THE PREVALENCE OF H5N1 INFLUENZA VIRUS ON POULTRY AT TRADITIONAL MARKET IN SEMARANG, INDONESIA

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ABSTRACT

Traditional market is a potential source of the spread of avian influenza virus. This research is aimed to determine the prevalence of avian influenza virus (AIV) subtype H5N1 on poultry sold in traditional market in Semarang City. Fifty-five poultry samples sold in six traditional markets in Semarang City; i.e. Karangayu market, Mangkang market, Gunungpati market, Rejomulyo market, Gayamsari market and Karimata market taken from its cloaca swab. Further, cloacal swab samples grown in pathogen-free chicken embryos aged nine days. Then, it incubated for four days at 37 °C. Allantoic fluid then collected and tested to agglutinate red blood cells. RNA extracted from the samples showed haemagglutination activity. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) applied to identify the subtype of AIV using the specific primer H5 and N1. The PCR product resulted from RT-PCR then visualized using electrophoresis. The results showed that four AIV subtype H5N1 isolates have successfully isolated from cloacal swab samples (7.27%) with the distribution of 2 isolates were from Mangkang Market (28.57%), 1 isolate was from Rejomulyo Market (7.69%) and 1 isolate was from Karimata Market. The prevalence of AIV subtype H5N1 was 28.57% in Mangkang market, 7.69% in Rejomulyo market and 9.09% in Karimata market. The prevalence values of each species were 7.89% in chicken and 9.09% in the wild duck. According to this result, poultry sold in traditional market in Semarang have a potential source of AIV subtype H5N1.

Key words: H5N1, poultry, prevalence and traditional market

INTRODUCTION

During the last years, world attention including Indonesia, were focused on the outbreak of avian influenza virus (AIV) subtype H5N1. The outbreak of AIV has caused mass death of poultry and even human. Nevertheless, the transmission of H5N1 from human to human has never been reported (Buxton *et al.* 2000; The Writing Committee WHO 2005; Kandun *et al.* 2006). The more the case of zoonotic transmission of human, the more the potential for pandemic issue. In addition, the AIV transmission to human is still considered as directly from chicken or the environment contaminated with AIV (Smith *et al.* 2006). The pandemic happens when new subtype of AIV can across the host barrier, such as between chicken and mammal, as well as human. H5N1 has potential as the cause of the influenza pandemic on human through two mechanisms. First, a direct virus infection by influenza strain will trigger reassortment causing AIV subtype H5, which transmit from human to human. Another alternative is direct mutation of H5N1 AIV that able to spread from human to human (Russell & Webster 2005).

Until recently, all highly pathogenic avian influenza (HPAI) virus plague is caused by an influenza from subtypes H5 and H7. The primary factor in the spread of HPAI virus is the distribution of poultry through human mobility (WHO 2004). Another factor is the bird migration (Capua & Marangon 2006; Chen *et al.* 2006). The analysis of global distribution of H5N1 AIV in Asia showed that 9 out of 21 viruses introduced into Asian countries was from poultry trading or poultry products. Bird migration also plays a role in the distribution and introduction of H5N1 AIV to 3 out of 21 Asian countries. In addition, the introduction of H5N1 AIV on 20 out of 23 European countries occurred through bird migration. In Africa, 2 out of 8 countries experienced introduction of H5N1 AIV through poultry trading and 3 out of 8 countries through bird migration (Kilpatrick *et al.* 2006). The introduction of H5N1 AIV to Indonesia happened more than once (Susanti *et al.* 2008a). The causes of AIV spreading is probably due to wild bird migration (Capua & Marangon 2006; Smith *et al.* 2006). In addition, it caused by the bird trading bird products (Smith *et al.* 2006) or inter-locations transportation (Kuiken *et al.* 2006).

