

# A Reassortant Virus of A Thai Swine Influenza Virus (SIV) and The Pandemic H1N1 of Pig Origin Did Not Induce Severe Disease in Experimental Ducks

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## Abstract

Currently, the pandemic H1N1 (pH1N1) 2009 influenza A virus and its reassortant pandemic H1N1 influenza (rH1N1) viruses have been circulating in pigs population in many countries around the world including Thailand. Many organizations and researchers have raised some concerns about interspecies transmission, particularly in aquatic avian species including domestic ducks commingling in backyard farming. In order to elucidate the pathogenesis of those viruses in domestic ducks, the study on pH1N1 and rH1N1 recently isolated from pigs was conducted to understand the susceptibility, clinical signs, viral shedding and lesions in experimental ducks. Twenty-one 6-week-old influenza A virus negative ducks were divided into 3 groups (2 challenged groups and 1 control group). In the challenged groups, the ducks in each group were individually inoculated with pH1N1 or rH1N1 as mentioned previously. All experimental ducks were observed for clinical signs and oropharyngeal and cloacal swabs were collected to investigate the viral shedding using a modified real time RT-PCR. Tissues samples were collected for pathological and immunohistochemistry examination. Results demonstrated that neither pH1N1 nor rH1N1 induced significant flu-like clinical signs. However, both viruses could infect the experimental ducks as varied gross and microscopic lesions as well as duration of virus shedding were observed. Interestingly, the detectable lesions and the extended shedding period were found in the group of rH1N1-infected ducks. In conclusion, inter-species transmission should be considered when commingling different animal species.

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**Keywords:** ducks, influenza, pandemic H1N1 2009, pathogenesis, pigs, reassortant

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## Introduction

Recently, pH1N1 2009 virus has rapidly spread causing at least 340,000 clinical cases reported and more than 4,000 deaths around the world including Thailand (Bai et al., 2011). The genome of the virus is closely related to the swine influenza virus (SIV), called swine-origin 2009 influenza A virus (H1N1) or swine flu (Mullen et al., 2009). The origin of the virus came from reassorted genome segments between the triple reassortant internal gene (TRIG) virus from North America and the avian-like Eurasian swine lineage from Europe (Dawood et al., 2009; Dubey et al., 2009; Forrest and Webster, 2010). This virus has been suspected to circulate in the swine population without undetectable clinical signs and to possibly transmit to humans, especially swine workers, with unclarified evidences (Arias et al., 2009; Peiris et al., 2009; Smith et al., 2009; Kitikoon et al., 2011<sup>b</sup>). In pigs, the first report of pH1N1-infection was found in Canadian pigs in April 2009 (Smith et al., 2009). Later, several reports were found in Hong Kong in October 2009 (Vijaykrishna et al., 2010) and in Thailand in December 2009 (Sreta et al., 2010). After the emergence of pH1N1, another novel reassortant H1N2 adopting the HA and NA genes from classical swine virus H1N2 and the remaining genes from pH1N1 was reported in the United Kingdom (Howard et al., 2011). In Thailand, the reassortant pH1N1 (H1 gene from the 2009 pH1N1 and N1 from an endemic Thai SIV) was found in a commercial farm in Central division of Thailand (Kitikoon et al., 2011a). Additionally, with the ability of the reassortant virus to survive and circulate in the swine population, transmission to humans or other commingled species in the future is possible (Dawood et al., 2009).

Since the emergence of pH1N1 in 2009, interspecies transmission has been of concern, particularly in aquatic avian species including domestic ducks (Kim et al., 2009). Previous studies reported that pH1N1 contained genes from avian-like Eurasian swine H1N1 lineage which were the avian-like swine virus and genes from TRIG which were closely related to the virus from duck origin (Arias et al., 2009; Forrest and Webster, 2010). Moreover, most of HA and NA surface antigens can be found in ducks with no clinical signs (Kim et al., 2009) and many species of ducks have seasonal migratory behaviors leading to the spreading of the virus (Keawcharoen et al., 2008; Kim et al., 2009).

