Original Article

นิพนธ์ต้นฉบับ

Comparison of Pathologic Manifestations of Influenza A H5N1 Disease in Humans and Domestic Ducks

Yavarace (Vongsivavilas) Young*

Thaweesak Songserm**

Mongkol Uiprasertkul***

*Department of Medical Services, Ministry of Public Health, Nonthaburi, Thailand

**Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen campus, Nakhon Pathom, Thailand

***Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract Recently, numerous outbreaks of highly pathogenic avian influenza, H5N1 subtype have occurred in various countries in Asia and Eastern Europe. This has caused a global concern that influenza pandemic is imminent. Since infected poultry is the source of virus for humans, mammals, and birds, early detection and control of this disease in poultry is mandatory to avoid expansion of the virus in the environment and reduce the risk of viral transmission to humans and other species. Besides molecular genetic studies, histopathology and immunohistochemical techniques are the important diagnostic tools for the detection of H5N1 disease in humans and animals. A comparative study of histopathologic changes in humans and ducks was carried out based on the postmortem materials obtained during the 2004 outbreak in Thailand. H5N1 virus caused lethal systemic infection in domestic ducks with viral antigen found in their various damaged organs and in macrophages within the spleen. Whereas pathologic changes were more limited in the fatal cases of human infection with lung involvement as the most prominent and consistent features.

Key words: **avian influenza, H5N1 virus, influenza, infectious disease, pneumonia, pathology**

In 1878, the term "fowl plague" (FP) was first used to describe a serious disease of chickens in Italy. In 1902, the causative virus was isolated for the first time but it was not identified as a member of influenza A virus family until 1955,[1]. Since then it has been called "avian influenza" (AI). AI viruses have been isolated from domestic poultry suffering from FP and from apparently healthy wild birds including waterfowl. They have also caused influenza outbreaks in mammals, such as

seals, whales, minks, pigs, and horses,[2-6]. Any AI virus regardless of its hemagglutinin designation meeting specified virulence criteria in the laboratory is designated highly pathogenic avian influenza (HPAI).

In 1996, the first known case of AI subtype H5N1 was found in a domestic goose in Guangdong, China,[7-8]. A year later, human infection with this virus was detected for the first time during the simultaneous outbreaks of H5N1 in poultry in Hong Kong,[9-11]. The 1997 outbreak resulted in 18 cases of human infection with 6 deaths and a territory-wide slaughter of more than 1.5 million chickens,[8,9]. In addition, there was a case of probable human to human transmission of the viruses which was detected by a positive anti-H5 antibody,[12]. The incident might be due to close physical contact with H5N1-infected patients. By 2000, the viruses had extended to domestic ducks which played a key role in the genesis of the subsequent outbreaks of H5N1,[7].

In late 2002, H5N1 viruses re-emerged and caused deaths among wild migratory birds and domestic waterfowl including ducks in two Hong Kong parks,[13]. In January of 2003, H5N1 disease was diagnosed in two patients with acute respiratory distress, one of them died,[13,14]. This was the first reported lethal outbreak of AI infection in wild aquatic birds since 1961,[13]. Since mid 2003, lethal outbreaks of H5N1 in poultry have occurred in several countries in Southeast Asia and China. In spring of 2005, more than 6000 migrating aquatic birds were found dead due to H5N1 infection at the Qinghai Lake in Qinghai, China,[15].

Recently, the viruses have spread rapidly from western China to Russia, Kazakhstan, Turkey, Romania, and Kuwait. So far more than 140 million birds have died of the disease or been killed. The various outbreaks in Asia were also followed by human cases of direct transmission of H5N1 viruses from chickens and a case of probable human to human transmission,[16-20]. As of October 2005, more than 100 confirmed cases of human H5N1 disease with 62 deaths have been reported, all in Asia. Antigenic and sequence analyses indicated that all genes of the H5N1 human isolates were of avian origin, the reassortants of multiple co-circulating AI viruses,[8, 21-26].

Meanwhile, H5N1 virus seems to be finding its way into more and more species. Once known to infect chickens, ducks, and occasional humans, the virus is now found in a wide range of birds including migratory birds and has infected tigers, leopards, cats, mice, ferrets, pigs and dogs,[27-29]. This causes an increasing concern that the virus is spreading unchecked. The wide host range suggests adaptation of the virus to various species, thus increases its chance of reassortment into a more virulent strain that can easily be transmitted among people and causes a deadly pandemic. At this time no one knows how long it takes for the virus to develop such capability.

The 2004 outbreak of H5N1 disease claimed the lives of more than 30 million birds and 12 human beings among 17 confirmed cases in Thailand. Since infected chickens are clearly the source of virus for humans and ducks, it is very important for physicians and pathologists to have the greater awareness of this disease in animals. Meanwhile, veterinarians should be interested in this disorder in humans as well. So far numerous researches have focused on the molecular genetic basis for biological characteristics of this virus but data on pathology of this disease is very limited. We did a comparative study of histopathologic changes in humans and domestic ducks from the 2004 outbreak in Thailand. Now it is time for us

to share the fruit of endeavor, and hopefully the study of these autopsy materials will help us explain the expansion of host range and lethality of the virus in humans and domestic ducks.

Methodology

Virology studies of the 2004 epidemics of poultry in Thailand confirmed the causative virus of being HPAI of H5N1 subtype. Postmortem examination was performed on 15,000 to 16,000 dead domestic ducks from 157 diseased duck flocks. They were of various ages ranged from 1 to 12 months. The average of 15 to 20 representative dead ducks from each flock was necropsied and their internal organs were removed for gross and microscopic examination. Tissue sections of various organs were embedded in paraffin and tissue blocks were cut at 4 μ, then stained with hematoxylin and eosin (H&E). Immunohistochemical stain of the internal organs was done in some selected cases.

