AVIAN INFLUENZA H5N1 IDENTIFICATION IN AVIAN SPECIES SURROUNDING AVIAN INFLUENZA H5N1 HUMAN CASES IN BEKASI, WEST JAVA, 2011)

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ABSTRACT

H5N1 subtype Avian Influenza (AI) virus is the causal agent of AI disease in humans. In Indonesia, the first human AI occurred in Tangerang 2005. Human AI in Indonesia has now spread into 12 provinces, including West Java, Jakarta, Banten, North Sumatra, East Java, Central Java, Lampung, South Sulawesi, West Sumatra, South Sumatra, Riau, and Bali. Until 2011, the total human AI cases were 182 cases with 150 deaths. This study was conducted to identify of H5N1 AI virus in birds in area surrounding a human AI human case in Bekasi city in March 2011 and to investigate its role in the spread of AI to humans using methods of Hemaglutination Inhibition (HI), and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The result showed that 80% of birds in the area surrounding AI surrounding H5N1 AI human case in Bekasi 2011 were antibody negative against H5N1-AI virus. Antibody against H5N1-AI virus with the titer less than 4 log 2 was detected in 4.4% of birds and with antibody titer 04 4-7 log 2 in 15% of birds. By RT-PCR, H5N1 AI virus was not detected in 47.6% of bird samples. H5 positive and N1 negative AI virus was detected in 30.2% samples. Only 11.2% samples showed positive for H5N1 AI virus. The results suggest that H5N1-AI virus affecting birds may have a positive role in transmitting to the virus to human in Bekasi 2011.

Keywords : H5N1subtype AI virus, birds, HI and RT-PCR

INTRODUCTION

Diseases that occur because of transmission between humans, wild animals, and pets have an important effect to public health general, economy, poultry, and protection of wildlife. Lately, results of surveys show there are over 1,400 species of pathogens in humans and over half known to be zoonotic (Taylor etal., 2001). Survey results show that pathogens considered to be the cause of the outbreak disease is most likely a pathogen which is zoonotic compared to pathogens which is not zoonotic (Woolhouse et al., 2001). H5N1 bird flu is an example of pathogens which is known as the cause of the occurrence outbreaks of disease in humans.

In 1997, the spread of bird flu H5N1 from poultry to humans first in report in Hongkong (Mounts et al., 1999; Shortridge et al., 2000). Next, the plague bird flu disease causes death in humans in Vietnam and Thailand with 22 people died from 33 cases of bird flu in humans that occurred in January 2004. The cause is the subtype bird flu virus H5N1 and is the biggest epidemic on commercial poultry (WHO, 2004) in a number other countries in Asia such as Cambodia, China, Indonesia, Japan, and Korea. Case of flu This bird causes more than 100 million birds die or be destroyed in two the first month of 2004 (Fleck, 2004, WHO, 2004).

In Indonesia, the case of bird flu in humans first occurred in Tangerang, Banten in 2005. The case of bird flu on humans in Indonesia today have spread across 12 provinces of West Java, DKI Jakarta, Banten, North Sumatra, East Java, Java Central, Lampung, South Sulawesi, Sumatra West, South Sumatra, Riau, and Bali. Since cases of bird flu in humans found in Indonesia in 2005, the cumulative number the case is 182, with resulting in 150 deaths up to November 2011 (MOH, 2011).

The death rate from flu High birds usually occur together with potential epidemics in humans, so greater attention is focused on humans because the bird flu virus is highly pathogenic on human. Natural reservoir of bird flu virus is

waterfowl. Waterfowl can become infected by avian or low strain avian flu virus pathogenic by showing clinical symptoms not severe or without symptoms clinical at all. Influenza virus is secreted along with poultry feces infected with bird flu and the transmission occurs directly or indirectly (Horimoto and Kawaoka, 2001).

Transmission of bird flu virus from poultry to humans occur directly or not directly. How to spread bird flu virus which is likely to be the cause cases of bird flu in humans in the city of Bekasi in March of that year causing one of its citizens to die. From the results of identification, the cause is a virus bird flu subtype H5N1. In connection with the research aimedto identify and identify viruses bird flu subtype H5N1 in the poultry be around the case of bird flu cases on human in Bekasi City in March year 2011 and how the role of poultry is within the spread of bird flu virus to humans. This research is done by method Hemaglutination Inhibition (HI), and Reverse Transcriptase-Polymerase Chain Reaction (RTPCR) which aims to know the distribution antibodies and genetic characteristics of the flu virus the H5N1 subtype bird.

