Jurnal Veteriner
pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional SK. No. 15/XI/Dirjen Dikti/2011

Juni 2016 Vol. 17 No. 2 : 205-210
DOI: 10.19087/jveteriner.2016.17.2.205
online pada http://ojs.unud.ac.id/php.index/jvet

Avian Influenza Virus H5N1 Remained Exist in Gastrointestinal Tracts of House Flies 24 Hours Post-infection)

(VIRUS FLU BURUNG H5N1 TETAP BERADA DALAM SALURAN PENCERNAAN LALAT RUMAH 24 JAM PASCAINFEKSI)

Tisna Tyasasmaya[1], Hastari Wuryastuty[2], Wasito[3], Kai Sievert[4]

[1]Post Graduated Student, Faculty of Veterinary Medicine,
[2]Department of Internal Medicine, [3]Department of Pathology,
Faculty of Veterinary Medicine, Gadjah Mada University,
Jl. Fauna No. 2, Karangmalang, Yogyakarta 55281,
Indonesia, Phone: (0274) 560861
Email : tisnatyasasmaya@yahoo.com
[4]Syngenta Crop Protection AG, Basel, Switzerland
Schwarzwaldallee 215, 4002 Basel, Switzerland
Phone: +41 613239509, Email : kai.sievert@syngenta.com

ABSTRACT

House flies (*Musca domestica* L.) are one of the major pests found in poultry farms resulting in not only annoyance and indirect damage to the poultry production but also transmitting many infectious organisms mechanically and biologically. A highly pathogenic avian influenza virus H5N1 (HPAIV H5N1) collected from field house flies in Java Island, have fully succeeded identified and isolated in 2008. The AIV H5N1 isolates were further used in the present study to determine the presence and persistence of the AIV H5N1 in the laboratory infected house flies. One hundred house flies from a free AIV poultry farm in Yogyakarta, Indonesia were used in this study. The collected house flies were fasted for 12 hours then divided equally in control and treated groups. The treated group was allowed to imbibe Dulbecco's modified eagle medium (DMEM) containing AIV H5N1 while the control group imbibed medium without virus for one hour. The flies from each group were collected at 12 and 24 hours post-exposure, respectively. All flies were immobilized at 4°C, immersed in absolute ethanol for a few seconds and dissected under the stereomicroscope to collect the gastrointestinal (GI) tracts. Based on the RT-PCR results, it is concluded that AIV H5N1 remained exist in GI tracts of house flies for at least 24 hours post-exposure.

Key words: house flies (*Musca domestica* L.), mechanical and biological vectors, avian influenza virus H5N1, gastrointestinal tracts, RT-PCR

ABSTRAK

Lalat rumah (*Musca domestica* Linnaeus) telah diketahui dapat mentransmisikan berbagai agen infeksius secara mekanis maupun biologis. *Highly pathogenic avian influenza virus* H5N1 (HPAIV H5N1) telah berhasil diidentifikasi dan diisolasi dari lalat rumah yang diambil di Pulau Jawa, Indonesia. Isolat virus flu burung H5N1 tersebut kemudian digunakan dalam penelitian ini untuk mengetahui keberadaan virus flu burung dalam saluran pencernaan lalat rumah yang diinfeksi dengan HPAIV H5N1 di laboratorium. Lalat rumah diambil dari satu peternakan ayam di Yogyakarta, Indonesia, digunakan dalam penelitian ini. Seratus lalat rumah dipuasakan selama 12 jam dan dibagi menjadi kelompok kontrol dan perlakuan. Kelompok perlakuan diberi *dulbecco's modified eagle medium* (DMEM) yang mengandung isolat virus H5N1, sedangkan kelompok kontrol diberi media tanpa isolat virus selama masing-masing satu jam. Lalat dari tiap kelompok dikoleksi setelah 12 dan 24 jam pascainfeksi, kemudian diimobilisasi pada suhu 4°C, disterilkan dengan ethanol absolut dan dipotong bagian abdomennya di bawah *stereomicroscope*. Dari hasil analisa RT-PCR dapat disimpulkan bahwa virus flu burung H5N1 tetap berada dalam tubuh lalat hingga 24 jam pascainfeksi.