There were 12 deaths among 17 confirmed human cases of H5N1 disease (70.59% mortality rate). A complete autopsy was performed in 2 cases and their pathology reports were published earlier,[16,17,20]. H&E stained slides of autopsy material of the 6-year-old boy (case 1) and an H&E stained slide of the lung of the 26-year-old woman

Table 1 Clinical manifestations of avian influenza H5N1 disease in humans and domestic ducks

Clinical Data	Domestic Ducks	Case 1 (Human)	Case 2 (Human)
Clinical Symptoms and Signs	Thirsty, inappetence, swollen & watery eyes, opaque cornea, decreased egg production, diarrhea, ruffled feathers, cyanosis of unfeathered skin, edema of head, face & neck, rales, sinusitis. Neurological signs: ataxia, torticollis, drowsiness, convulsion, & fatal	Fever, cough, dyspnea, and drowsiness Right lower lobe pneumonia Acute respiratory distress syndrome Multiple organ failure	Fever, Pneumonia, and acute respiratory distress syndrome
Blood tests	Leukopenia, lymphopenia, thrombocytopenia	Same as ducks	Same as ducks
Chest X-rays	None	Right lower lobe infiltrates	Bilateral lower lobe consolidation
Exposure to poultry or poultry products	Occurred during outbreaks in poultry	Contacted with dead chickens	No history of exposure to poultry but providing unprotected nursing care for her daughter who was a probable case of H5N1 infection
Treatment	None	Multiple broad-spectrum antibiotics, granulocyte colony-stimulating factor then Oseltamivir and methylprednisone	Antibiotics
Outcome	Died within 24 to 48 hours of the onset of illness	Died at 17 days after the onset of illness	Died at 9 days after the onset of illness

Table 2 Histopathologic findings of avian influenza H5N1 disease in humans and domestic ducks

Pathology	Domestic Ducks	Case 1 (Human)	Case 2 (Human)
Hematoxylin & Eosin stain	Brain: non-suppurative encephalitis, peri-vascular cuffing with mononuclear cells, gliosis, neuronal degeneration, meningitis, & myelitis Heart: fibrinoid necrosis, myocarditis Lungs: edema, congestion, interstitial pneumonitis, DAD, diffuse hemorrhage Pancreas: necrotizing hemorrhagic pancreatitis, Liver: fatty changes Intestine: foci of mucosal necrosis Spleen & Harderian gland: focal necrosis, lymphoid depletion Kidneys: tubular necrosis Nasolacrimal gland: focal necrosis & inflammation	Brain: edema, necrotic foci, meningitis Lungs: proliferative to organizing phase of DAD, interstitial pneumonitis, focal hemorrhage, bronchiolitis, pneumocytes type 2 hyperplasia & superimposed aspergillosis Liver: mild fatty changes, activated Kupffer cells & slight lymphoid infiltrates in the portal areas Bone marrow & lymph nodes: slight histiocytic hyperplasia Spleen: congestion, lymphoid depletion, focal & subcapsular hemorrhage Other organs: no significant changes	Lungs: exudative phase of DAD, interstitial pneumonitis, hemorrhage, pneumocyte hyperplasia, bronchiolitis & pleuritis pleuritis Liver: cholestasis, congestion & hemophagocytosis Spleen: congestion & lymphoid depletion
Immunohisto-chemical stain	Positive staining for H5-specific monoclonal antibody in various organs, i.e., neurons, glia cells in the brain, airway epithelia & alveolar cells, myocardium, hepatocytes, a few number of pancreatic acinar cells, renal tubular cells, cells of nasolacrimal gland, intestinal mucosal epithelium, macrophages in spleen	Positive staining for influenza A antigen in pneumocytes type 2 H5-specific RNA was detected in the lungs, spleen and intestine by RT-PCR Electron microscopy: viral particles in pneumocytes & macrophages	Positive staining for influenza A antigen in the desquamated cells of the airways H5N1 was detected by RT-PCR in the lung only

Abbreviation: DAD = Diffuse alveolar damage, RT-PCR = Reverse transcription polymerase chain reaction

(case 2) were available for a review. We also did a thorough review of research data with emphasis on histopathologic features of the H5N1 disease,[16-20].

Results

HPAI subtype H5N1 caused lethal systemic infection among chickens and domestic ducks with 100 percent mortality. Clinical and pathologic manifestations in domestic ducks were similar to those in chickens but conjunctivitis and eye discharge was less severe than those in chickens. Clinical manifestations in humans ranged from mild or asymptomatic infection to fever, leukopenia, lymphopenia, pneumonia, acute respiratory distress syndrome, multiple organ failure and death. The mortality rate in humans was 70.59 percent. The detailed clinical manifestations and histopathogic findings of human cases and domestic ducks are shown in table 1 and 2. The picture of dead, dying and convulsing ducks is shown in figure 1. Gross pathologic features of necropsied

ducks are shown in figure 2 and 3. Microscopic features of the various organs of necropsied ducks are shown in figures 4 to 8. Histopathologic findings of human cases are shown in figures 9 to 14.

Discussion

Since 1980, there have been several outbreaks of HPAI of various subtypes in domestic poultry throughout the world with occasional human infections,[30-36]. However, the H5N1 outbreak is unprecedented in its geographic reach, its extremely high pathogenic in poultry, wild birds, and mammals, and its persistence despite efforts for eradication. Human H5N1 disease is associated with higher mortality rate when compared to the other influenza subtypes.

It was once believed that AI viruses could not jump species. However, the outbreaks of H5N1 since 1997 have established that AI viruses can directly transmit to and cause lethal infection in humans, mammals, and birds. These incidents have fundamentally changed our concepts and views of HPAI. The acquisition of ability to cross-species transmission of this virus is not clearly understood. Perhaps, the virus has gained this capability as a result of being a product of natural reassortment among the genes of multiple avian species. More research is needed to clarify this.