In Indonesia, the poultry farm is mainly managed by a traditional system causing the spreading potential of AIV in Indonesia. Many poultry and waterbirds farming use the house environment without caging system. The AIV prevalence on waterfowl in domestic rearing system in West Java is relatively high. The prevalence was 6.67% in poultry, 4.85% in ducks and 4.04% in wild ducks (Susanti *et al.* 2008b). The large number of waterbirds as reservoir of H5N1 AIV has risen the risk of virus transmission to land birds and mammals including human (Xue *et al.* 2007). The transmission of AIV from waterfowl to other birds has often proven from poultry market. At the market, the contact between waterfowl to other birds is unavoidable (Capua & Marangon 2006; Gilbert *et al.* 2006; Xue *et al.* 2007). The traditional poultry markets sell chickens, ducks, wild ducks, geese, and birds that come from many areas. Start with the markets, the potential infectious AIV will spread to other fields. In the market, the poultry was placed densely in selling area. This condition facilitated the AIV spread between birds (Antara *et al.* 2009). Remembering that market is a potential place for the spread of AIV, the prevalence of AIV subtype H5N1 on poultry sold in traditional market in Semarang city, Central Java, Indonesia need to be carried out

MATERIALS AND METHODS

Sample preparation

This study was an exploratory study. In this study, the samples were chicken and waterfowl sold in 6 traditional markets in Semarang, i.e. Karangayu, Mangkang, Gunungpati, Rejomulyo, Gayamsari, and Karimata. Randomly, fifty-five samples were taken from its cloacal swab (Table 1). The cloacal swabs were rubbed and then inserted into the tube containing transport medium i.e. glycerol-phosphate buffer saline (WHO 2002). The tubes were labeled and then they placed in the cool box containing ice packs.

Table 1. Cloaca swab samples from each market by bird type

Market	Species				Number of samples
	Indigenous chicken	Duck	Wild duck	Goose	
Karangayu	8	-	-	-	8
Mangkang	7	-	-	-	7
Gunungpati	6	-	2	-	8
Rejomulyo	5	4	4	-	13
Gayamsari	8	-	-	-	8
Karimata	4	-	5	2	11
Total	38	4	11	2	55

Virus propagation

The samples were cultured in the specific pathogen-free embryo aged nine days. Firstly, the egg swabbed with alcohol 70% then bored by the egg puncher. Inoculum was then inserted into the egg cavity that have been incubated in the room temperature for 30 minutes. The swabs were inoculated in the allantoic cavity of the egg and incubated at a temperature of 37 °C and daily observed for four days. The allantoic fluid was collected to identify the capability to agglutinate the red blood cell (WHO 2002). A quick agglutination test was performed by mixing one drop of allantoic fluid with 5% chicken red blood cell (v/v). The existence of the virus was shown by red blood cell agglutination within 15 seconds after mixing. Allantoic fluid that showed the positive result were tested using U bottom microplate (Nunc). The hemagglutination test of allantoic fluid was conducted based on prevailing standard procedure (WHO 2002).

RNA Virus Extraction

Positive-tested allantoic fluid based on HA test was extracted for its RNA. The RNA isolation applied in this study was Trizol®LSReagent (Invitrogen) and performed based on the producer's instruction. RNA solution stored at a temperature of -20 °C until RT-PCR performed.

Identification of AIV subtype using RT-PCR

AIV subtypes H5 and N1 were recognized using reverse transcriptase-polymerase chain reaction (RT-PCR) using primer H5-1 (5'GCCATTCCACAACATACA CCC'3) and H5-3 (5'CTCCCCTGCTCATTGCTATG'3) (WHO 2005) as well as CU-N1F (5'GTTTGAGTCT GTTGCTTGGTC'3) and CU-N1R (5'TGATAGTGTC TGTTATTATGCC'3) (Payungporn *et al.* 2004). Samples that not included as H5N1 were further identified out by Newcastle disease virus (NDV) using primer NDVF (5'GGTGAGTCTATCCGGARGATACAAG'3) and NDVR (5'TCATTGGTTGCRGCAATGCTCT'3) (Creelan *et al.* 2002). The size of PCR products was 219 bp for H5, 131 bp for N1 and 202 bp for NDV.