In Thailand, domestic ducks are the most important influenza reservoirs that play an important role in the spreading of the disease. Four duck raising systems exist in most Asian countries, including Thailand; close biosecurity system, open house system, grazing system and backyard ducks (Songserm et al., 2006). Interestingly, the grazing and backyards ducks are suspected to be the major virus reservoir for other domestic species. Since ducks roam freely in the environment in these two types of raising systems, the chance of exposure among ducks and other susceptible species, especially pigs which are the main source of pH1N1 and rH1N1, increases. Therefore, the pathogenesis of pH1N1 and rH1N1 in experimental ducks should be considered for the information of

possible virus transmission events. In order to elucidate the pathogenesis of those emerging viruses in ducks, the study of pH1N1 and rH1N1 isolated from pigs was of interest to understand the susceptibility, clinical signs, viral shedding and lesions in the experimental ducks. This investigation will provide the information beneficial to the epidemiology, disease control and prevention of those emerging influenza viruses in the future.

## Materials and Methods

**Virus preparation:** Virus stocks of the pandemic H1N1 (pH1N1) influenza virus, A/swine/Thailand/CU-RA29/2009 (H1N1) and its reassortant pH1N1 (rH1N1) influenza virus, A/swine/Thailand/CU-SA43/2010 (H1N1) were prepared by passaging in 9-day-old embryonated chicken eggs. Allantoic fluid was harvested after 72 h post infection. Infectivity of stock viruses was determined in Mardin-Darby canine kidney (MDCK) cells according to a standard procedure routinely performed at the Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL) (Charoenvisal et al., 2013). The 50% tissue culture infectious dose (TCID<sub>50</sub>) was calculated by Reed and Muench method. Concentration of both viruses, which were used in this study, was 10<sup>4</sup> TCID<sub>50</sub>/ml. All experiments involving live viruses were conducted under biosafety level 2 containment (BSL-2) and the viruses were aliquoted and kept in -80°C until used.

**Animals:** Six-week-old cross-bred ducks were obtained from a commercial farm considered as influenza A viruses negative farm. Serum samples, oropharyngeal and cloacal swabs were collected from all experimental ducks 1 week before starting the experiment. The sera were analyzed by using a commercial ELISA H1N1 kit (HerdChek H1N1ELISA; IDEXX Laboratories, Westbrook, Maine) according to the manufacturer's instructions. The swabs were tested for the presence of influenza A viruses using matrix (M) gene real time reverse transcription polymerase chain reaction (rt RT-PCR) (Spackman et al., 2002). Absence of pre-existing influenza A virus antibodies and M gene were expected in the sera and swabs of all animals.

All ducks were divided into 3 groups separately in the animal facility biosafety level 2, with 3 animals in the control group and 9 animals each in two challenged groups. In the challenged groups, the ducks were individually inoculated intratracheally and intraesophageally either with 3 ml containing 10<sup>4</sup> TCID<sub>50</sub>/ml of the pH1N1 or the rH1N1 in the assigned group as mentioned previously. In the control group, the animals were mock inoculated with 3 ml of minimal essential medium (MEM) media. Animal care and the experimental procedures were approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University (Protocol No. 11310052).

**Clinical observation and sampling:** All experimental ducks were observed for clinical signs for 12 d post infection (DPI). The oropharyngeal and cloacal swabs were collected at 0 to 12 DPI in the viral transport

media. At each time point (3, 5 and 13 DPI), 3 ducks from each challenged group and 1 duck from the control group were randomly euthanized using intravenous administration of pentobarbital administration overdose. Tissues including air sac, trachea, lung, brain, pancreas, liver, jejunum, colon, spleen and kidney were collected. All swabs and collected tissues were performed using a modified real time reverse transcriptase-polymerase chain reaction (rtRT-PCR) to detect the presence of both influenza viruses and the collected tissues were fixed in 10% buffer formalin for pathologic examination.