Currently, the true prevalence of H5N1 infection in humans is not known. It may have been

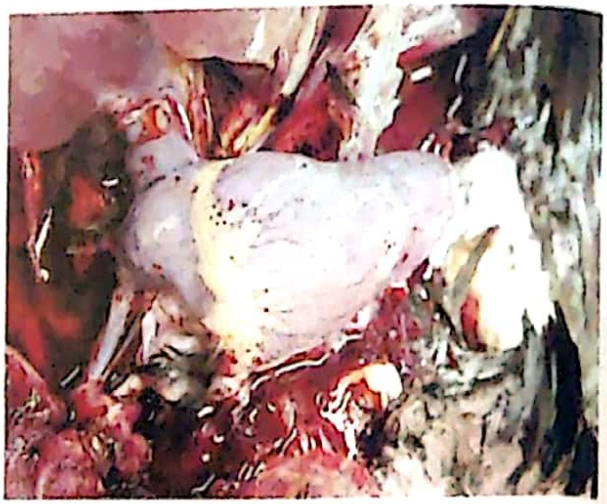

Fig. 2 A necropsied duck showing congested and edematous heart with extensive petticheal hemorrhage in the pericardium.

Fig. 1 A small cluster of recently dead, dying, and convulsing H5N1 infected ducks. The 2 sitting ducks with ruffled feathers appear drowsy. Cyanosis of the ducks' unfeathered skin is quite noticeable.

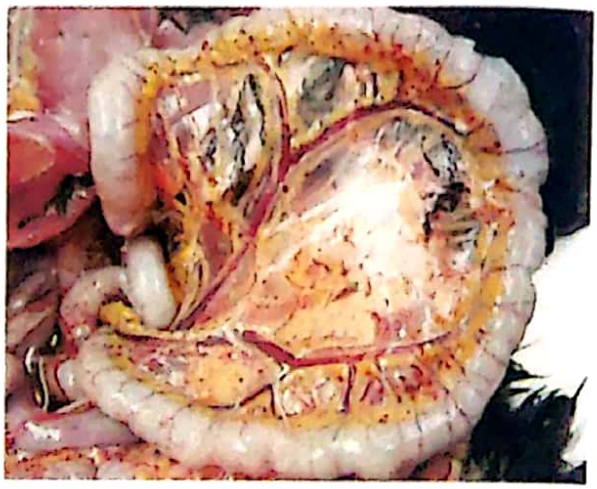

Fig. 3 A necropsied duck showing an intestine with bluish discoloration of the intestinal wall, diffuse petticheal hemorrhage, and prominent vascular congestion.

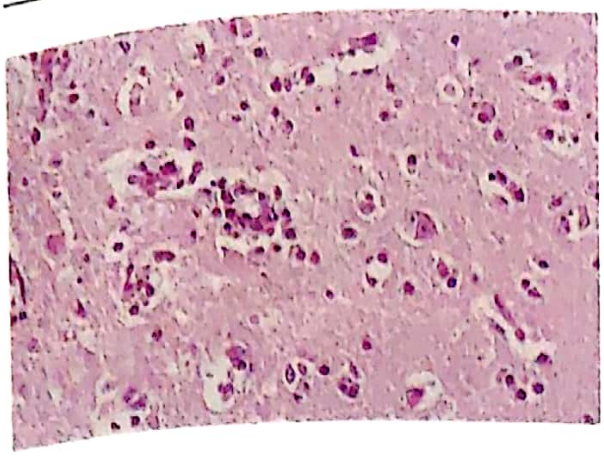

Fig. 4 Section of the duck's brain showing typical non-suppurative encephalitis, peri-vascular lymphocytic cuffing, neuronal degeneration, and gliosis, (H&E section X 400).

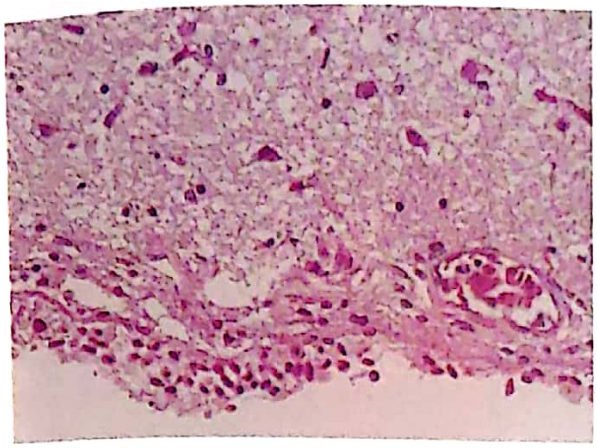

Fig. 5 Section of the duck's meninges showing hemorrhage and mononuclear cell infiltrate in the meninges, compatible with viral meningitis, (H&E section X 400).

underreported due to limited surveillance, a lack of facilities for diagnostic tests or political reason. Some cases may escape from detection due to mild or absence of symptoms, inappropriate collection of clinical samples for diagnostic tests, confusing with other mimicking ailments or otherwise misdiagnosed. H5N1 outbreak in poultry and humans serves as a "wake-up call" that the devastating "avian flu" pandemic is imminent. Therefore, we should not fall into a sense of false security that H5N1 disease is merely a hypothetical threat. Even though, efficient transmission among people has not yet been observed, pandemic preparedness is a must.

Pathologic lesions along with the presence of viral antigen in the many organs of domestic ducks indicate a wide tissue tropism which in turn is responsible for an increase in pathogenicity. Histopathologic findings in the ducks indicate lethal systemic infection. The most striking and consis-

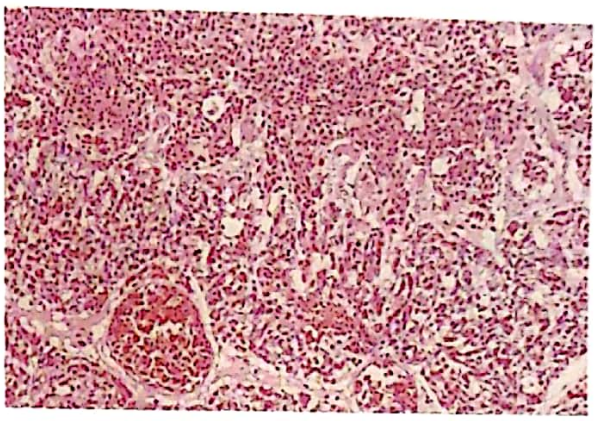

Fig. 6 Section of the duck's lung showing diffuse hemorrhage, interstitial pneumonitis, and diffuse alveolar damage, (H&E section X 200).