In this study, RT-PCR conducted was using the SuperscriptTM III One-step RT-PCR system. PCR reaction made by using composition of 25 µl 2x reaction mix, 2 µl primer forward (10 µM), 2 µl primer reverse (10 µM), 2 µl Superscript III RT/Platinum Taq Mix, 3 µl RNA sample and ultrapure H_2O until reaching the volume of 50 µl. The Primer used to amplify the H5, and N1 genes are presented in Table 3. The RT-PCR program started by reverse transcription 45 °C for 60 minutes, continued by pre-denaturation at 95 °C for 5 min, 35 cycles consisted of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 40 seconds, and finally, the post extension at 72 °C for 10 minutes (Payungporn *et al.* 2004; WHO 2005). In order to identify virus subtypes, the isolate was amplified using H5 and N1 primers, whereas the NDV identification was performed by PCR using specific primer for NDV with annealing at 48 °C (Creelan *et al.* 2002). The particular DNA band as the result of PCR was identified using electrophoresis on 2% agarose gel.

RESULT

The hemagglutination test of 55 allantoic fluid samples showed that five isolates reacted positively and could agglutinate chicken red blood cell at titer of 10^7-10^{11}. The hemagglutination activity marked with agglutination of erythrocytes with look like the sand particle. The result of agglutination test showed that 5 out of 55 inoculums contained virus with over the threshold number that can grow in the embryonic egg (Beato *et al.* 2007; Terregino *et al.* 2007). Based on the results of red blood cell agglutination ability, five isolates were identified as *Orthomyxoviridae* (influenza virus) or *Paramyxoviridae* (ND) (OIE 2004). Both avian influenza and Newcastle disease viruses can agglutinate erythrocytes, for this reason HA test can not be used to differentiate AI virus and ND virus based on their subtype. RT-PCR confirmed the presence of AIV and to determine the AIV subtype. Beside RT-PCR technique, a sequencing of the genetic material, that encode HA and NA, could be carried out to characterize AIV subtypes (Dharmayanti *et al.* 2005; Suwarno *et al.* 2006).

The present results showed that four isolates were positive as H5 subtype (Figure 1a), and five isolates were positive for N1 subtype (Figure 1b). Further, the analysis showed that 4 out of 55 (7.27%) samples were positive for H5N1 avian influenza virus, and one sample (1.82%) was positive HxN1. Four isolates identified as AIV H5N1 subtypes, 2 isolates (28.57%) were from Mangkang market,

1 isolate (7.69%) was from Rejomulyo market, and 1 strain (9.09%) was from Karimata market, whereas the HxN1 isolate was from Gunungpati market. HxN1 isolate is an AIV type A with subtype N1 other than H5. In this study, four strains of avian influenza virus subtype H5N1 obtained consists of 3 chicken isolates and 1 wild duck isolate, with the prevalence of 7.89 % and 9.09 %, respectively (Table 2).

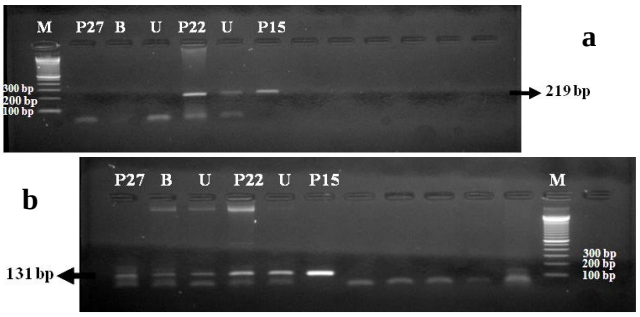

Figure 1. The visualization of the RT-PCR amplification product in 1.2% agarose gel electrophoresis. a) Amplification using haemagglutinin 5 (H5) primer pair, the positive-tested samples showed 219 bp PCR product, b) amplification using neuraminidase 1 (N1) primer pair, the positive-tested samples showed 131 bp PCR product. M= Marker, P15, P22, and P27= samples from traditional market, U and B= samples from the other places, K= positive controls.