RESEARCH METHODS

Sampling

Blood serum samples and cloacal swabs poultry, cage swabs, and cutting board obtained from around the location of residence the victim who died and identified positive H5N1 subtype bird flu. Blood serum samples were taken from chickens and swans, cloacal swabs from chickens, geese, and some ornamental birds, cage swabs taken from cages or bird cages ornamental birds, while the cutting of the cutting board was obtained from the cutting board which is used to cut the product poultry in the market. The sample used consisted of 45 serum samples, 18 cloacal swab samples, 45 swab samples, and one cutting board. Complete sample data are presented in Table 1.

Cloacal swab samples, cage swabs, and subsequent cutting of the cutting board is stored in the media transport Dulbecco's Modified Eagle Medium (DMEM) and antibiotics (penicillin 2000 units / mL, sterptomycin 2 mg / mL). Serum samples, swabs cloaca, wipe the cage, and wipe the cutting board immediately checked after incubation for 1-2 hours on room temperature. If the sample is not allows to be done as soon as possible, then the sample is kept at 4oC during temperature four days. More sample storage of the four days stored at a temperature of -80oC (OIE, 2008).

Hemaglutination Inhibition (HI) Test

Serological examination by HI test done to know the existence formation of antibodies against bird flu virus H5N1 subtypes that can be observed on day-to- 7 to 10 post-infections. HI test performed according to the standard (OIE, 2008). Titer HI is calculated on the basis of the highest dilution serum samples are still inhibiting agglutination of red blood cells perfectly, and expressed in log 2.

Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) Test

RNA extraction is done with using QIAmp RNA viral mini kit (Qiagen) commercially available and its use according to the instructions of use with slight modifications. RT-PCR reaction performed using Superscript III one Step RT-PCR system (Life Technology). Primer H5, and the RT-PCR program used in accordance with Lee et al., (2001) while the primary N1 and RT-PCR programs which is used in accordance with Wright et al., (1995). The amplification results are visualized with UV transilluminators and documented.

Table 1. Obtaining the number of blood serum samples and swabs of poultry originating from around the case bird flu in humans in Kelurahan JakaMulya , South Bekasi district, Kota Bekasi in 2011

No	RT/RW Kelurahan	Type of Animal	Number of Sample			
			Serum	Cloacal swab	Cage swab	Cutting board swab
1	**Wet Market**	Ayam Broiler	33	11	-	1
		Ayam Kampung	9	3	-	-
2	RT 1/RW 4	Burung Kacer	-	-	1	-
		Burung Kerocokan	-	-	2	-
		Burung Decu	-	-	2	-
		Burung Gondoijo	-	-	1	-
		Burung Perkutut	-	-	3	-
		Burung Kenari	-	-	1	-
		Burung Beo	-	-	1	-
		Burung Wambi	-	-	1	-
		Burung Jalak Suren	-	-	2	-
		Burung Jalakijo	-	-	1	-
		Burung Prenjak	-	-	1	-
3	RT 2/RW 4	Burung Perkutut	-	-	1	-
		Burung Anis	-	-	1	-
4	RT 4/RW 4	Burung Puter	-	1	3	
		Burung Tekukur	-	1	-	-
		Burung Perkutut	-	-	2	-
		Burung Kerocokan	-	-	2	-
		Burung Jalak Kebo	-	-	2	-
		Burung Cucak Rawa	-	-	1	-
		Burung Kutilang	-	-	1	-
		Burung Jalak Nias	-	-	1	-
		Burung Kacer	-	-	3	-
		Burung Srindit	-	-	1	-
		Burung Cucak Biru	-	-	1	-
		Burung Pok Mandarin	-	-	1	-
		Burung Beo	-	-	1	-
		Burung Puter Kapas	-	-	2	-
5	Jl.Bojong	Ayam Kampung	-	1	-	-
		Burung Puter	-	-	1	-
		Burung Ciblek	-	-	1	-
		Burung Kenari	-	-	1	-
		Burung Kerocokan	-	-	1	-
		Burung Cucakijo	-	-	1	-
		B.Titok	-	-	1	-
		Angsa	3	1	-	-
Total sample obtained			45	18	45	1

Ayam : Chicken; *Burung* : Bird; *Angsa* : Goose;

RESULT AND DISCUSSION

HI test results from poultry samples obtained from around the house of the victim of the flu case H5N1 subtype birds are presented in Table 2.