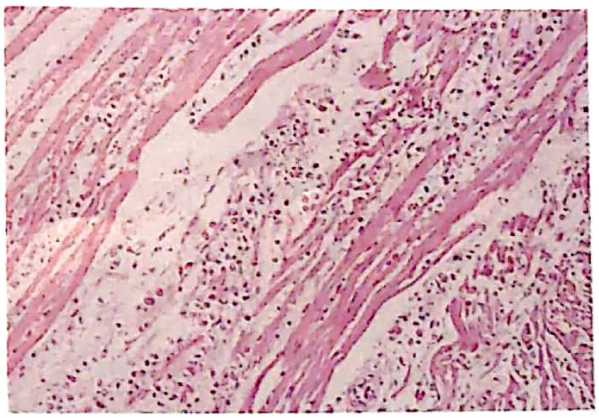

Fig. 7 Section of the duck's heart showing fibrinoid necrosis of myocardial fibers with inflammatory infiltrates and myocarditis, (H&E section X 400).

tent findings are in the brain, spinal cord, lungs, and pancreas. The less common findings are fibrinoid necrosis of cardiac muscle fibers and myocarditis. The least common abnormalities are fatty changes in the liver, multifocal necrosis of the intestine, renal tubular necrosis, focal necrosis in the spleen and naso-lacrimal gland. The authors believe the frequency and severity of the lesions in each organ were probably related to tissue tropism, host species, and how long the ducks had survived before their deaths occurred. In some cases, death might have come before a full-blown inflammatory response was mounted.

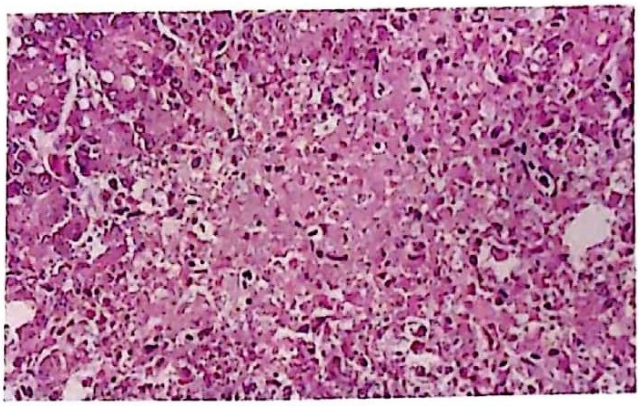

Fig. 8 Section of the duck's pancreas showing a large area of necrosis with inflammatory infiltrates, (H&E section X 400).

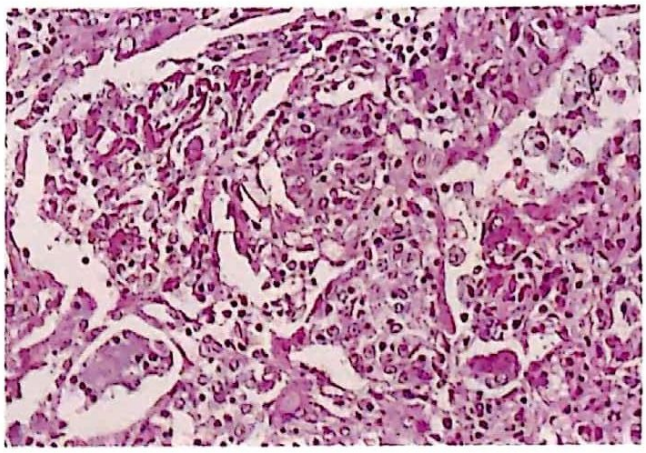

Fig. 9 Lung of human case 1 showing proliferative phase of diffuse alveolar damage, pneumocyte hyperplasia, and interstitial pneumonitis, (H&E X 200).

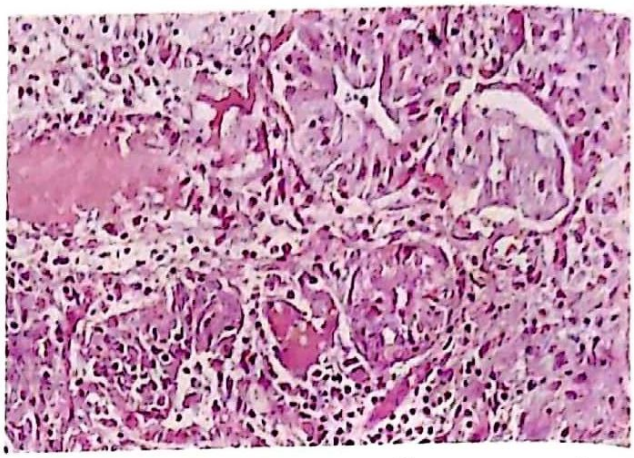

Fig. 10 Another area of the lung of human case 1 showing organized phase of diffuse alveolar damage, prominent squamous metaplasia, interstitial pneumonitis, and focal fibrosis, (H&E X 200).

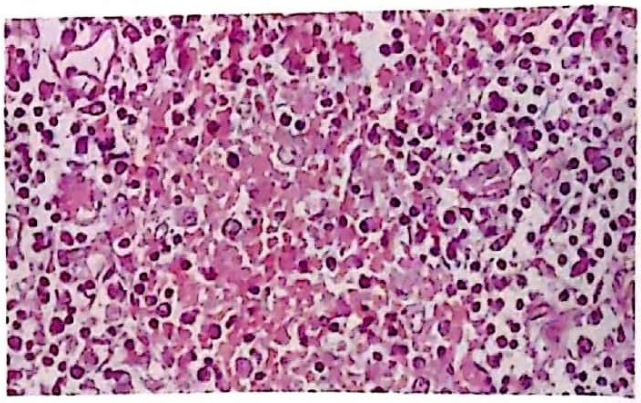

Fig. 11 Spleen of human case 1 showing focal hemorrhage and lymphoid depletion, (H&E X 200).