**Pathological study and Immunohistochemistry:** The tissue samples were fixed in 10% buffered formalin and embedded in paraffin wax. The 4 µm thick tissue sections were prepared for histological analysis by hematoxylin and eosin (H&E) staining and for detecting Influenza A virus antigen by immunohistochemical technique using an anti-influenza A nucleoprotein monoclonal mouse antibodies (HB654404 B.V.EUROPEAN VETERINARY LABORATORY, the Netherlands) as a primary antibody. The biotinylated rabbit anti-mouse IgG antibody and envision polymer (Envision Polymer DAKO®, Denmark.) were used as a secondary antibody. The reactions were developed in 3, 3'-diaminobenzidine tetrahydrochloride (DAB) as the substrates and counterstained with Mayer's hematoxylin. The SIV infected pig lung tissue section from the previous experimental study was used as a positive control. The Influenza A virus positive antigen was evaluated using the following protocol (average finding of 10 high power fields/slide): (-) no viral positive antigen could be detected, (+) 1-2 viral positive cells detected in the whole tissue, (++) 1-2 viral positive cells detected per 1 high power field, (+++) 3-10 viral positive cells detected per 1 high power field and (+++++) more than 10 viral positive cells detected per 1 high power field (Haines et al., 1993; Sreta et al., 2009).

**Modified real time RT-PCR (rtRT-PCR) for influenza A virus detection:** Viral RNA was extracted from the oropharyngeal and cloacal swabs and from all tissues using the Nucleospin® RNA virus (Machery-nagel, Duren, Germany). The rtRT-PCR assays were performed on Corbett Rotor-Gene™ 6000 (Qiagen) using SuperScript™ III Platinum® One-Step Quatitative RT-PCR System (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. Primers and probes were taken from a recent publication (Spackman et al., 2002) specifically to amplify a portion of the M gene of influenza A virus with some modifications (forward primer (MF3; 5'TGATCTTCTTGAAAATTTGCAG 3'), reverse primer (MR1+; 5' CCGTAGMAGGCCCTCTTTTCA 3') and M-64probe (FAM-TTGTGGATTCTTGATCG-TAMRA) (Payungporn et al., 2006). The positive CT value was 1-40 and more than 40 was determined as a negative result (Charoenvisal et al., 2013).

The positive control of rtRT-PCR was obtained from the positive sample based on viral isolation and titration in the MDCK cell line. The viral antigen was identified using the Influenza A nucleoprotein monoclonal antibodies (HB654404), a

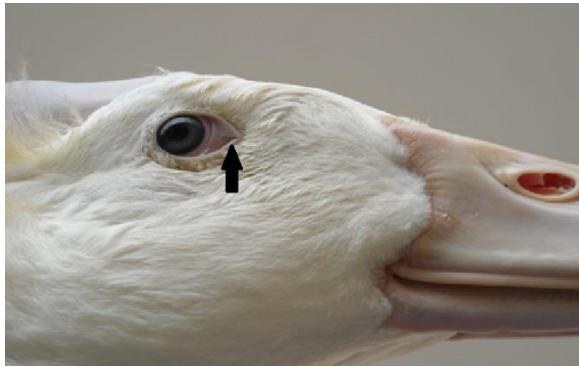
rabbit anti-mouse IgG conjugated horseradish peroxidase (Dako Cytomation, USA) and developed with chromogen aminoethyl carbazole (AEC) substrate. Virus titration (TCID<sub>50</sub>/ml) was performed using the routine procedure of the CU-VDL (Sreta et al., 2009) and calculated using Reed and Muench method (1938).

**Serological study:** Serum samples were collected from all experimental ducks 1 week before starting the experiment and from all animals before euthanized at day 2, 4 and 12. The serum sample were analyzed using a commercial ELISA H1N1 kit (Avian Influenza virus antibody test kit H1N1 ELISA; IDEXX Laboratories, Westbrook, Maine) according to the manufacturer's instructions and hemagglutination inhibition test (HI test) as described previously (Sreta et al., 2013). The serum samples obtained from 1 week before starting the experiment must have no pre-existing influenza A virus antibodies and the swabs must show no influenza positive sample from all animals. The serum samples from all ducks before euthanasia were used for the antibody measurement to the influenza virus during the study.