Table 2. The number and prevalence of AIV subtype H5N1 isolated from the traditional markets

Market	Isolate of H5N1 AIV				Total
	Chicken	Duck	Wild Duck	Goose	
Karangayu	0	0	0	0	0
Mangkang	2 (28.57%)	0	0	0	2 (28.57%)
Gunungpati	0	0	0	0	0
Rejomulyo	1(20,00%)	0	0	0	1(7.69%)
Gayamsari	0	0	0	0	0
Karimata	0	0	1(20,00%)	0	1(9,09%)
Total	**3(7.89%)**	**0**	**1(9.09 %)**	**0**	**4(7.27%)**

DISCUSSION

Reverse transcription polymerase chain reaction (RT-PCR) is now routinely used for diagnosis of avian influenza virus subtype. This method has high sensitivity and high specificity even in a small amount of the virus genetic materials (WHO 2002; Payungporn *et al*. 2004; OIE 2005). In this study, the subtyping method were used the primer pair of H5-1 and H5-3 (WHO 2005a) and also CU-N1F and CU-N1R (Payungporn *et al*. 2004). In the case of the virus subtype that not classified as H5 and N1, the primer pair of Newcastle disease virus (NDV) is used for further identification (Creelan *et al*. 2002). The PCR product of these three primer pairs are small and precise, i.e. 219 bp for H5, 131 bp for N1 dan 202 bp for NDV (Payungporn *et al*. 2004).

According to the results of this study, the prevalence of AIV subtype H5N1 on chicken and wild duck sold in the traditional markets in Semarang was relatively high. The prevalence in chicken was 7.89% and in the wild duck was 9.09%. This data showed that poultry sold in traditional market in Semarang was potentially as source of transmission of AIV H5N1 between birds and human. The same case also reported in Hongkong, i.e. the prevalence of H5N1 in the poultry market in Hongkong (1997) was 19.5% in chicken, followed by 2.5% in geese and 2.4% in duck (Shortridge 1997). The high prevalence AIV in traditional market in Semarang was probably caused by placement pattern of various poultry (land bird and water bird) in all ages in one place. For this reason, the chance of virus transmission is also high. Mixing the poultry from different ages and types in a single location will increase the rate of AIV transmission and can cause AIV persistent in that place (Swayne & Halvorson 2003). In water bird, AIV subtype H5N1 could not produce clinical symptom because water bird is a natural host of AIV. In land bird, it will be more highly pathogenic (Hulse-Post *et al.* 2005). In 2006, someone in Guangzhou (China) was suspected to have H5N1 virus infection after visiting poultry market. The same case has also reported in Hanoi, Vietnam in 2001, where H5N1 virus was also detected in domestic poultry market (Wang *et al.* 2006).

In the present study, duck and geese were not infected by AIV and showed a 0% of AIV prevalence. The little number of AIV prevalence in waterfowl was probably due to the lower number of sample of geese and duck in the market. In point of fact, it supported by previous researchers, the prevalence of H5N1 was higher in waterfowl than in chicken. A study by Susanti *et al.* (2008a) showed that the prevalence of AIV on waterfowl in West Java was 6.67% in geese, 4.85% in ducks and 4.04% in wild ducks. In between of 2002 and 2004, the prevalence of H5N1 in South China was greater in domestic waterbird (2.94%) than in the land bird (0.89%) (Guan *et al.* 2002; Li *et al.* 2004; Chen *et al.* 2006; Smith *et al.* 2006). More, in Nanchang poultry market, in China, the prevalence of AIV was high in duck (1.3%), followed by chicken (1.2%), quail (0.8%) and then pigeon (0.5%) (Liu *et al.* 2003). In addition, the prevalence of HPAI virus subtypes H5, H7, H9 in water birds in Minnesota was 21.5%. The subtypes H3, H4, and H6 reached 63.8%. In addition, the prevalence of AIV subtypes H5 and H9 were 0.4% each and H7 was 0.7% (Hanson *et al.* 2003). Xue *et al.* (2007) studied the prevalence of AIV on domestic duck in live poultry market in China during 2002-2006. The result showed that the prevalence of H5 was 27.2%, H3 and H6 were 15.42% and 11.23%, respectively. In China, H5N1 was isolated for 2.5 years (January 2004 to June 2006). The results showed that the prevalence of H5N1 in duck was 3.03%, and then in the wild duck was 2.68% (Chen *et al.* 2006).