Table 2. HI test results of poultry serum samples obtained from approximately subtype bird flu cases H5N1 in humans in Kelurahan JakaMulya, South Bekasi District, Bekasi City, West Java in 2011

No	Address	Spesies	Number of Sample	Titer HI (log 2)			
				-ve	<4	4-7	>7
1	Wet Market	Ayam Broiler	33	27	2	4	0
		Ayam Buras	9	9	0	0	0
2	Jl.Bojong	Angsa	3	0	0	3	0
	Total		45	36	2	7	0

Ayam : Chicken; *Burung* : Bird; *Angsa* : Goose;
Explanation : ve is negative antibody titer

HI test results on 45 serum samples poultry showed that 36 serum samples react negatively to bird flu antigen subtype H5N1 but reacts positively with titers <4 log 2 for two serum samples, and as many seven serum samples with titers 4 to 7 log 2. The picture shows that serum birds taken from poultry around most victims' homes do not have antibody titer against bird flu (36 serum) but has a bird flu antibody titer low (2 serum), titer of bird flu antibody medium (7 serum) and no samples showed high titer of bird flu antibody. RT-PCR test result of swab sample cloaca, wipe the cage, and wipe the environment poultry around the H5N1 flu burng subtype case in humans is presented in Table 3.

Result of RT-PCR test to sample from wipe the cloaca, wipe the cage, and rub the environment that comes from the poultry around the victim's house of the flu case bird subtype H5N1 in humans shows that as many as 30 samples which comes from cloacal swabs and cage swabs poultry shows negative results against primary H5 so it is not continued to further RT-PCR testing with primary N1. There are, however, 19 samples collected from cloacal swabs, and wipe the cage showed a positive result on H5 primer but negative to primary N1. Nevertheless there are seven samples of swabs cloaca, wipe the cage, and wipe the environment identified as bird flu subtype H5N1 because it is able to amplify H5 and primers N1, as it obtained fragments of 545 bp with H5 primer and 616 fragment fragments bp with primary N1.

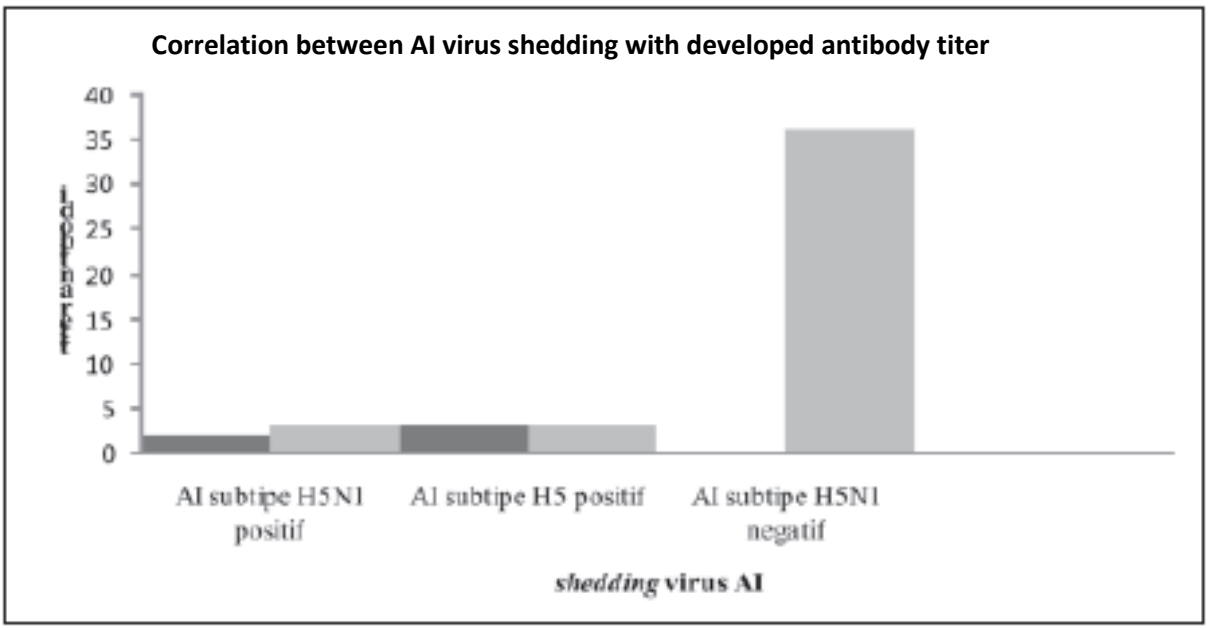
Generally, poultry or wild birds can be infected by viruses influenza. These birds include chickens, turkey, duck, pearl chicken, goose pet, quail, wild bird, chicken forests, seagulls, sea birds, birds beach. Some poultry infected with influenza virus may present with clinical symptoms or without clinical symptoms (Easterday et al., 1997; Webster and Kawaoka, 1988). Condition the area around where the victim lived died from infected bird flu subtype H5N1 shows that most the community keeps the ornamental birds as pets and there are several people which keeps the ornamental birds for sale back. The ornamental bird is stuck in a cage and mostly hanged in front of or on the terrace of that house have a great chance to get in touch with the surrounding community. Although the victim who died from subtype bird flu cases H5N1 does not keep poultry included ornamental birds in his home but neighbors around the victim's home included his mother-in-law keeps the ornamental birds stuck in a cage and hung on yard or terrace of the house. Victims of H5N1 subtype H5 flu cases over time certain also make a visit home his father-in-law. Before infected, the victim as well make visits to traditional markets with the purpose of shopping for daily needs. About 100 meters from the victim's home, There is a traditional market that sells poultry life that operates