## Results

**Clinical observation and gross pathology:** The ducks in both experimental challenged groups remained healthy with no clinical signs observed. However, the pH1N1-infected ducks showed the sign of conjunctivitis at 6 (3/3), 7 (3/3) and 10 (2/3) d post infection (DPI) (Fig 1). Macroscopic findings of pH1N1-infected ducks at 2 (3/3), 4 (2/3) and 12 DPI (1/3) predominantly showed mild airsacculitis. In addition, hemorrhage was also present in the tracheal epithelium (1/3), lung (1/3), and liver (1/3) at 2 DPI. Serosanguineous fluid in the pericardial sac (1/3) was also found at 2 DPI. Similarly, in the rH1N1-infected ducks, consistent gross lesion was found and was mild airsacculitis. Other gross lesions including multifocal white foci in caudal lobe of liver at 2 DPI (1/3) and 4 DPI (1/3) were observed. Serosanguineous fluid in the pericardial sac (1/3), lung congestion (2/3), petechial hemorrhages in the pancreas, and multifocal white foci on the surface of the spleen (1/3) and on the air sac membrane (1/3) were seen at 4 DPI. One duck from the rH1N1-infected group was removed after inoculation because of concurrent bacterial infection. None of the sham-inoculated group exhibited significant clinical signs, macroscopic findings or mortality during the study period.

**Histopathology and immunohistochemistry:** In the pH1N1-infected ducks, frequent microscopic lesions included mild to moderate multifocal interstitial pneumonia at 2, 4, 12 DPI (9/9), mild to moderate diffuse degenerative changes of the liver at 2, 4, 12 DPI (6/9), none to severe multifocal lymphocytic airsacculitis at 2, 4, 12 DPI (7/9) and mild interstitial nephritis at 2, 4, 12 DPI (8/9). Other detected lesions included none to mild pancreatic (4/6) and splenic (3/6) degeneration at 2 and 12 DPI, moderate enteritis at 4 DPI (1/3) and mild hepatitis at 2 DPI (1/3). There



**Figure 1** Mild conjunctivitis found in the pH1N1-challenged duck

were no remarkable microscopic lesions observed in the trachea, jejunum or brain. No influenza antigen staining was found in the tissues of the ducks from this group.

The ducks inoculated with rH1N1 virus displayed mild to moderate multifocal interstitial pneumonia at 2, 4, 12 DPI (8/8), mild to moderate diffuse enteritis at 2, 4, 12 DPI (6/8), mild to severe diffuse degenerative changes of the liver at 2, 4, 12 DPI (5/8), and none to mild multifocal lymphocytic airsacculitis at 2, 4, 12 DPI (5/8). Moderate focal pancreatitis at 4 DPI (1/3), moderate multifocal interstitial nephritis at 2 DPI (1/3) and mild hemorrhages of the spleen at 12 DPI (1/2) were also observed. Neither specific histological lesions nor specific influenza antigen staining were presented in all collected tissues of the ducks from this group.

No significant histopathological lesions or antigen staining were presented in all collected tissue of the control ducks.

**Viral shedding:** Each duck from all groups were swabbed every day from the oropharyngeal and the cloacal routes. The real time RT-PCR was performed on both collected routes. In the pH1N1-infected ducks, small amounts of virus were individually detected sporadically of oropharyngeal swabs at 1 DPI, 2 DPI, 5 DPI and 6 DPI (detection levels ranged between 0 and  $2.37 \times 10^8$  viral RNA copies/ml) and cloacal swabs at 5 DPI ( $1.02 \times 10^7$  viral RNA copies/ml) (Table 1). Among the rH1N1-inoculated ducks, viral shedding varied substantially in the individual ducks, which was observed from 1 DPI to 6 DPI in oropharyngeal swabs (detection levels ranged between 0 and  $5.56 \times 10^8$  viral RNA copies/ml) and from 1 DPI to 7 DPI in cloacal swabs (detection levels between 0 and  $2.40 \times 10^8$  viral RNA copies/ml) (Table 2). No viral RNA or viral infectivity was detected from the collected tissues in both infected groups and in the control group.