H5N1 isolated from traditional markets in Semarang need to be characterized molecularly and biologically to know its virulence in poultry and mammals (including human). Based on the previous study in South China, H5N1 isolated from healthy duck was molecularly and biologically pathogenic for chicken and mammal (mouse). The molecular-based on pathogenicity and the ability to transmit inter-species (from poultry to mammal) were apparently involved various virus genes, including haemagglutinin gene (HA) (Chen *et al.* 2004).

In addition, the finding of AIV other than subtype H5N1 in this research (i.e. HxN1) also need further review. Cocirculation of AIV H5N1 increases the risk of emergence of new virus strain due to reassortment. The genotyping of AIV H5N1 isolated from healthy waterbird in South China showed that the virus was a result of reassortment between HA gene of A/Gs/Gd/1/96 virus and AIV Eurasia. The reassortment formed 9 genotypes (Chen *et al.* 2004). The emergence of G genotype of AIV subtype H5N1 from Vietnam duck isolate (Dk/VNM/568/05) was the result of reassortment of AIV Z genotype with PB2 gene from other AIV (Smith *et al.* 2006).

Other researchers also reported the role of traditional market as the place of AIV transmission. Leung *et al.* (2007) stated that live poultry market plays a significant role in conservation proliferation and transmission of AIV. The poultry market is also the risk factor of the AIV H5N1 spread to human (Shortridge 1997; Mount *et al.* 1999; Badiwangsa 2007; Dinh *et al.* 2007). The condition is possible due to human mobility to buy live poultry and poultry products in the market. Asian traditional market is a contact point for human to the poultry, so the place is potential as the primary source of infection and virus proliferation (Webster 2004). In addition, it was reported that many AIV cases on human happen on those who work or live near the living poultry (WHO 2004). The increase of AIV infection and proliferation in Asia during 2003 to 2004 related to the distribution of waterbird as the AIV reservoir. Also, the living poultry market as the source of larger environmental contamination (Sims *et al.* 2005). The traditional poultry market in Phitsanulok Province, Thailand, is one of the chains for the HPAI (H5N1) distribution (Paul *et al.* 2013). The risk of AIV infection on traditional poultry market suppressed by measures from seller who apply AIV response management. The management such as not mixed various kind of bird in one cage, and doing routine disinfection on the cage used. Jacob *et al.* (2003) stated that the high risk of AIV infection through poultry market was due to inadequate sanitation. The biosecurity condition on the poultry market in Tasikmalaya district showed that 4.5% placed in the same cage (Sugiarti 2009).

Minnesota Board of Animal Health stated that there are many factors considered in controlling AIV on live poultry, i.e. suppliers, brokers, customers, markets, and one-way traffic. The poultry origin also determines the distribution pattern in the poultry market. Poultry sold by the seller can become from various areas. This poultry source can show the source of AIV infection if AIV case found in the market. The other markets as the place of the seller to sell the bird can also explain the origin of the AIV source. Virus distributed to those areas through brokers and the equipment used by the seller in selling the poultry. The trade pattern in traditional market potential to spread AIV to other fields (Antara *et al.* 2009). Yulisma (2012) showed that one of critical points of AIV contamination in traditional market in Aceh Besar is chicken and duck that placed in a temporary cage.