Kata-kata kunci : lalat rumah (*Musca domestica* L.), vektor mekanis dan biologis, virus flu burung H5N1, traktus gastrointestinal, RT-PCR

INTRODUCTION

The avian influenza virus (AIV) is a type A influenza belongs to Orthomyxovirdae virus family, and has been an important pathogen for the chicken in poultry industry worldwide for many years (Spackman, 2008). Based on the survey done by food and agriculture organization of The United Nations, Indonesia is the worst country hit by AIV. Highly pathogenic avian influenza virus has entered Indonesia since 2003 which is now endemic in 31 of 33 provinces and has the potential to cause significantly economic loss for the poultry producer and also consumers (Patrick *et al*., 2008). It was reported that there is a decrease mortality number of poultry infected by AIV in Indonesia from 2006 (2751 cases) to 2014 (306 cases), and the total number of human cases for AIV from 2003 to 2015 is 197 cases with 165 deaths (WHO, 2013). The causes of AIV outbreak are still complicated by several factors. One of the important factor is "the back-yard poultry system" (Sector 4) in Indonesia that is developed by most people who live in the villages and sub-urban areas for economic reasons who generally lack of knowledge about AIV. It is likely that Sectors 2 and 3 also play an important role in AIV outbreak. Sector 2 or known as medium-scale commercial poultry system relies on natural airflow through the shed and chickens are kept in wire cages. Sector 3 or small-scale commercial poultry system uses local building materials consisting of timber or mud bricks and bamboo where chicks are brooded, pullets are reared and layers are kept in a floor based system or cages (Glatz and Pym, 2006). These conditions may give an opportunity to another birds species for invading the farm and become the main factor of AI transmission. The low biosecurity in sector 4 may be able also to cause the AI virus spread easily between the sick and healthy poultry.

It is known that house flies (*Musca domestica* Linnaeus) are the most dominant insect in poultry farm and are able to transmit more than 65 pathogens, such as parasites, bacteria and viruses (Greenberg, 1973; Axtell, 1999). In the previous field research study done by Wuryastuty *et al*. (2008), it was reported that house flies (*M. domestica* L.) collected from poultry farms in three different provinces, East Java (Malang, Blitar and Tuban), South Sulawesi (Sidrap) and Central Java (Karanganyar), Indonesia were positive for the AIV H5N1 after being assayed with immunohistochemical streptavidin biotin and reverse transcriptase polymearse chain reaction assays (RT-PCR) (Wuryastuty and Wasito, 2013). The AIV H5N1 of Indonesian isolates from this previous study were used in the present study. The objective of this study is to determine the presence and persistence of the AIV H5N1 in the laboratory infected *M. domestica* L. *in vivo*.

RESEACH METHODS

House Flies

Adult house flies (*M. domestica* L.) both females and males were randomly collected from a poultry farm (battery layer farm) Yogyakarta, Indonesia which never had AIV outbreak before. House flies were collected using cotton plugs which were already immersed in saturated sugar and placed in falcon tubes under the chicken cages to attract the flies. House flies were then adapted for days before infection.

Flies Infection

A total of 100 house flies were fasted for 12 hours and divided equally into control and treated groups. The treated group was allowed to imbibe high glucose dulbecco's modified eagle medium (DMEM) containing AIV H5N1 while the control group imbibed DMEM without virus for one hour. The AIV H5N1 isolates used in the present study were obtained from a field case in the previous study (Wuryastuty *et al*., 2008). The virus isolates were already inoculated and propagated in embryonated chicken eggs and analyzed using RT-PCR. The research was done in the Department of Internal Medicine at Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia.

NP, N1 and H5 Influenza A Virus Gene Detection from House Flies

Flies from each group were collected 12 and 24 hours post infection respectively. House flies were then immobilized at 4°C and dipped in absolute ethanol for a few second, to make sure that AIV found in samples were only from inside house fly bodies. After that, flies were immersed in sterile aquadest to avoid any false negative during the RT-PCR because ethanol can inhibit the enzymes used in RT-PCR. Flies were then dissected under stereomicroscope to collect the gastrointestinal (GI). The collected GI tract were extracted using Trizol solution and analyzed

molecularly using RT-PCR technique for NP (552 bp), N1 (616 bp) and H5 (290 bp) gene, followed by gel electrophoresis.

RESULT AND DISCUSSION

Based on the previous research done by Wuryastuty *et al*. (2008) from the field cases in Indonesia, it was found that house flies are able to harbor the AIV outside and inside their body. It was visualized by immunohistochemical staining using monoclonal antibody anti nucleoprotein that AIV appeared in house flies body surface, muscle and also in reproductive tract. The objective of this present study was to study the ability of house flies to take up and harbor AIV H5N1 experimentally. According to the RT-PCR result of the present study it showed that GI tract from treated house flies were positive for NP (552 bp), N1 (616 bp) and H5 (290 bp) respectively (**Figure 1**). It means that the AIV remained exist inside GI tract of house flies for at least 24 hours post infection. In this case, house flies may act as a mechanical vectors and have the possibility to be biological vectors, but it needs further researh by inoculating the virus in embryonated chicken eggs to find out if the virus is viable inside GI tract of house flies.

