CHAPTER VI

EVALUATION OF H5N1 SPECIFIC ANTIBODY IN HIGH RISK PEOPLE IN THAILAND

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Abstract

The outbreak of H5N1 HPAI virus affected the economic lass and public health concerns throughout Asia, including Thailand. Most of backyard poultry farms were responsible for the outbreak situation. Moreover, cross species transmission was occurred from avian to felids and human. This study aimed to investigate the seroprevalence in people with high risk in H5N1 exposure and determine the risk actors accounted for cross-species transmission and probabilityin feline-to-human transmission by using microneutralization (MN) assay and confirmed by hemagglutination inhibition (HI) assay. The results showed that there was 3.47% of high risk population with specific antibody against H5N1 influenza virus. Most of those were male and worked in poultry farms in Northern Central of Thailand. Approximately 83% of seropositive people obtained serum in 2008, a year after H5N1 outbreak in the same area, suggested that H5-specific antibody can be existed more than a year after infection or there might be sporadic H5N1 infection in the same area after 2007. Moreover, H5N1 surveillance in epidemiology and seroprevalence should be monitored continuously due to the accumulation of influenza virus mutation.

Introduction

From 1996-2008, there were sporadic outbreaks of Highly Pathogenic Avian Influenza Virus (HPAI) H5N1 subtype in many countries, causing many economic detriments and concerns of public health. The first case of species-transmission of H5N1 virus to human was reported in Hong Kong [66-68], and suddenly occurred throughout Asian countries, such as China, Thailand, Indonesia, Cambodia and Viet Nam, and then invaded into European countries [71,157]. Beyond 2008, there were 385 human cases confirmed the H5N1 infection with 243 deaths around the world, suggesting very high fatality rate (more than 60%) [158]. Although the large outbreaks of

H5N1 HPAI were decreased, many sporadic outbreaks of H5N1 always appear. In this situation, avian-to-human transmission can be resulted in most cases. In contrast, human-to-human transmission of H5N1 virus has been limited [72-75], also the efficiency of cross-species transmission from other mammal to human remains unknown.

For H5N1 circumstance in Thailand, the H5N1 infection was originally reported in 2003, followed by 3 major outbreaks therefore; January – March, 2004, July – December, 2004 and from October to December, 2005. The H5N1 outbreak in Thailand affected the great number of poultry culling in many provinces, such as NakornPathom, NakornNayok, NakornSawan, Supanburi, Nontaburi and Kanchanaburi [159]. In Thailand, 25 human cases with H5N1 HPAI infection were found, and 17 cases were dead. The backyard poultry farming is major responsible for the spread of H5N1 in Thailand [72-75]. Interestingly, our previous study reported the first case of species transmission from avian to felids, two tigers (*Panthera tigris*) and two leopards (*P. pardus*) at a tiger zoo in Supanburi province were dead. The PCR results and histopathologic and immunohistochemical evidences presented the existence of avian influenza virus particle clearly [76]. The investigation revealed that these felids were fed by poultry carcasses from local slaughter house. That finding extended the host range of H5N1 virus, besides the previous finding of H5N1 infection in domestic cat. This host range restriction comes up to the question that whether or not, the H5N1 infection can be transmitted between felids to human, also like avian – to – human transmission and one way to identify the H5N1 infection in human is to evaluate the specific antibody response to H5N1 virus by using microneutralization (MN) test and hemagglutination inhibition (HI) test. For MN test, the direct method to validate the amount of antibody against virus, is a highly sensitive technique using the strain-specific antibody to inhibit

the ability of virus in host cell entry, resulted in blocking of virus replication while HI test, which is more widely used and less time consume, is the technique using the property of HA protein of influenza virus which can agglutinate the erythrocyte by the adherence between surface HA protein and sialic acid receptor [160]. This study investigated the seroprevalence of antibody against H5N1 HPAI in people who were the first rank of contact with infected animals, poultry and tiger which can be a record of the next outbreak and can brings us to determine the mode of transmission, the risk factors affiliated the H5N1 infection and possibility of feline – to – human transmission of this virus by using microneutralization test and hemagglutination inhibition test.