**Serological examination:** All ducks sera collected at the beginning of the experiment and from the necropsied ducks at 3, 5 and 10 DPI were tested negative for influenza A antibody by the commercial ELISA test kit and all ducks sera were test negative for pH1N1 antibody by HI test.

## Discussion

Based on the results, the studied ducks seemed resistant to either the pH1N1 or rH1N1 infection. Both challenged groups exhibited asymptomatic to mild clinical signs, with low viral RNA level detected by the real time RT-PCR. However, major lesions were located only in the respiratory tract. The results found in this study were similar to previous studies using low pathogenic avian influenza virus (LPAI) inoculation in experimental ducks (Itoh et al., 2009; Brown et al., 2012; Wibawa et al., 2013). Most distinguished lesions of LPAI were observed at 2-5 DPI and mainly located in the respiratory tract such as air sacs, bronchi and trachea (Franca et al., 2012). Interestingly, the rH1N1-infected ducks in this study had more lesions than those of the pH1N1-infected ducks including the lesions in pancreas and spleen, but the lesions were limited and only found at 2-4 DPI, similar to other LPAI viruses (Brown et al., 2012; Franca et al., 2012; Brojer et al., 2013).

Aquatic poultry, especially ducks, are suspected to be the main reservoir of the influenza A virus (Shoham, 2006). Most of the infected ducks showed none to mild clinical signs (Kida et al., 1980; Bao et al., 2010) similar to the pH1N1 or rH1N1-challenged ducks in this study. There are many explanations why the ducks were not susceptible to the studied viruses. Firstly, ducks have higher body temperature than pigs. The higher body temperature could affect the normal replication cycles and the virus ability to survive in the host cells (Hatta et al., 2007; Beato et al., 2012). Secondly, there are variations in the expression of the sialic acid (SA) receptors in the host. The 2, 6 sialogalactoside receptors are usually found in the respiratory epithelium of human and mammalian including pigs. This receptor is compatible to HA from the mammalian virus, including both studied viruses, which received HA gene from the classical swine lineage from North America. The avian species, including ducks, had more expression  $\alpha$  2, 3 sialogalactoside in the epithelium of gastrointestinal tract. Possibly, the receptors of the experimental ducks were compatible to HA from the avian virus than those of the mammalian viruses (Neumann and Kawaoka, 2006; Imai and Kawaoka, 2012). Thirdly, a previous study found a rapid cell death mechanism response to the influenza virus infection, demonstrating that duck cells had faster apoptotic mechanism after influenza virus infection than chicken cells (Kuchipudi et al., 2012). This suggested the limitation of the viral replication and the resistance to the virus in duck cells, leading to undetectable virus antigen in the cells.

This study showed that both challenged groups had variable shedding patterns between 1-7 DPI in the oropharyngeal (OP) and cloacal (CL) routes based on the rRT-PCR. It was consistent with a previous study indicating that the LPAI infected ducks could shed the virus between 1-7 DPI (Franca et al., 2012). The highest number of shedding pattern of both infected group were at 3-5 DPI and the shedding ceased by 7 DPI. Interestingly, the rH1N1-challenged ducks could shed

**Table 1** Viral detection from oropharyngeal and cloacal swabs of ducks in pH1N1 group using a modified real time RT-PCR

Duck ID.	Viral Detection												
	1DPI	2DPI	3DPI	4DPI	5DPI	6DPI	7DPI	8DPI	9DPI	10DPI	11DPI	12DPI	13DPI
<b>Oropharyngeal swab</b>													
1	+	-	-	-	+	+	-	-	-	-	-	-	N
2	-	-	N										
3	-	-	-	-	-	-	-	-	-	-	-	-	N
4	+	-	-	-	N								
5	-	+	-	-	-	-	-	-	-	-	-	-	N
6	-	-	N										
7	-	-	-	-	N								
8	-	-	N										
9	-	-	-	-	N								
<b>Cloacal swab</b>													
1	-	-	-	-	-	-	-	-	-	-	-	-	N
2	-	-	N										
3	-	-	-	-	+	-	-	-	-	-	-	-	N
4	-	-	-	-	N								
5	-	-	-	-	-	-	-	-	-	-	-	-	N
6	-	-	N										
7	-	-	-	-	N								
8	-	-	N										
9	-	-	-	-	N								