This result becomes important since it is known that house flies pick up pathogenic organisms from garbage, sewage and other filthy sources, then transferred it to human and animal food (Arroyo, 2011) which may facilitate the disease transmission.

House flies also have a high intake of food which makes them deposit feces repeatedly. Flies feces contains pathogen which can contaminate food merely by landing on it because house flies defecate and regurgitate whenever they come to rest. It is also correlated to the flying distance of house flies which can reach 12 km in one day (Wanaratana *et al*., 2011). It means that there is a possibilty for house flies to shed the virus while they are flying.

It is also possible for the poultry to get infected with AIV by ingesting flies because poultry are able to eat flies even when the flies are flying (Sawabe *et al*., 2011). Poultry are also able to get infected by direct contact to contaminated feces or vomited matter from infected flies (Sawabe *et al*., 2011). In order to be infective and transmissible, high concentration of AIV inside house flies body is needed and able to attach on its host target receptor (Wuryastuty *et al*., 2008).

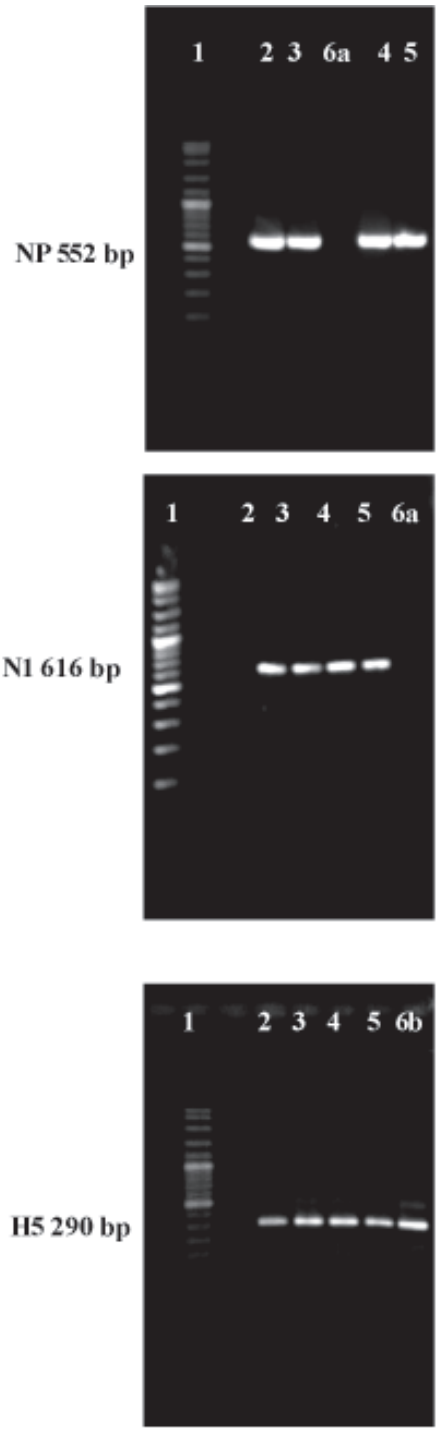

Figure 1. Agarose gel electrophoresis of A). NP gene (552 bp), B). N1 gene (616 bp), C). H5 (290 bp) influenza A virus using RT-PCR. Lane 1, DNA marker 100 bp; Lane 2, Positive control (blood serum of infected chicken); Lane 3, GI tract of infected flies 12 hours post infection (p.i); Lane 4, GI tract of infected flies 24 hours p.i; Lane 5, GI tract of infected flies 24 hours p.i; Lane 6a, Negative control, Lane 6b, GI tract of infected flies 12 hours post infection (p.i).