daily (Figure 2). Based on observations, poultry that around the victim's home birds in humans no one showing clinical symptoms of bird flu. However, this needs to be wary of because birds are infected with bird flu virus not always showing clinical symptoms but still able to secrete AI virus through fesesnya.

Table 3. Result of RT-PCR test of swab sample of cloaca, swab the cage and wipe the poultry environment obtained from approximately cases of H5N1 subtype bird flu in humans in Kelurahan JakaMulya, South Bekasi District, Bekasi City, West Java in 2011

No	Address	Species	Number of Sample	Result of RT-PCR	
				Subtipe H5	Subtipe N1
1	Wet Market	Ayam Broiler	11	8	2
		Talenan	1	1	1
		Ayam Buras	3	3	3
2	RT1/RW4	Burung Kacer	1	0	0
		Burung Kerocokan	2	1	0
		Burung Decu	2	2	0
		Burung Gondolijo	1	0	0
		Burung Perkutut	3	1	1
		Burung Kenari	1	1	0
		Burung Beo	1	1	0
		Burung Wambi	1	1	0
		Burung Jalak Suren	2	1	0
		Burung Jalak Ijo	1	0	0
		Burung Prenjak	1	0	0
3	RT 2/RW 4	Burung Perkutut	1	0	0
		Burung Anis	1	0	0
4	RT 4/RW 4	Burung Puter	5	1	0
		Burung Kerocokan	2	0	0
		Burung Perkutut	2	2	0
		Burung Kutilang	1	0	0
		Burung Jalak Kebo	2	0	0
		Burung Jalak Nias	1	1	0
		Burung Kacer	3	1	0
		Burung Srindit	1	0	0
		Burung Cucak Biru	1	0	0
		Burung Poksai Mandarin	1	0	0
		Burung Beo	1	0	0
		Burung Tekukur	1	1	0
		Burung Cucak Rawa	1	0	0
5	Jl.Bojong	Ayam Buras	1	0	0
		Burung Puter	1	0	0
		Burung Ciblek	1	0	0
		Burung Kenari	1	0	0
		Burung Kerocokan	1	0	0
		Burung Cucakijo	1	0	0
		Burung Titok	1	0	0
		Angsa	1	0	0
	Total		63	26	7