Poultry market is potential as AIV transmission from waterbird to the landbird, where the contact between the waterbird and other birds is unavoidable (Wheaver 2005; Gilbert *et al.* 2006). The important factor that trigger pandemic is the dense population of poultry, pig, and human because this play role in AIV evolution. In the traditional market, the chickens, ducks, wild ducks, geese, and pig are sold in the market. Various bird placed in very close area. This condition facilitates AIV transmission between the animals (Antara *et al.* 2009).

The AIV transmission from birds to others animal has been proven to happen in the poultry market, where the contact between chicken, quail and other birds is unavoidable (Capua & Marangon 2006; Gilbert *et al.* 2006; Xue *et al.* 2007). With the high potential of traditional market as a source of AIV subtype H5N1 transmission, it is needed to improve the rearing management and poultry trading. The avoidance measures of HPAI H5N1 virus can be done by regulating the live poultry market so that the seller did not mix various bird in one location (Capua & Marangon 2006; Cristalli & Capua 2007).

REFERENCE

Antara IMS., Suartha IN., Wiryana IKS., Sukada IM., Wirata W., *et al.*, 2009. Pola Distribusi Unggas dari Pasar Tradisonal Berperan dalam Penyebaran Virus Flu Burung. *Jurnal Veteriner* 10 (2):104-110.

Badiwangsa IGN. 2007. *Penyidikan Faktor-Faktor RisikoTertular Flu Burung Desa Desa Di Kabupaten Klungkung, Bali*. Dinas Peternakan, Perikanan dan Kelautan Kabupaten Klungkung.

Beato MS., Toffan A., Nardi R De., Cristalli A., Terregino C., *et al.*, 2007. A conventional, inactivated oil emulsion vaccine suppresses shedding and prevents viral meat colonisation in commercial (Pekin) ducks challenged with HPAI H5N1. *Vaccine* 25: 4054-4072

Buxton BC., Katz JM., Seto WH., Chan PK., Tsanq D., *et al.*,. 2000. Risk of influenza A (H5N1) infection among healt care workers exposed to patients with influenza A (H5N1) HongKong. *J Infect Dis* 181: 344-348

Capua I. & Marangon S., 2006. Control of avian influenza in poultry. *Emerg Infec Dis* 12 (9): 1319-1324.

Chen H., Deng G., Li Z., Tian G., Li Y., *et al.*, 2004. The evolution of H5N1 influenza viruses in ducks in southern China. *Proc Natl Acad Sci* USA 101: 10452-10457

Chen H., Smith GJD., Li KS., Wang J., Fan XH., *et al.*, 2006. Establishment Of Multiple Sublineages Of H5N1 Influenza Virus In Asia: Implications For Pandemic Control. *Proc Natl Acad Sci USA* 103 (8): 2845-2850.

Creelan JL., Graham DA., McCullough SJ., 2002. Detection and differentiation of pathogenecity of avian paramixovirus serotype 1 from field cases using one-step reverse transcriptase-polymerase chain reaction. *Avian Pathol* 31: 493-499

Cristalli A., & Capua I. 2007. Practical problems in controlling H5N1 high pathogenicity avian ifluenza at village level in Vietnam and introduction of biosecurity measures. *Avian Dis* 51: 461-462

Dharmayanti NLPI., Indriani R., Damayanti R., & Wiyono A., 2005. Isolasi dan Identifikasi Wabah Avian Influenza pada Bulan Oktober 2004-Maret 2005 di Indonesia. *Biol. Indon*. III (9):341-350.

