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)

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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


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 Orthomyxoviridae 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 polymerase 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.

RESEARCH 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 research 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 possibility 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](image_url)
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.

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