The house fly has been considered as a potential agent for disease transmission and able to trasmit more than 65 pathogens such as bacteria, protozoa, virus also parasite (Greenberg, 1973; Fasanella *et al*., 2010). It is also supported that there are some factors which affect the AIV occurence in Indonesia. First, the open system management of poultry farm and "back-yard poultry farm system" (Sector 4) which is developed by most people in Indonesia who live in the village and sub-urban areas. The low biosecurity system in Sector 4 and backyard farm with unlimited access for people to enter this poultry farms may cause the disease transmissions easily occurred, mainly AIV. It may also lead to AIV in human because generally people live side by side between the sick and healthy poultry. It is also known that most of poultry farmers in Indonesia who are lack of knowledge about biosecurity system, often maximize their poultry waste products for some economic reasons. In fact that poultry excrement is a perfect breeding media for house flies. Second factor is caused by the warm temperature in Indonesia as a tropical country which is suitable for house flies development. In warm temperature, house flies can complete their development from egg to adult at least in seven days. This tropical climate influences the mating rate, preoviposition period and oviposition of house flies which result in the high number of house flies (Keiding, 1986: Jin and Jaal, 2009). Third factor is that structurally, house fly is well adapted for collecting pathogens. The proboscis of house fly has a profusion of fine hairs that is able to collect environmental detritus (Fasanella *et al*., 2010). House flies also transmit pathogens through their mouthparts, vomit droplets, feces and body surface (Greenberg, 1973). According to Kobayashi *et al*. (1999), to be referred as a mechanical vector, it needs only a direct contact with contaminated legs or mouthparts and by the excreta or regurgitated fluid in a short time after exposure to pathogens. Pathogens that stick to the surfaces of house fly bodies may survive only for only a few hours but those are ingested with food may survive in fly's gut for several days (Keiding, 1986). In order to be referred as a biologic vector, the virus should be able to replicate inside house fly bodies before it is transmitted to the host target.

CONCLUSION

The present research showed that avian influenza virus was still exist inside house fly's bodies for at least 24 hours post infection. This result can be used as a standard control in biosecurity to control the dissemination of AIV.

SUGGESTION

In order to find out whether the virus replicate inside house fly bodies or not, it needs a further study by culturing and isolating the virus in embryonated chicken eggs. Furthermore, the viability of AIV inside house flies should be followed more than 24 hours up to the absence of AIV in house fly bodies to find out the survival time of AIV in house flies.

ACKNOWLEDGEMENT

The author would like to thank to Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia and Syngenta Crop Protection AG, Basel, Switzerland for their scientific advices and discussion. This project was supported by a grant from Novartis Animal Health Inc., Basel, Switzerland.

REFERENCES

Ahmed S, Zia K, Akhtar I. 2005. Responses of House Fly, *Musca domestica* L. to a Baiting System. Department of Agric. Entomology. University of Agriculture. Pakistan. *Int J Agri Biol* 7(3): 424-426

Albarrak AS. 2009. Comparative Studies on House Fly, Musca domestica L. Population in Different Animal Farms in Relation to Attractants and Control at Hail Province, Saudi Arabia. Department of Biology. Faculty of Sciences. University of Hail. Saudi Arabia. *Pak Entomol* 31(2): 142-148

Arroyo SH. 2011. *House Fly Musca domestica Linneaeus*. Entomology and Nematology Department. Univesity of Florida. Institute of Food and Agricultural Sciences. Gainesville.

Axtell RC. 1999. Poultry Integrated Pest Management: Status and Future. *Integrated Pest Manage Rev* 4: 53-73

Banjo AD, Lawal OA, Adeduji OO. 2005. Bacteria and Fungi Isolated from Housefly (*Musca domestica* L.) Larvae. Department of Biological Sciences. Olabisi Onabanjo University. Ogun State. Nigeria. *Afr J Biotech* 4(8): 780-784.

Barin A, Arabkhazaeli F, Rahbari S, Madani SA. 2010. The housefly, *Musca domestica*, as a Possible Mechanical Vector of Newcastle Disease Virus in the Laboratory and Field. Department of Parasitology. Faculty of Veterinary Medicine. University of Tehran. Iran. *Med Vet Entomol* 24: 88–90.

Butler JF, Garcia-Maruniak A, Meek F, Maruniak J. 2010. Wild Florida House Flies (Musca domestica) as Carriers of Pathogenic Bacteria. Department of Entomology and Nematology. University of Florida. Gainesville. Florida. *Florida Entomologist* 93(2).

Fasanella A, Scasciamacchia S, Garofolo G, Giangaspero A, Tarsitano E, Adone R. 2010. Evaluation of The House Fly *Musca domestica* As A Mechanical Vector for An Anthrax. *Plos One* 5(8): e12219

Gauthier-Clerc M, Lebarbenchon C, Thomas F. 2007. Recent Expansion of Highly Pathogenic Avian Influenza H5N1: A Critical Review. Station Biologique de la Tour du Valat. Le Sambuc. France. *Ibis* 149: 202–214.

