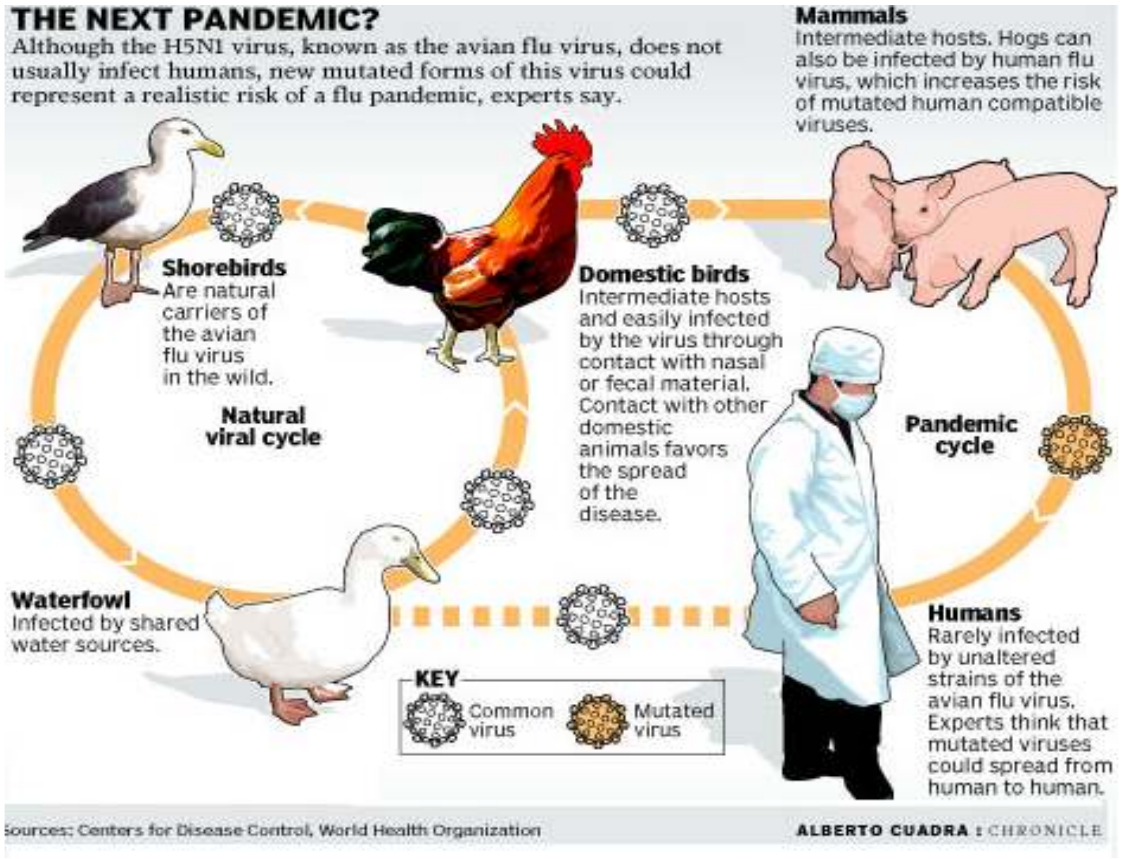

Fig. 3

CONCLUSION

The H5N1 is an avian influenza virus that has infected wild birds and poultry in several places in the world, becoming a serious economic problem to many countries. This virus has caused cases of serious and frequently fatal illness in humans, but the transmission has been limited and virtually always has involved contact with infected birds. Several lines of evidence indicate that the threat of H5N1 pandemic is real and that careful planning should be done in order to implement effective measures to reduce the public health impact of H5N1. Monitoring and rapid detection of H5N1 is necessary to combat the spread of this virus. Recent human isolates resistant to amantadines and neuraminidase inhibitors, as well as the difficulty for vaccine production against H5N1 illustrate the need for more research about the epidemiology and biology of H5N1 virus. This article presents an overview of avian H5N1 influenza virus biology and highlights currently available information about prevention and control.

ACKNOWLEDGEMENTS:

We are grateful to Professor Debesh Chandra Majumdar, Chairman of Gupta College of Technological Sciences and Professor Kalyan Kumar Sen, Principal of Gupta College of Technological Sciences for their kind help and inspiration for writing this review.