Correlation between flu virus presence H5N1 subtype birds with antibody reactions against the H5N1 subtype avian influenza antigen formed shows that as many as two positive samples of avian influenza subtype H5N1 virus, and antibodies react positively to the antigen bird flu subtype H5N1 while as much three samples showed positive for virus bird flu subtype H5N1 but no antibodies reacting to the subtype bird flu antigen H5N1. The sample identified the flu virus H5 subtype birds and antibodies react positively against the H5N1 subtype H5 avian influenza antigen as many as three samples, while the sample is positive against the H5 flu virus subtype H5 but negative antibodies against avian influenza antigen H5N1 subtypes of three samples. A number 36 samples showing negative results against H5N1 and subtype H5N1 avian influenza viruses no antibodies to flu antigen are formed bird subtype H5N1. Influenza virus is secreted from the channel indigestion of poultry infected through feces for seven days maybe even up to 21 (Webster et al., 1978; Kida et al., 1980). Avian influenza virus transmission can occur through aerosols and other contaminated materials bird flu virus. When poultry is infected Bird flu emits bird flu virus in large quantities through his feces, some materials such as feed, water, equipment, and the cage is contaminated with bird flu virus and contribute to the spread of the flu virus birds (Ito et al., 1995).
Explanation : Black graph -> positive antibody