The cerebrum, cerebellum, and spinal cord of the ducks showed edema, perivascular cuffing with lymphocytes and glia cells, gliosis, neuronal degeneration, myelitis, and meningitis. These findings are consistent with non-suppurative meningoencephalitis and myelitis of viral etiology. Enterovirus encephalitis, Newcastle disease or Marek's disease can produce similar lesion in the brain, but widespread systemic infection along with simultaneous outbreak of H5N1 in poultry makes the presumptive diagnosis of HPAI most correct. In these ducks, the diagnosis was con-

firmed by immunohistochemical stain of the tissue in paraffin blocks showing a positive staining for H5-specific monoclonal antibody in the parenchymal cells in various organs and in macrophages in spleen as shown in Table 2. Definite viral identification can be done with the use of culture from various organs and reverse transcription polymerase chain reaction (RT-PCR) technique. Virology studies also confirmed the diagnosis of H5N1 infection.

Microscopic lesions in the lungs were edema, congestion, early exudative phase of diffuse alveolar damage (DAD), interstitial pneumonitis, and hemorrhage. Similar lesions were also recognized in the lungs of human cases with this disorder but late exudative, proliferative or organizing phase of DAD were seen as humans survived a little longer after the infection. Moderate degree of pleural effusion was seen in some ducks.

Another striking finding was in the ducks' pancreas where necrotizing hemorrhagic pancreatitis was quite extensive but H5-specific monoclonal antibody reaction was seen only in a few numbers of pancreatic acinar cells. In addition to the cytopathic effect of the virus, extensive necrosis in the pancreas could very well be due to a release of pancreatic enzymes that further caused damages to the pancreatic acini.

Cardiac lesion of the ducks was grossly visualized in the pericardium where peticheal hemorrhage was quite extensive. Microscopic findings in the myocardium included loss of striation, homogeneity, and fibrinoid necrosis of cardiac muscle fibers, and myocarditis. These similar changes have previously been described in influenza infection in other avian species such as chickens and turkeys,[37-39], and also in humans,[40-42].

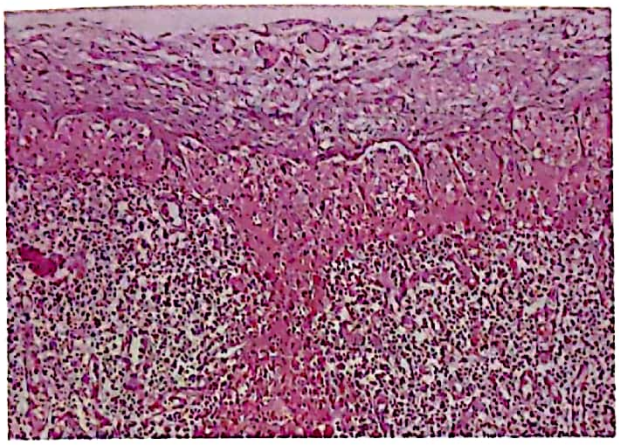

Fig. 12 Spleen of human case 1 showing edema, subcapsular hemorrhage, and lymphoid depletion, (H&E X 200).

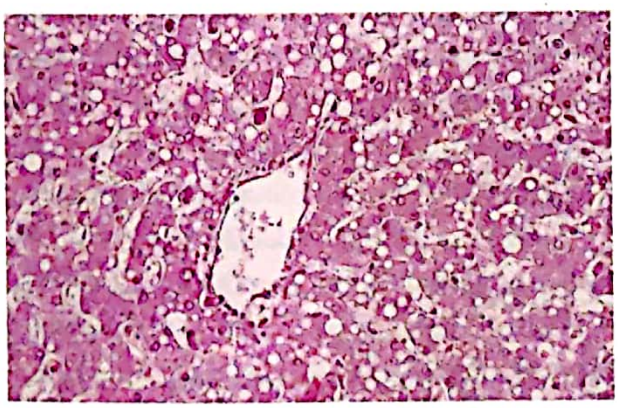

Fig. 13 Liver of human case 1 showing fatty changes, (H&E X 200).

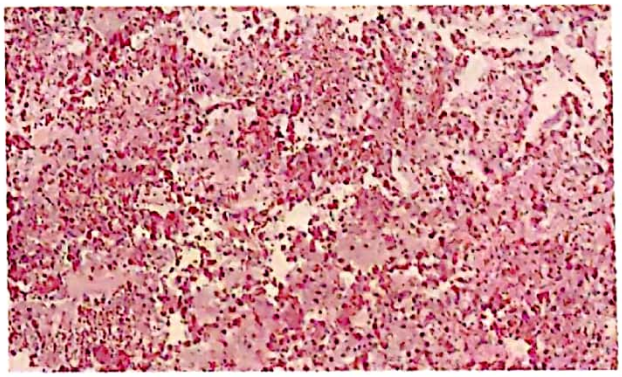

Fig. 14 Lung of human case 2 showing diffuse hemorrhage, exudative phase of diffuse alveolar damage, and interstitial pneumonitis, (H&E X 200).

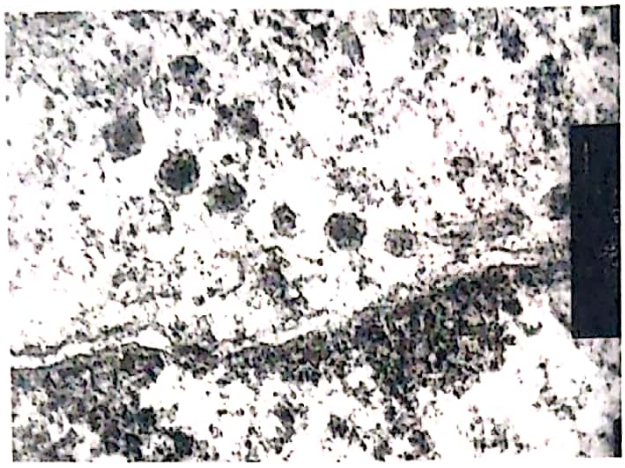

Fig. 15 Electron microscopy of the lung tissue from human case 1 showing pnuemocyte with intracellular particles, morphologically consistent with influenza viral particles, (EM X 13200).

So far cardiac involvement has not been detected in human cases with H5N1 infection.