REFERENCES

1. (WHO), W.H.O. Cumulative Number of Confirmed Human Cases of Avian Influenza A/ (H5N1) Reported to WHO, (2006).

2. FAO Avian Influenza Disease Emergency Situation Update, (2008).
3. Wright P.F., R.G. Webster, Orthomyxoviruses. In: D.E. Griffin, et al., Editors Fields Virology. Philadelphia, PA, USA: Lippincott Williams & Wilkins, pp. 1533-79, (2001).
4. (ICTV). Orthomyxoviridae. In: ICTVdB - The Universal Virus Database, version 3. Büchen-Osmond, C. (Ed), ICTVdB Management, Columbia University, New York, USA, (2004).
5. Lamb R.A., Krug R.M., Orthomyxoviridae: The viruses and their replication. In: D.M. Knipe, et al. Editors Fields Virology. Philadelphia, PA, USA: Lippincott Williams & Wilkins, pp. 1487-531, (2001).
6. Noda T., H. Sagara, A. Yen, et al. Architecture of ribonucleoprotein complexes in influenza A virus particles. Nature; 439(7075): 490-2, (2006).
7. Takeda M., G.P. Leser, C.J. Russell, et al. Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. Proc Natl Acad Sci U S A; 100(25):14610-7, (2003).
8. Chen, J., K.H. Lee, D.A. Steinhauer, et al., Structure of the hemagglutinin precursor cleavage site, a determinant of influenza pathogenicity and the origin of the labile conformation. Cell, 95(3):409-17, (1998).
9. Kido H., M. Murakami, K. Oba, et al. Cellular proteinases trigger the infectivity of the influenza A and Sendai viruses. Mol Cells, 9(3):235-44, (1999).
10. Gamblin S.J., L.F. Haire, R.J. Russell, et al. The structure and receptor binding properties of the 1918 influenza hemagglutinin. Science, 303(5665):1838-42, (2004).
11. Steinhauer D.A. Role of hemagglutinin cleavage for the pathogenicity of influenza virus. Virology, 258(1):1-20, (1999).
12. Li Z., Y. Jiang, P. Jiao, et al. The NS1 Gene Contributes to the Virulence of H5N1 Avian Influenza Viruses. J Virol, (2006).
13. Salomon R., J. Franks, E.A. Govorkova, 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, 203(3):689-97, (2006).
14. Fouchier R.A., V. Munster, A. Wallensten, et al. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J Virol, 79(5):2814-22, (2005).
15. Lipatov A.S., E.A. Govorkova, R.J. Webby, et al. Influenza: emergence and control. J Virol, 78(17):8951-9, (2004).
16. Arruda E., O.A.L. Cintra, F.G. Hayden. Respiratory Tract Viral Infections. In: R.L. Guerrant, D.H. Walker, and P.F. Weller, Editors Tropical infectious Diseases: Principles, Pathogens & Practice. Philadelphia, PA, USA: Elsevier Churchill Livingstone, pp. 637-79, (2006).
17. Al Faress S., G. Cartet, O. Ferraris, et al. Divergent genetic evolution of hemagglutinin in influenza A H1N1 and A H1N2 subtypes isolated in the south-France since the winter of 2001-2002. J Clin Virol, 33(3):230-6, (2005).
18. Ghedin E., N.A. Sengamalay, M. Shumway, et al. Large-scale sequencing of human influenza reveals the dynamic nature of viral genome evolution. Nature, 437(7062):1162-6, (2005).
19. Guan Y., M. Peiris, K.F. Kong, et al. H5N1 influenza viruses isolated from geese in Southeastern China: evidence for genetic reassortment and interspecies transmission to ducks. Virology, 292(1):16-23, (2002).
20. Li K.S., Y. Guan, J. Wang, et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature, 430(6996):209-13, (2004).
21. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. "Avian flu: influenza virus receptors in the human airway". Nature, 440 (7083): 435–436, (2006).
22. Van Riel D, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, Kuiken T. "H5N1 Virus Attachment to Lower Respiratory Tract". Science, (2006).