Materials and Methods

Serum Samples

This study was conducted between 2003-2009, one hundred and seventy-three single serum samples were collected from three sources;

- Fifty-eight workers in tiger zoo, Supanburi province, Thailand, which was reported the tigers and leopard deaths in 2003, serum samples were assembled during the investigation.

- Forty-six workers and executioners in poultry farms in Lopburi province, Thailand, which was stated the outbreak of H5N1 in 2008, serum samples were subjected within 14 day after the investigation.

- Sixty-nine workers in the same poultry farms in Lopburi, which was stated the outbreak of H5N1 in 2008, serum samples were subjected 1 year later after the

investigation and there was no H5N1 outbreak in these farms during 2008-2009. The sera obtained from workers in 2009 were not the same subject in 2008.

All participant completed questionnaires for collection information about sex, age, period of employment and exposure to infected or dead animals (poultry or felids). All serum samples were kept in dry ice during transportation to Center of Excellence in Clinical Virology, Faculty of Science, Chulalongkorn University, Bangkok and were kept in -70° C until H5N1-specific antibody testing was performed.

Serologic Testing

All serum samples were checked for quality before performed the serologic assays. Protocol for MN test was established following the Centers for Disease Control and Prevention (CDC) protocol [133,160,161] while the protocol for HI assay was modified from World Health Organization and our previous study [132,139,161]. Highly pathogenic avian influenza H5N1 subtype isolate A/Thailand/NK-165/2005 (accession No. DQ372591-DQ372598) was used as a virus antigen to perform serologic testing. Before HI assay was performed, serum samples were reacted with receptor-destroying enzyme (RDE) as previous study [144] to reduce the possibility of false positive results and H5N1 virus was titrated into 8 HAU by using the hemagglutination (HA) test [161]. In correspondence to modified protocol, the neutralizing specific antibody titer to H5N1 which is $\geq$ 40 were considered to be positive results as previous study [133,163,164].

Statistical evaluation

The mean, median proportions associated with participant's age and sex and the positive percentage of infected subjects were calculated by using the SPSS statistical software program version 17.0 (IBM, NY) for statistical evaluation.

Results

Study Population

The population in this study consisted of 173 participants which were categorized following 3 sample sources;

Group 1: 58 workers in tiger zoo, Supanburi province, Thailand in 2003 (33.53% of participants)

Group 2: 46 workers and slaughters of the backyard poultry farms, upper central provinces of Thailand in 2008 (26.59%)

Group 3: 69 workers in backyard poultry farms in upper central provinces of Thailand, one year later after the H5N1 outbreak (39.88%). The male:female ratio of the study population was 1.18:1 with median of age = 29 years old. Typically, there were no significant differences among three groups of the study in demographic characteristics and history of illness. Most of them (43.4%) were between 15-29 years old and mostly (39.3%) were on duty as animal feeders (poultry/tiger). The characteristics of the study population were shown in Table15, separated by age range, duty and sex.

Serologic Analysis

All serum samples were examined by Microneutralization (MN) assay and Hemagglutination Inhibition (HI) assay. When the cut-off MN titer value $\geq$ 40 was used to determine positive neutralization antibody against H5N1 influenza virus infection, the MN results showed that 6 from 173 participants (3.47%) were seropositive and the HI titers were correlated with MN titers. Details of the seroprevalence of participants were demonstrated in Table16 and the characteristics of subjects with seropositive against H5N1 avian influenza virus were showed in Table17.