This study demonstrated the H5N1 viruses caused more tissue damage and wider organ distribution range in ducks than in humans. In humans, the most striking pathologic findings are in the lungs with virus antigen found in pneumocytes type 2 in case 1 and in desquamated bronchial epithelium in case 2. So far, human autopsy cases have not shown viral spread beyond the lungs except case 1 whereby H5N1 viruses were isolated from the lungs, intestine, and spleen but evidence of viral replication was found only in the lungs and intestine,[16]. Undetectable viruses in the other organs could be related to limited tissue tropism, low viral titers, or the viruses were cleared up by neutralizing antibody produced by human host. Since pathologic changes occurred in the organs beyond the lungs and these changes probably represented direct cytopathic effect of the virus, we cannot help thinking of a hit and run behavior of the virus when they were not recovered from these organs.

Lesions in the lungs of human cases included pulmonary edema, congestion, DAD, interstitial pneumonitis, focal hemorrhage, and bronchiolitis. Case 1 died 17 days after the onset of illness with progressive worsening of pneumonia and acute respiratory distress syndrome. Lung lesions were compatible with these clinical manifestations. The lungs showed various phases of DAD from late exudative, proliferative and organizing phases with fibrosis and squamous metaplasia. These changes indicated that DAD did not occur at the same time. Lung lesion probably occurred at some spots in the early stage and later spread to the other parts of the lung. Pulmonary Aspergillosis in case 1 was a secondary infection and could be a complication of viral pneumonia and/or corticosteroid administration. The changes in the brain of case 1 included edema, some necrotic foci, and minimal focal lymphocytic infiltrate in the meninges. Some pathologic changes were also found in the spleen, liver, lymph nodes, and bone marrow but no significant changes were detected in the other organs.

Case 2 showed the most prominent lesions in the lungs that were closely similar to those in case 1 except exudative phase of DAD. Changes were reported to involve liver and spleen too. Although the lesions in the lungs are non-specific, they are typical lesions of pneumonia of viral etiology. Ancillary tests are needed to identify specific causative organism. Virology studies confirmed the diagnosis of H5N1 infection in these 2 human cases. The genomic sequences of the Thai H5N1 isolates from chicken and humans of the same 2004 outbreak were closely similar,[43]. The study also demonstrated that the Thai H5N1 virus was a member of the 2000's H5N1 lineage with most of the genetic sequences closely related to the Influenza A/Duck/China/E319.2/03 (H5N1),[43]. It belonged to a single genotype, des-

ignated as genotype Z,[16,44].

According to the limited number of autopsy cases available in English literatures, the most striking and consistent pathologic findings of human H5N1 disease have been in the lungs. The 4 postmortem cases of the 1997 and the autopsy case of the 2003 outbreaks in Hong Kong showed prominent lung lesions and hemophagocytosis as the most striking features,[9,14]. Therefore, it was postulated that in those 5 cases, initial viral replication in respiratory tract might trigger hypercytokinemia resulting in reactive hemophagocytotic syndrome,[9,14].

These 2 Thai cases also showed evidence of cytokine dysfunction but wider range of organ involvement when compared to the previous 5 Hong Kong cases. Case 1 showed viral mRNA and tumor necrotic factor-alpha (TNF-α) in the lung,[16]. The simultaneous presence of viral antigen and cytokine TNF-α in the same organ suggests a direct induction of cytokine in the viral infected cells. This finding concurs with previous observations that human H5N1 isolates initiate the production of cytokines, most prominently TNF-α, in cultured human macrophages in vitro,[45].

When doing an autopsy of infectious disease, pathologist usually requires ancillary tests beyond gross and microscopic examination in order to establish the organism-specific diagnoses. Viral culture is valuable for definitive diagnosis and to yield viruses for molecular epidemiological study. Viral genotyping has proved to be an invaluable tool in assessing epidemiological links between viruses from the various outbreaks. The virus isolates can be stored and retrieved for later research.

Immunohistochemical stain can be done on fresh frozen cryostat tissue or paraffin block of tissue and can be useful as a diagnostic test especially when fresh specimens are not available for culture. The stain permits visualization of specific substances such as viral antigen in morphologically preserved tissue, thus providing us a better understanding regarding the extent of viral infection relative to the host inflammatory responses. One must be well aware that a positive staining confirms the diagnosis but a negative result does not preclude the presence of viral antigen either,[46]. Actually, both viral culture and immunohistochemical techniques are complimentary diagnostic tools. Presently, immunohistochemical stain has become an integral part of a pathology laboratory.

Conclusions

H5N1 virus caused lethal systemic infection in domestic ducks with viral antigen found in their various damaged organs and in macrophages within the spleen. Whereas pathologic changes were more limited in the fatal cases of human infection with lung involvement as the most prominent and consistent features. Since infected poultry is the source of virus for humans, mammals, and birds, early detection and control of this disease in poultry is mandatory to avoid expansion of the virus in the environment and reduce the risk of viral transmission to humans and other species.

Acknowledgements

We would like to thank Mrs. Patra Moolchit and Ms. Ame-on Amesombun for technical preparation of the H&E slides of necropsied ducks and an autopsy case 1.