DPI = Day post inoculation, N = Necropsy, real time RT-PCR result (+ = Ct value&lt;40, - = Ct value≥40)

**Table 2** Viral detection from oropharyngeal and cloacal swabs of ducks in rH1N1 group using modified real time RT-PCR

Duck ID.	Viral Detection												
	1DPI	2DPI	3DPI	4DPI	5DPI	6DPI	7DPI	8DPI	9DPI	10DPI	11DPI	12DPI	13DPI
<b>Oropharyngeal swab</b>													
1	+	+	N										
2	-	-	+	+	+	+	-	-	-	-	-	-	N
3	+	-	N										
4	+	-	-	+	N								
5	+	+	+	+	N								
6	+	+	+	-	N								
7	-	-	N										
8	+	-	+	+	+	+	-	-	-	-	-	-	N
9	+	-	+	+	+	-	-	-	-	-	-	-	N
<b>Cloacal swab</b>													
1	+	+	N										
2	+	+	+	+	+	+	+	-	-	-	-	-	N
3	+	-	N										
4	+	+	+	+	N								
5	+	-	-	+	N								
6	+	+	+	+	N								
7	-	-	N										
8	-	-	+	-	+	-	+	-	-	-	-	-	N
9	+	+	+	+	-	-	-	-	-	-	-	-	N

DPI = Day post inoculation, N = Necropsy, real time RT-PCR result (+ = Ct value&lt;40, - = Ct value≥40)

the virus in large quantity comparing to the pH1N1-challenged ducks from both routes. In addition, the rH1N1 showed better replication and shedding via both respiratory and gastrointestinal tracts than the pH1N1. In contrast to a previous investigation of both studied viruses in commingled experiment of sentinel ducks with infected pigs, the pH1N1-infected ducks had more pathogenicity and viral shedding levels than the rH1N1-infected ducks (Charoenvisal et al., 2013).

The result from the previous study indicated that the pH1N1 had higher potential for transmitting to other hosts via the environment from the pH1N1-infected pigs. The differences in the pathogenicity and transmission potential of both studied viruses could be due to the differences between the NA gene of rH1N1 and pH1N1. The rH1N1 obtained the NA gene from the endemic Thai swine lineage, whereas the pH1N1 obtaining it from the Eurasian swine lineage. Even

though the role of NA gene in the pathogenesis of SIV is not clearly elucidated at present, a study about the NA gene related to the virulence of the reassortant virus has been reported. The viruses in this study generated from pH1N1 and some of the genes including NA genes were obtained from the seasonal influenza A virus (H3N2). The results showed that the NA gene of the reassortant virus tended to induce greater pathogenic than the original pH1N1 (Schrauwen et al., 2011).

As known previously, ducks play an important role as the main reservoir of the influenza A virus (Keawcharoen et al., 2011). Most of the HA and NA subtypes can be found in ducks especially for avian influenza H5N1 and H7N9 (Kim et al., 2009; Gao et al., 2013). Ducks can reverse the highly pathogenic to low pathogenic avian influenza H5N1 virus after the infection but the viruses still continue, circulate and transmit to other avian species consequently (Hulse-Post et al., 2005; Keawcharoen et al., 2008). In 2012-2013, the novel avian influenza virus H7N9 emerged and caused severe respiratory signs and mortality in humans mostly in China. Many studies suggested that the origin of the virus originated from the reassortant among avian influenza viruses from ducks, chicken and wild aquatic birds in China (Gao et al., 2013; Stein, 2013). There were no clinical signs in those avian species. These demonstrate that aquatic birds, especially domestic ducks, are the important host generating the novel influenza A virus or maintain and transmit the virus to both avian and mammalian hosts.