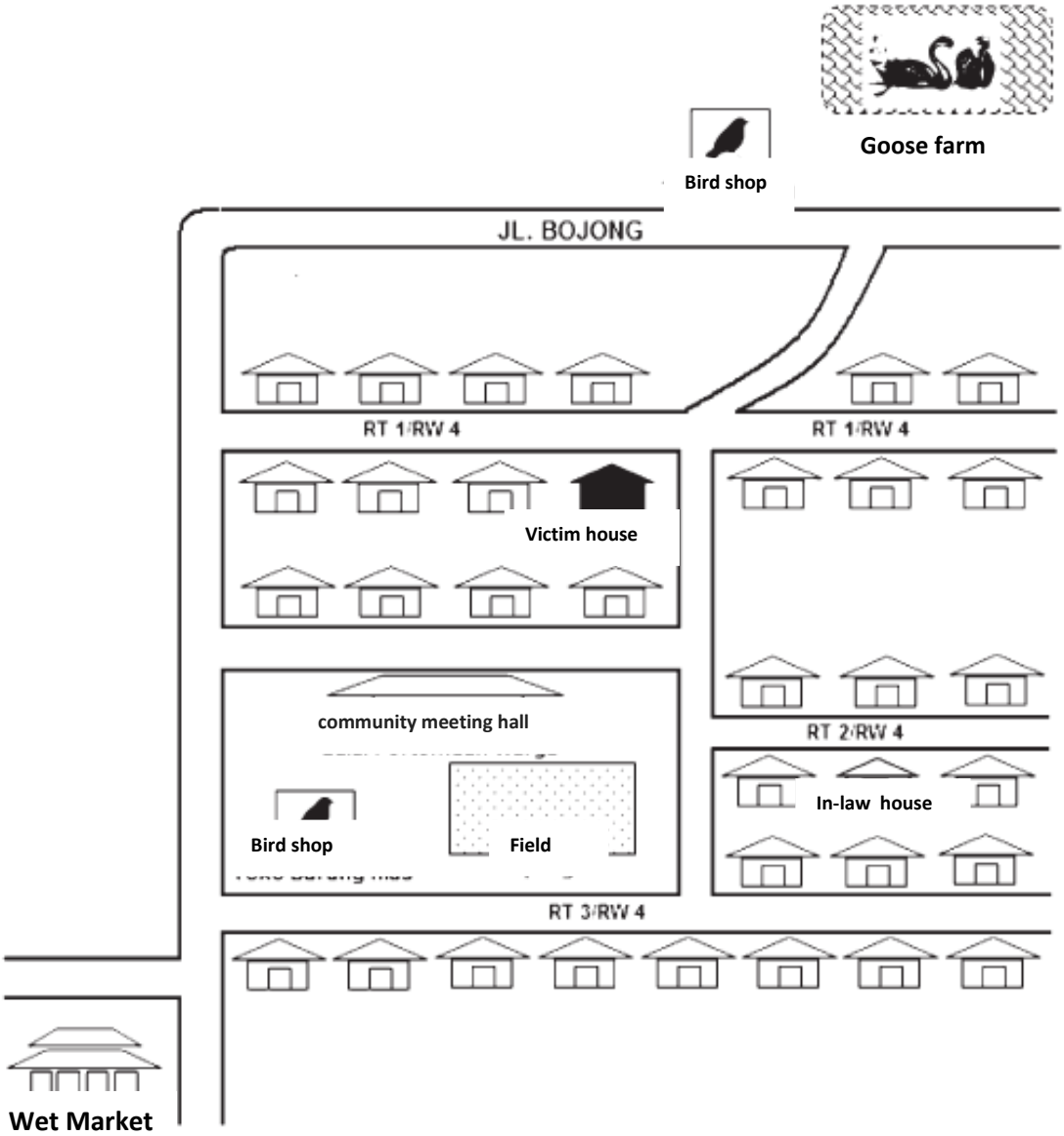


Grey graph -> negative antibody

Picture 1. The correlation shedding of H5N1 subtype H5N1 influenza virus and antibody reaction against H5N1 subtype H5 avian influenza antigen.

Most of the people who are keep ornamental birds and poultry traders living in the traditional markets do not use disinfectant when cleaning the cage or cage. Cage cleaning is done by using water periodically with different frequencies between each owners. This condition is possible bird flu virus can survive deep a longer period of time on the material such as equipment and cages that are not disinfected periodically. According to reports Lombardi et al., (2008), some disinfectants such as 5% acetic acid, citric acid 1% and 3%, calcium hypochlorite 750 ppm, sodium hypochlorite 750 ppm effective to inactivate the flu virus birds on objects with porous surfaces or nonporous. Conventional detergent with varying concentrations as well known to be able to activate the flu virus good birds on objects with surfaces porous and nonporous.

Implementation of strict biosecurity such as use of disinfectants as well as programs vaccination are the factors that play a role to minimize the spread AI virus to the environment. Cage poultry in around bird flu casualties in humans, no received a deep disinfectant treatment cleaning cages and equipment as well the poultry is not getting vaccinated bird flu so the secretion of bird flu virus of birds infected with bird flu and presence of bird flu virus in the environment can take place in more time long. Research on the development of a prototype vaccine inactive bird flu subtype H5N1 local and isolate its application is reported Indriani et al., (2005) that the secretion of the virus challenges bird flu from body of a broiler chicken vaccinated bird flu Inactive H5N1 is no longer detected seventh day. Lierz et al., (2007) reported that the bird flu vaccination program is applied able to provide protection on eagle through the shedding of that virus decreased, and decreased the risk of bird flu virus transmission to other types of poultry and human.



Picture 3. Plan the location of H5N1 subtype bird flu cases in humans in Kelurahan JakaMulya, Kecamatan Bekasi Selatan, Kota Bekasi, West Java in 2011.

Generally the bird flu virus does not replicate efficiently in humans. This matter indicates that direct transmission bird flu virus from humans is rare. For example, bird flu virus in dosage height required to be able to replicate in the human body (Beare and Webster, 1991). During the poultry outbreak that occurred in Pennsylvania 1983-1984, no report cases of diseases that resemble influenza in high-risk humans exposed by HPAI virus. This condition lasts so long that an emerging allegations that the control program strict bird flu virus growth able to prevent pandemic danger influenza caused by new strains influenza virus. Nevertheless, this assumption broken with the discovery of virus isolates bird flu subtype H7 isolated from human with conjunctival symptoms in Hongkong in 1996. Source of this virus has to do with the existing waterfowl around the scene. In 1996 also, avian flu virus subtype H9N2 can isolated from two children with symptoms mild influenza. The virus is genetic have close kinship with H5N1 subtype bird flu virus and gengen the virus plays a role in the spread bird flu virus from poultry to humans. Surveillance of live poultry markets in Hong Kong in 1997 reported that the H5N2 subtype bird flu virus in addition the H5N1 subtype is isolated from the market (Guan et al., 1999; Kurtz et al., 1999; Peiris et al., 1999). Bird flu outbreaks that occurred in Hong Kong this is unique and indicates that possible bird flu virus pandemic can occurs through direct and / or transmission reassortment or adaptation in the human body.
However, how is transmission bird flu virus in humans still not certainly.