Dinh PN., Long HT., Tien NTK., Hien NT., Mai LTQ., *et al.*, 2007. Risk Factor for Human Infection with Avian Influenza A H5N1, Vietnam. Emerg Infect Dis 13 (9):

Gilbert M., Chaitaweesub P., Parakamawongsa T., Premashthira S., Tiensin T., *et al.*, 2006. Free-Grazing Ducks And Highly Pathogenic Avian Influenza, Thailand. *Emerg Infec Dis* 12 (2):227-234.

Guan Y., Peiris M., Kong KF., Dyrting KC., Ellis TM., *et al.*, 2002. H5N1 influenza viruses isolated from geese in southeastern Cina: evidence for genetic reassortment and interspecies transmission to duck. *Virology* 292: 16-23

Hanson BA., Stallknecht DE., Swayne DE., Lewis LA., Senne DA., 2003. Avian influenza in Minnesota ducks during 1998-2000. *Avian Dis* 47: 867-871

Hulse-Post DJ., Sturm-Ramirez KM., Humberd J., Seiler P., Govorkova EA., *et al.*, 2005. Role Of Domestic Ducks In The Propagation And Biological Evolution Of Highly Pathogenic H5N1 Influenza Viruses In Asia. *Proc Natl Acad Sci USA* 102 (30): 10682-10687

Jacob JP., Butcher GD., Mather FB., Miles RD., 2003. Avian Influenza in Poultry. University of Florida. http://edis.ifas.ufl.edu.

Kandun IN., Wibisono H., Sedyaningsih ER., Yusharmen, Hadisoedarsuno W., Purba W., *et al.*. 2006. Three Indonesian clusters of H5N1 virus infection in 2005. *N Engl J Med* 355: 2186-2194

Kilpatrick AM., Chmura AA., Gibbons DW., Fleischer RC., Marra PP., Daszak P., 2006. Predicting the global spread of H5N1 avian influenza. *Proc Natl Acad Sci USA* 103: 19368-19373

Kuiken T., Holmes EC., McCauley J., Rimmelzwaan GF., Williams CS., Grenfell BT., 2006. Host species barriers to influenza virus infections. *Science* 312: 394-397

Li KS., Guan Y., Wang J., Smith GJ., Xu KM., *et al.* 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 430: 209-213

Liu M., Guan Y., Peiris M., He S., Webby RJ., *et al.* 2003. The Quest Of Influenza A Virus For New Host. *Avian Dis* 47: 849-856.

Leung YHC., Zhang LJ., Chow CK., Tsang CL., Chi-Fung NG., *et al.* 2007. Poultry Dringking Water Used For Avian Influenza Surveillance. *Emerg Infec Dis*. 13 (9) :

Mounts AW., Kwong H., Izurieta HS., Ho Y., Au T., Lee M., 1999. Case Control Study of Risk Factor for Avian Infuenza A (H5N1) Disease. Hong Kong, 1997. *J Infec Dis*. 180:505-508.

[OIE] Office international des Epizooties. 2004. Manual of diagnostic test and vaccines for terrestrial animal. Avian Influenza. 5th Edition. http://www.oie.int/[21 Oktober 2006]

Paul M., Baritaux V., Wongnarkpet S., Poolkhet C., Thanapongtharm W., *et al.*, 2013. Practices associated with Highly Pathogenic Avian Influenza spread in traditional poultry marketing chains: Social and economic perspectives. *Acta Trop*. 126(1):43-53

Payungporn S., Phakdeewirot P., Chutinimitkul S., Theamboonlers A., Keawcharoen J., *et al.*, 2004. Single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) for influenza A virus subtype H5N1 detection. *Viral Immunol* 17(4): 588-593

Russell CJ. & Webster RG. 2005. The genesis of a pandemic influenza virus. *Cell* 123:368-371

Shortridge KF. 1997. Poultry ang the influenza H5N1 outbreak in Hong Kong, 1997: abridged chronology and virus isolation. *Vaccine* 17: 826-829

Sims LD., Domenech J., Benigno C., Kahn S., Kamata A., *et al.*, 2005. Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *Vet Rec*. 157(6):159-64