References

1. Schafer W. Vergleichende sero-immunologische Untersuchungen uber die Viren der Influenza und

klassischen Geflugelpest. Z Naturforsch. 1995; 10b: 81.
2. Webster RG, Hinshaw VS, Bean WJ, van Wyke KL, Geraci JR, Petursson G. Characterization of an influenza A virus from seals. Virology 1981; 113: 712-24.
3. Hinshaw VS, Bean WJ, Geraci J, Fiorelli P, Early G, Webster RG. Characterization of two influenza A viruses from a pilot whale. J Virol 1986; 58: 655-6.
4. Klingeborn B, Englund L, Rott R, Juntti N, Rockborn G. An avian influenza A virus killing a mammalian species-the mink. Brief report. Arch Virol 1985; 86 (3-4): 347-51.
5. Scholtissek C, Burger H, Bachmann PA, Hannoun C. Genetic relatedness of hemagglutinins of the H1 subtype of influenza A virus isolated from swine and birds. Virology 1983; 129: 521-3.
6. Guo Y, Wang M, Kawaoka Y, Gorman O, Ito T, Saito T, et al. Characterization of a new avian-like influenza A virus from horses in China. Virology 1992 May; 188 (1): 245-55.
7. Sims LD, Domenech J, Benigno C, Kahn S, Kamata A, Lubroth J, et al. Origin and evolution of highly pathogenic H5N1 avian influenza in Asia, Vet Rec 2005 Aug 6; 157 (6): 159-64.
8. Cauthen AN, Swayne DE, Schultz-Cherry S, Perdue ML, Suarez DL. Continued Circulation in China of Highly Pathogenic Avian Influenza Viruses Encoding the Hemagglutinin Gene Associated with the 1997 H5N1 Outbreak in Poultry and Humans. J Virol 2000 Jul; 74 (14): 6592-9.
9. Chan PK. Outbreak of Avian Influenza A (H5N1) Virus Infection in Hong Kong in 1997. CID 2002; 34 (Suppl 2): S58-63.
10. Isolation of avian influenza A (H5N1) viruses from humans-Hong Kong, May-December 1997. Morb Mortal Wkly Rep 1997; 46: 1204-7.
11. Anonymous. Influenza A (H5N1). Wkly Epidemiol Rec 1998; 73: 24.
12. Katz JM, Lim W, Bridges B, Rowe T, Hu-Primmer J, Lu X, et al. Antibody response in individuals infected with avian influenza A (H5N1) viruses and detection of anti-H5 antibody among household and social contacts. J Infect Dis 1999; 180: 1763-70.
13. Sturm-Ramirez KM, Ellis T, Bousfield B, Bissett L, Dyrting K, Rehg JE, et al. Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. J Virol 2004 May; 78 (9): 4892-901.
14. Peiris JS, Yu WC, Leung CW, Cheung CY, Ng WF, Nicholls JM, et al. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet 2004; 363:617-9.
15. Liu J, Xiao H, Lei F, Zhu Q, Qin K, Zhang XW, et al. Highly pathogenic H5N1 influenza virus infection in migratory birds. Science. 2005 Aug 19; 309 (5738): 1206.
16. Uiprasertkul M, Puthavathana P, Sangsiriwut K, Pooruk P, Srisook K, Peiris M, et al. Influenza A H5N1 replication sites in humans. Emerg Infect Dis. 2005 Jul; 11 (7): 1036-41.
17. Uiprasertkul M. Pathological Findings in Influenza A (H5N1) Infection. Siriraj Med J 2005 Jul; 57 (7): 291-92.
18. Susiva C, Limprayoon K. First fatal avian influenza A (H5N1) in Siriraj hospital: a case report. Siriraj Med J 2005 Jul; 57 (7): 272-74.
19. Hien TT, Liem NT, Dung NT, San LT, Mai PP, Chau NV, et al. Avian influenza A (H5N1) in 10 patients in Vietnam. N Engl J Med 2004 March; 350 (12): 1179-88.
20. Ungchusak K, Auewarakul P, Dowell SF, Kitphati R, Auwanit W, Puthavathana P, et al. Probable person to person transmission of avian influenza A (H5N1). N Engl J Med 2005 Jan 27; 352 (4): 333-40.
21. Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, et al. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 1998; 279: 393-6.
22. Guan Y, Shortridge KF, Krauss S, Webster RG. Molecular characterization of H9N2 influenza viruses: were they the donors of the "internal" genes of H5N1 viruses in Hong Kong? Proc Acad Sci USA 1999 Aug; 96 (16): 9363-7.
23. Hiromoto Y, Yamazaki Y, Fukushima T, Saito T, Lindstrom SE, Omoe K, et al. Evolutionary characterization of the six internal genes of H5N1 human influenza A virus. J Gen Virol 2000; 81: 1293-303.
24. Shortridge KF, Gao P, Guan Y, Ito T, Kawaoka Y, Markwell D, et al. Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective. Vet Microbiol 2000; 74: 141-47.
25. Bender C, Hall H, Huang J, Klimov A, Cox N, Hay A, et al. Characterization of the surface proteins of influenza A (H5N1) viruses isolated from humans in 1997-1998. Virology 1999 Feb 1; 254 (1): 115-23.
26. Zhou NN, Shortridge KF, Claas EC, Krauss SL, Webster RG. Rapid evolution of H5N1 influenza viruses in chickens in Hong Kong. J Virol 1999 April; 73 (4): 3366-74.
27. Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RA, Amornsin A, Payungporn S, et al. Avian influenza H5N1 in tigers and leopards. Emerg Infect Dis. 2004 Dec; 10 (12): 2189-91.
28. Amonsin A, Payungporn S, Theamboonlers A, Thanawongnuwech R, Sudadhat S, Pariyothorn N, et al. Genetic characterization of H5N1 influenza A viruses isolated from zoo tigers in Thailand. Virology (serial online) 2006, [cited 2006 Mar 7]; 344: [12 screens].

Available from:URL:http://library.searo. who.int/modules.php? op=modload&mane=websis& file=avflulit&from=avflulit1& show=1537&with=Tigers.