Gerberich JB. 1952. The Housefly (*Musca domestica* Linn.), as a Vector of *Salmonella pullorum* (Retteger) Bergy, The Agent of White Diarrhea of Chicken. Department of Biology. University of Minnesota. Duluth Branch. *The Ohio J Sci* 52(5): 287.

Glatz P, Pym R. 2006. *Poultry Housing and Management in Developing Countries*. University of Queensland. Queensland. Australia

Greenberg B. 1973. *Flies and Disease. Vol. II. Biology and Disease Transmission*. Princeton. NJ: Princeton University Press.

Gubler DJ. 2009. Vector-borne Diseases. Program in Emerging Infectious Diseases. Medical School Singapore. Singapore. *Rev. Sci Tech Off Iint Epiz* 28(2): 583-588.

Hamm RL, Scott JG. 2009. A High Frequency of Male Determining Factors in Male *Musca domestica* (Diptera: Muscidae) from Ipswich, Australia. Department of Entomology. Comstock Hall. Cornell University. Ithaca. New York. *J Med Entomol* 46(1): 169-172.

Holt PS, Geden CJ, Moore RW, Gast RK. 2007. Isolation of *Salmonella enteritica* serovar enteritis from House Flies (*Musca domestica*) Found in Rooms Containing *Salmonella* serovar enteritis-challenged Hens. *Appl. Environ. Microbiol* 73: 6030–6035.

Jin BL, Jaal Z. 2009. Linn (Diptera: Muscidae) in Poultry Farms in Penang, Malaysia. Vector Control Research Unit. School of Biological Sciences. Universiti Sains Malaysia. Penang. Malaysia. *Trop Biomed* 26(2): 140-148.

Keiding J. 1986. *The Housefly, Biology and Control. Division of Control of Tropical Diseases*. World Health Organization. Geneva. Switzerland. Hlm. 302-323

Kobayashi M, Sasaki T, Saito N, Tamura K, Suzuki K, Watanabe H, Agui N. 1999. Houseflies: Not Simple Mechanical Vectors of Enterohemorrhagic Eschericia coli O157:H7. *Am J Trop Med Hyg* 635-629.

Nazni WA, Seleena B, Lee HL, Jeffery J, Rogayah TAR, Sofian MA. 2005. Bacteria Fauna from the Housefly, *Musca domestica* (L.). medical Entomology Unit. Infectious Disease Research Centre. Institute for Medical Research. Kuala Lumpur. Malaysia. *Trop Biomed* 22(2): 225–231.

Patrick I, Jubb T, Rolfe P. 2008. *A Scoping Study Investigating Opportunities for Improving Biosecurity on Commercial Poultry Farms in Indonesia*. Canberra. Australia. Australian Center for International Agricultural Research (ACIAR).

Sawabe K, Hoshino K, Isawa H, Sasaki T, Hayashi T, Tsuda Y, Kurahashi H, Tanabayashi K, Hotta A, Saito T, Yamada A, Kobayashi M. 2011. Detection and Isolation of Highly Pathogenic H5N1 Avian Influenza A Viruses from Blow Flies Collected in the Vicinity of An Infected Poultry Farm in Kyoto, Japan, 2004. 2006. Department of Medical Entomology. National Institute of Infectious Diseases. Toyama. Shinjuku-ku. Tokyo. Japan. *Am J Trop Med Hyg* 75(2): 327-332.

Spackman E. 2008. *Methods in Molecular Biology : Avian Influenza Virus*. Southeast Poultry Research Laboratory. US Department of Agriculture. Agricultural Research Service. Athens. GA.

Wanaratana S, Panyim S, Pakpinyo S. 2011. The Potential House Flies to Act as A Vector of Avian Influenza Subtype H5N1 Under Experimental Condition. Department of Veterinary Medicine. Faculty of veterinary Science. Chulalongkorn University. Bangkok. Thailand. *Med and Vet Entomol* 25: 58-63.

WHO. 2013. Cummulative Number of Confirmed Human Cases for Avian Influenza A (H5N1) Reported to WHO (2003-2013). www.who.int/influenza/human_animal_interface/H5N1_cummulative_table_archives/en/. Accessed in July 2013

Wuryastuty H, Widyarini S, Wasito R. 2008. Research report: *Peran Lalat Rumah (Musca domestica) sebagai Vektor Biologik Virus Avian Influenza*. Gadjah Mada University. Yogyakarta. Indonesia

Wuryastuty H, Wasito R. 2013. Molecular Identification of Avian Influenza A Virus in House Flies (Musca domestica Linnaeus) Collected from Different Poultry Farms in Indonesia. *J Sains Vet* 31(1): 1-7.