In Indonesia, the H5N1 subtype bird flu virus which can be isolated from poultry around the case bird flu subtype H5N1 in humans still recognize avian receptor (á2,3) and not yet know human receptor (á2,6) so infection in humans is likely to be infected of birds that were previously infected with the virus bird flu subtype H5N1. Bird flu virus isolated from chickens around the case of bird flu H5N1 subtype in humans has genetic characters on the NS1 are interesting so chances are correlated with adaptation of the virus in humans. Virus reassortant possibilities are also found at Indonesia obtained from bird flu virus
Indonesian H5N1 subtype and avian influenza virus subtype H3N2 Hongkong who experienced genetic reassortment. This reassortant virus has the HA, NA, and M genes originating from avian influenza virus subtype H5N1 Indonesia, while the NS1 protein comes from the flu virus H3N2 Hongkong subtype birds. Character virus bird flu that infects humans have differences with bird flu virus which only infects poultry. Substitution amino acids typical of M1 and M2 proteins only found on viruses that come from human or bird virus isolated around the human bird flu case most likely a virus that causing infection in humans. Virus origin poultry that is not isolated from infection cases human bird flu has no substitution the. H5N1 subtype bird flu disease the endemic in Indonesia requires our vigilance, the possibility genetic reassortment between avian influenza viruses subtypes of H5N1 and H1N1 novels and viruses other influenza such as seasonal H1N1 / H3N2 flu that is likely to cause a virus H5N1 subtype bird flu is easier adapt to humans (Dharmayanti, 2009; Dharmayanti et al., 2011).

The correlation between the presence of viral shedding bird flu subtype H5N1 with antibodies formed also observed in this study. Poultry newly infected with bird flu virus subtype H5N1, the examination shows the existence of shedding of H5NI subtype H5 flu virus, but antibodies to AI subtype antigens H5N1 has not yet formed, while the poultry is no H5N1 subtype bird flu virus was found but serology results indicate a reaction antibodies against subtype avian influenza antigen H5N1. This indicates that poultry was once infected with bird flu virus. The condition to watch out for is if birds are identified as having flu virus bird subtype H5N1 and antibodies against H5N1 subtype H5N1 avian influenza antigen reacts positive, then this situation will cause shedding the avian influenza virus subtype H5N1 high numbers, and high risk of possible outbreak of H5N1 subtype bird flu. Case of H5N1 subtype bird flu in humans usually found along with outbreaks bird flu subtype H5N1 in poultry. There shedding H5N1 and subtype H5N1 avian influenza viruses the formation of H5N1 subtype H5 avian influenza antibodies which are found, require special attention against the danger of shedding the H5N1 high flu virus subtype virus. This situation is likely to be a risk factor which is high against the transmission of bird flu virus subtype H5N1 to surrounding environment included human.

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The live poultry market becomes a place which provides an optimal opportunity against the transmission and evolution of the disease zoonosis due to being the main contact spot between humans and live poultry (Guan et al., 2007; Webster, 2004). That part of the neighbourhood often contaminated is the process slaughter of poultry and cutting process carcasses after such poultry slaughter on sales and waste disposal. This contamination may occur due to the process cuts produce waste possibly containing virus particles and potential internal organ disposal contains a large number of viruses. Although slaughter is done on the spot apart, contamination may occur in the process sales and waste disposal through carcass and internal organ expenditure usually done in slaughterhouses poultry and kiosks selling poultry meat (Indriani et al., 2010). The existence of a cutting board used meat traders in the market is one of the risk factors of flu virus transmission birds from birds to humans because of cutting boards usually used as a base for cutting meat and discharging internal organs poultry when the buying and selling process occurs between traders and buyers.

CONCLUSION

H5N1 subtype avian influenza virus can identified from poultry and the environment at around the case of bird flu in humans. The existence of H5N1 subtype H5 virus shows the correlation with the formation antibodies against subtype avian influenza antigen H5N1 in birds around the case of bird flu in humans. Circulation of bird flu virus H5N1 subtype in birds around bird flu cases in humans play a role in the transmission of bird flu virus from poultry to humans both directly or indirectly.

RECOMMENDATION

Strategy of controlling the spread of flu virus birds that have become the program the government should always be real applied in the field in order efforts to cut out the spreading link AI virus from poultry to humans. Other than that, good cooperation between each agencies related to health animals and people and society should always nurtured in control and effort eradication of the spread of bird flu virus
from birds to humans.

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