29. Kuiken T, Rimmelzwaan G, van Riel D, van Amerongen G, Baars M, Fouchier R, et al. Avian influenza in cats. Science 2004 Oct 8; 306 (5694): 241.
30. McNulty MS, Allan GM, McCracken RM, and McParland PJ. Isolation of a highly pathogenic influenza virus from turkeys. Avian Pathol. 1985; 14: 173-6.
31. Lin YP, Shaw M, Gregory V, Cameron K, Lim W, Klimov A, et al. Avian to human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. Proc Natl Acad Sci ASA. 2000 Aug 15; 97 (17): 9654-8.
32. Campitelli L, Mogavero E, De Marco MA, Delogu M, Puzelli S, Frezza F, et al. Interspecies transmission of an H7N3 influenza virus from wild birds to intensively reared domestic poultry in Italy. Virology. 2004 May 20; 323 (1): 24-36.
33. 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: 587-93.
34. Peiris M, Yeun KY, Leung CW, Chan KH, Ip PLS, Lai RMW, et al. Human infection with influenza H9N2. Lancet. 1999 Sept 11; 354: 916-7.
35. Cameron KR, Gregory V, Banks J, Brown IH, Alexander DJ, Hay AJ, et al. H9N2 subtype influenza A viruses in poultry in Pakistan are closely related to the H9N2 viruses responsible for human infection in Hong Kong. Virology 2000 Dec 5; 278 (1): 36-41.
36. Capua I, Alexander DJ. Avian influenza and human health. Acta Trop 2002 Jul; 83 (1): 1-6.
37. Uys CJ, Becker WB. Experimental infection of chickens with influenza A/Tern/South Africa/1961 and A/Chicken/Scotland/1959 viruses II. Pathology. J Comp Pathol 1967; 77: 167-74.
38. Jungherr EL, Tyzzer EE, Brandly CA, Moses HE. The comparative pathology of fowl plague and Newcastle disease. Am J Vet Res 1946; 7: 250-88.
39. McKenzie BE, Easterday BC, Will JA. Light and electron microscopic changes in the myocardium of influenza-infected turkeys. Am J Pathol 1972; 69: 239-54.
40. Oseasohn R, Adelson L, Kaji M. Clinicopathologic study of 33 fatal cases of Asian influenza. N Engl J Med 1959; 260: 509-18.
41. Finland M, Parker F, Barnes MW, Joliffee LS. Acute myocarditis in influenza A infection. Two cases of nonbacterial mycocarditis with isolation of virus from the lungs. Am J Med Sci 1945; 209: 455-68.
42. Lucke B, Wright Q, Kim E. Pathologic anatomy and bacteriology of influenza. Arch Intern Med 1919; 24: 154-237.
43. Viseshakul N, Thanawongnuwech R, Amonsin A, Suradhat S, Payungporn S, Keawchareon J, et al. The genome sequence analysis of H5N1 avian influenza A virus isolated from the outbreak among poultry populations in Thailand. Virology 2004 Oct 25; 328 (2): 169-76.
44. Li KS, Guan Y, Wang J, Smith GJ, Xu KM, Duan L, et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 2004; 430: 209-13.
45. 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; 360: 1831-37.
46. Guarner J, Shieh W, Dawson J, Subbarao K, Shaw M, Ferebee T, et al. Immunohistochemical and in situ hybridization studies of influenza A virus infection in human lungs. Am J Clin Pathol 2000; 114: 227-33.

บทคัดย่อ **การเปรียบเทียบพยาธิสภาพของโรคไข้หวัดนกสายพันธุ์ H5N1 ในมนุษย์และเป็ดเลี้ยง**

เยาวเรศ (วงศ์ศิวะวิลาส) ยัง*, หวีสักดี่ ส่งเสริม, มงคล อุยประเสริฐกุล*****
*กลุ่มงานพยาธิวิทยา สถาบันโรคทรวงอก กรมการแพทย์ กระทรวงสาธารณสุข, **มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จังหวัดนครปฐม, ***คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

วารสารวิชาการสาธารณสุข ๒๕๔๙; ๑๕:๔-๑๖.

เมื่อเร็ว ๆ นี้ โรคไข้หวัดนกชนิดรุนแรงสายพันธุ์ H5N1 ได้ระบาดหลายครั้งในหลาย ๆ ประเทศแถบทวีปเอเชียและยุโรปตะวันออก เหตุการณ์นี้ทำให้นานาชาติหวั่นวิตกว่าโรคไข้หวัดใหญ่ระบาดทั่วโลกใกล้จะเกิดขึ้นอย่างแน่นอน เนื่องจากว่าสัตว์ปีกที่ติดเชื้อเป็นแหล่งต้นตอของเชื้อไวรัสชนิดนี้ที่จะแพร่ไปสู่มนุษย์ สัตว์เลี้ยงลูกด้วยน้ำนมและสัตว์ปีกดังนั้นจึงควรวินิจฉัยโรคให้ได้ตั้งแต่ระยะเริ่มแรกกับทั้งควบคุมโรคนี้ในสัตว์ปีก ทั้งนี้เพื่อหลีกเลี่ยงการแพร่กระจายของเชื้อไวรัสในสิ่งแวดล้อมและลดความเสี่ยงของการแพร่เชื้อไวรัสมาสู่มนุษย์และสัตว์อื่น ๆ นอกเหนือจากการศึกษาพันธุกรรมในระดับโมเลกุลแล้ว เทคนิคทางด้านการตรวจชิ้นเนื้อและการย้อมพิเศษอิมมูโนจากชิ้นเนื้อก็เป็นเทคนิคที่สำคัญที่ใช้ในการวินิจฉัยโรคไข้หวัดนกสายพันธุ์H5N1 ในมนุษย์และสัตว์ คณะผู้วิจัยได้ศึกษาเปรียบเทียบพยาธิสภาพของชิ้นเนื้อในมนุษย์และสัตว์ จากการชันสูตรศพและชันสูตรซากเป็ดที่เสียชีวิตจากโรคไข้หวัดนกเมื่อครั้งการระบาดปี ๒๕๔๗ ในประเทศไทย จากการศึกษานี้พบว่าไวรัสสายพันธุ์ H5N1 ทำให้เกิดการติดเชื้ออย่างรุนแรงในอวัยวะทุกระบบของเป็ดเลี้ยงจนเป็นเหตุถึงตาย พร้อมทั้งได้ตรวจพบเชื้อไวรัสในอวัยวะต่าง ๆ ที่มีการเปลี่ยนแปลงทางพยาธิวิทยาและใน macrophages ภายในม้าม ส่วนในมนุษย์ผู้เสียชีวิตนั้นพบพยาธิสภาพได้ในบางอวัยวะเท่านั้นและพบพยาธิสภาพในปอดเด่นชัดที่สุดและพบในทุกราย

คำสำคัญ: **ไข้หวัดนก, ไวรัส H5N1, ไข้หวัดใหญ่, โรคติดเชื้อ, โรคปอดอักเสบ, พยาธิวิทยา**