Smith GDJ., Naipospos TSP., Nguyen TD., Jong MD je, Vijaikrishna D., *et al.*, 2006. Evolution and adaptation of H5N1 influenza virus in avian and human hosts in Indonesia and Vietnam. *Virology* 350: 258-268

Sugiarti D. 2009. Kondisi Biosekuriti Pada Tempat Penjualan Unggas Hidup di Pasar Tradisional di Kabupaten Tasikmalaya dan Risikonya Terhadap Penyebaran Avian Influenza [Skripsi]. Bogor: Bogor Agriculture Institute

Susanti R., Soejoedono RD., Mahardika IGNK., Wibawan IWT. & Suhartono MT. 2008a. Filogenetik dan

struktur antigenik virus avian influenza subtipe H5N1 isolat unggas air. *Jurnal Veteriner* 9 (3): 99-106

Susanti R., Soejoedono RD., Mahardika IGNK., Wibawan IWT. 2008b. Prevalence of Avian Influenza Virus Subtype H5N1 in Waterfowl in West Java Province of Indonesia. *International Journal of Infectious Diseases*. 12. Supplement 1: e127

Suwarno, Rahardjo AP., Fauziah & Srihanto EA. 2006. Karakterisasi Virus Avian Influenza dengan Uji Serologik dan Reverse Transcriptase-Polymerase Chain Reaction. *Media Kedokteran Hewan* 22 (2):74-78.

Swayne D. & Halvorson D. 2003. Terjemahan Artikel "Diseases of Poultry" Edisi ke-11. Paeco Agung, Surabaya

Terregino C., Toffan A., Beato MS., De Nardi R., Drago A., Capua I. 2007. Conventional H5N9 vaccine supresses shedding in specific-pathogen-free birds challenged with HPAI H5N1 A/Chicken/Yamaguchi/7/2004. *Avian Dis* 51: 495-497

The Writing Committee of the World Health Organization (WHO) Consultation of Human Inflenza A/H5. 2005. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 353: 1374-1384

Wang M., Di B., Zhou DH., Zheng BJ., Jing H., *et al.*, 2006. Food Markets with Live Birds as Source of Avian Influenza. *Emerg Infec Dis* 12 (11): 1773-1775.

Weaver T. 2005. Avian influenza surveys in waterfowl part I: The Role Of Wild And Domestic Waterfowl In Avian Influenza Outbreaks In Domestic Poultry. *NAHSS Outlook*. February 2005. On line at *www.aphis.usda.gov/* [diakses tanggal 30 April 2009].

Webster RG. 2004. Wet markets-a continuing source of severe acute respiratory syndrome and influenza? *Lancet*. 363:234–236

[WHO] World Health Organization. 2002. WHO manual on animal influenza. Diagnosis and surveillance. On line at *www.who.int/* [diakses tanggal 27 September 2009].

[WHO] World Health Organization. 2004. Avian flu: avian influenza-Thailand update. World Health Organization. Southeast Asian section. November 2004. [cited 2004 Nov 26] Available from http://w3.whosea.org/en/Section10/Section1027_6761.htm

[WHO] World Health Organization. 2005. Recommended laboratory tests to identify avian influenza A virus in specimens from humans. http://www.who.int/ [Juni 2005]

Xue F., Peng D., Peng Y., Gu M., Qian Z., Zhang X., Liu X. 2007. Latent infection of avian influenza viruses in domestic ducks in Eastern China and the molecular genetic evolution of H5N1 influenza A viruses. *In*: Zhou J & Yan H (Ed). The 15th World Veterinary Poultry Congress Abstract Book. Beijing 11-14 September 2007: 124

Yulisma R. 2012. Identifikasi cemaran virus avian influenza di pasar tradisional Kabupaten Aceh Besar Provinsi Aceh [Tesis]. Yogyakarta: Gadjah Mada University.