Table15. Characteristics of population study of high risk people of h5N1 avian influenza virus infection in Thailand

Characteristics	Group 1: Tiger Zoo 2004	Group 2: Poultry Farm 2007	Group 3: Poultry Farm 2008	Total Population (%)
Age group (year)				
15-29	35	12	28	75 (43.4)
30-44	20	14	15	49 (28.3)
45-59	3	19	16	38 (22.0)
60-74	-	1	5	6 (3.5)
Missing	-	-	5	5 (2.9)
Duty				
Feeder	15	12	41	68 (39.3)
Slaughter	6	22	14	42 (24.3)
Animal Trainer	1	-	-	1 (0.58)
Photographer	8	2	-	10 (5.8)
Show and Moderator	11	-	-	11 (6.4)
Carcasses handle	9	7	4	20 (11.6)
Veterinarian	4	3	5	12 (10.4)
Others	4	-	-	4 (2.3)
Missing	-	-	5	5 (2.9)
Sex				
Male	20	25	34	79 (45.7)
Female	38	21	35	94 (54.3)

Table 16. Neutralizing antibody titers against avian influenza virus (H5N1) among high risk people in Thailand, determined by microneutralization (MN) assay

Group of Participant	No. of Participant	No. of Participants by antibody titers (MN titers)								
		< 10	10	20	40	80	160	320	640	1280
Group 1: Tiger Zoo 2004	58	57	1	0	0	0	0	0	0	0
Group 2: Poultry Farm 2007	46	43	2	0	0	1	0	0	0	0
Group 3: Poultry Farm 2008	69	57	3	4	3	2	0	0	0	0

Table 17. Characteristics of H5N1 antibody positive participants

Subject Sex, Age (year)	Group	Duty	Period of Duty	Serologic Response (Antibody titer)	
				MN titer	HI titer
Male, 44	2	Poultry Slaughter	3 years	80	40
Male, 60	3	Feeder	Missing	80	40
Female, 16	3	Poultry Slaughter	1 year	80	80
Male, 40	3	Missing	Missing	40	80
Male, 22	3	Poultry Slaughter	2 years	40	80
Male, 15	3	Poultry Slaughter	Missing	40	40

Discussion

One way to identify the evidence of infection records of individuals exposed to H5N1 virus is the detection of H5-specific antibody. This study aimed to investigate the seroprevalence in high risk people in contact with infect or dead animal (avian/felid)

during the outbreak of H5N1 influenza virus in Thailand and determine the possible risk factors responsible for H5N1 infection in human. After the examination by using MN and HI assays, 3.47% of study population were founded neutralizing antibody against H5N1 virus. From six persons, four of them were the poultry slaughters and most of them (83.3%) were male. Many previous studies have been reported the seroprevalence of possible occupations exposed to H5N1 influenza virus ranged from 0-12% [165]. Some factors were responsible for various results, such as the different cut-off level to identify the positive titer, virus evolution and the diversity of time in serum sampling. It is uncertain if the specific antibody has been persisted in the blood until the subject sampling occurs. Therefore, it would be underestimated if the sampling was obtained after specific antibody were activated or disappeared.

In this study, seropositive subjects were distributed in all age range and most of seropositive subjects were male, correlated with the previous study in Cambodia, which men were more highly exposed to H5N1 influenza virus than women [166]. When the seropositive subjects were separated following the group of study, the results showed that most of them (83.3%) were in group 3, represent people who worked in poultry farm in 2008, a year after H5N1 outbreak. The results suggested that the H5-specific antibody can be existed more than a year after infection or there might be sporadic H5N1 infection in the same area after 2007 and these participants might have subclinical or asymptomatic infections. If the subclinical or asymptomatic infection can be occurred, it might be said that the H5N1 avian influenza virus has limited efficiency to replicate in human host cell and the high fatality rate (59%) from H5N1 infection might be overestimated value. However, only the seroprevalence cannot absolutely define the ability of H5N1 virus. The lack of acute serum samples and the disable to isolate and culture virus from samples can limit the further investigation.

Moreover, H5N1 surveillance in epidemiology and seroprevalence should be monitored continuously due to the accumulation of influenza virus mutation. Interestingly, the results showed that none of workers in tiger farm during the outbreak in 2004 had seropositive against H5N1. The participant might be well-prepared to protect the exposure between human and infected animal. In addition, no evidence had been showed the feline-to-human transmission while the first priority of H5N1 cross-species transmission was from poultry contact via direct contact with bodily fluids, such as take care of poultry, culling or using poultry carcasses in food preparation [165,167].