This study indicated that the experimental ducks were not susceptible, but could be infected by both pH1N1 and rH1N1 viruses. However, the rH1N1 could possibly induce more obvious pathogenicity than the pH1N1. Both studied viruses caused mild clinical signs, but the relation to the influenza-induced lesions could not be confirmed due to undetectable influenza antigen. It should be noted that both viruses could be shed to the environment in low level via oropharyngeal and cloacal routes until 7 days after inoculation. It is of our concern that the study about the interspecies transmission and the influenza surveillance program of swine influenza virus should be carried out routinely in both ducks and pigs not only to prevent the spread of any novel reassortant virus to other areas, but also to prevent generation of a novel future pandemic influenza virus.

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## บทคัดย่อ

# เชื้อใช้หวัดสุกรที่กลายพันธุ์และเชื้อใช้หวัดใหญ่สายพันธุ์ใหม่ที่เพาะแยกได้จากสุกรไม่ก่อให้เกิด ความรุนแรงในเป็ดทดลอง

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ตั้งแต่ปี 2009 เชื้อใช้หวัดใหญ่สายพันธุ์ใหม่และเชื้อใช้หวัดสุกรที่กลายพันธุ์ได้มีการระบาดและวนเวียนในฝูงสุกรในหลายพื้นที่ทั่วโลก รวมถึงประเทศไทย นักวิจัยและหลายองค์กรต่างให้ความสำคัญในแง่ของการแพร่เชื้อระหว่างสายพันธุ์ โดยเฉพาะอย่างยิ่งในกลุ่มนก-น้ำ ซึ่งรวมถึงเป็ดไล่ทุ่งและเลี้ยงหลังบ้าน เพื่อจะศึกษาพยาธิกำเนิดของไวรัสสองตัวนี้ในเป็ด จึงได้ทำการทดลองให้เชื้อไวรัสในเป็ดทดลอง โดยศึกษาในแง่ของความสามารถในการรับเชื้อ อาการทางคลินิก รอยโรค และการแพร่เชื้อของเป็ดทดลอง แบ่งเป็ดทดลองอายุ 6 สัปดาห์ ที่ผ่านการตรวจสอบว่าปราศจากการติดเชื้อใช้หวัดใหญ่ออกเป็น 3 กลุ่ม (กลุ่มทดลอง 2 กลุ่ม และกลุ่มควบคุม 1 กลุ่ม) ให้เชื้อใช้หวัดใหญ่สายพันธุ์ใหม่และใช้หวัดใหญ่สุกรกลายพันธุ์ในกลุ่มทดลอง ตามลำดับ สังเกตอาการเป็ดทดลองทุกตัวและเก็บตัวอย่างสารคัดหลั่งเพื่อตรวจการแพร่เชื้อด้วยวิธี modified real time RT-PCR รวมถึงเก็บชิ้นเนื้อจากการผ่าซาก เพื่อทำการศึกษาทางพยาธิวิทยา ผลการทดลองแสดงให้เห็นว่าเป็ดทดลองติดเชื้อไวรัสทั้ง 2 ชนิด แต่ไม่แสดงอาการทางคลินิก รวมถึงมีรอยโรค ผลทางพยาธิวิทยา และระยะเวลาการแพร่เชื้อที่แตกต่างกันไป โดยเชื้อใช้หวัดสุกรกลายพันธุ์มีรอยโรคและระยะเวลาการแพร่เชื้อที่ยาวนานกว่าเชื้อใช้หวัดสายพันธุ์ใหม่ การทดลองนี้แสดงให้เห็นถึงความสำคัญของการติดตามชนิดสัตว์ของเชื้อใช้หวัดใหญ่สุกรในปัจจุบัน

**คำสำคัญ:** เป็ด เชื้อใช้หวัดใหญ่ เชื้อใช้หวัดสายพันธุ์ใหม่ พยาธิกำเนิด สุกร เชื้อใช้หวัดสุกรกลายพันธุ์

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