23. Forbes.com., "Studies Spot Obstacle to Human Transmission of Bird Flu", (2006).
24. Food and Agricultural Organization of the United Nations, "Wild birds and Avian Influenza", (2005).
25. Brstilo M. "Highly Pathogenic Avian Influenza in Croatia Follow-up report No. 4", (2006).
26. The World Health Organization Global Influenza Program Surveillance Network. "Evolution of H5N1 avian influenza viruses in Asia". Emerging Infectious Diseases, 11 (10), (2005).
27. Kou Z, Lei FM, Yu J, Fan ZJ, Yin ZH, Jia CX, Xiong KJ, Sun YH, Zhang XW, Wu XM, Gao XB, Li TX. "New genotype of avian influenza H5N1 viruses isolated from tree sparrows in China". J. Virol., 79 (24): 15460–15466, (2005).
28. Li KS, Guan Y, Wang J, Smith GJ, Xu KM, Duan L, Rahardjo AP, Puthavathana P, Buranathai C, Nguyen TD, Estoepangestie AT, Chaisingh A, Auewarakul P, Long HT, Hanh NT, Webby RJ, Poon LL, Chen H, Shortridge KF, Yuen KY, Webster RG, Peiris JS. "Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia". Nature, 430 (6996): 209–213, (2004).
29. World Health Organization "H5N1 avian influenza: timeline", (2005).
30. Beigel JH, Farrar J, Han AM, Hayden FG, Hyer R, de Jong MD, Lochindarat S, Nguyen TK, Nguyen TH, Tran TH, Nicoll A, Touch S, Yuen KY; Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. "Avian influenza A (H5N1) infection in humans". N. Engl. J. Med., 353 (13): 1374–1385, (2005).
31. Pandemic.org.au, H5N1 Transmission Update.
32. Gambaryan A, Tuzikov A, Pazynina G, Bovin N, Balish A, Klimov A. "Fatal Evolution of the receptor binding phenotype of influenza A (H5) viruses". Virology, 344 (2): 432–438, (2006).
33. Couch, R. "Chapter 58. Orthomyxoviruses Multiplication", in Baron, S. (ed.): Medical Microbiology. Galveston, Texas: The University of Texas Medical Branch at Galveston, (1996).
34. www.fluwikie.com
35. The prevention and treatment of viral respiratory disorders, (2007).
36. CIDRAP article Germany finds H5N1 in frozen duck meat, (2007).
37. Hot Water Burn & Scalding Graph., (2006).
38. Avian flu biofacts. CIDRAP.
39. Avian Influenza, including Influenza A (H5N1), in Humans: WHO Interim Infection Control Guideline for Health Care Facilities
40. The Threat of Global Pandemics. Council on Foreign Relations, (2005).
41. Vietnam to unveil advanced plan to fight bird flu", Reuters, (2006).
42. Chen H, Deng G, Li Z, Tian G, Li Y, Jiao P, Zhang L, Liu Z, Webster RG, Yu K. "The evolution of H5N1 influenza viruses in ducks in southern China". Proc. Natl. Acad. Sci. U. S. A., 101 (28): 10452-10457, (2004).
43. World Health Organization (W.H.O) [About.com: infectious diseases]
44. Oseltamivir (Tamiflu). National Institutes of Health, (2000).
45. CIDRAP article Study: Inhibiting cytokine response might not reverse H5N1 infections, 2007.
46. Alan Sipress. "Bird Flu Drug Rendered Useless: Chinese Chickens Given Medication Made for Humans", Washington Post, (2005).
47. WHO sees role for older antivirals in some H5N1 cases", CIDRAP, (2006).
48. CIDRAP article Pandemic Influenza, (2006).
49. IFPMA glossary.
50. Eurekalert article Universal flu vaccine being tested on humans, (2007).
51. MedImmune And National Institutes Of Health Begin Clinical Testing Of A Live, Attenuated Intranasal Vaccine Against An H5N1 Avian Influenza Virus.
52. FDA approval letter.

53. The Pandemic Vaccine Puzzle - Part 3: H5N1 poses major immunologic challenges.

54. The Pandemic Vaccine Puzzle - Part 4: The promise and problems of adjuvants.