SEROPREVALENCE OF AVIAN INFLUENZA A/H5N1 AMONG POULTRY FARMERS IN RURAL INDONESIA, 2007

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Abstract. Since 2003, about a third (>150 cases) of human cases of highly pathogenic avian influenza (HPAI) A/H5N1 worldwide are reported from Indonesia. We measured the seroprevalence of H5N1 among Indonesian poultry farmers and assessed the risk factors for and knowledge of H5N1 infection. In 2007, poultry workers and farm residents were interviewed about risk factors for knowledge of and then examined for the seropositivity of H5N1 virus. Four hundred ninety-five of 622 farmers (80%) from 12 farms participated in the study. Of these, 71% were male, with a median age of 29 years. None tested positive for H5N1 virus. Masks were never worn by 54% of participants; 86% were afraid of becoming infected. For the preceding six months, 1 farm was confirmed as having poultry infected with H5N1 virus. No evidence of subclinical infection with avian influenza A/H5N1 virus was found among poultry farmers, although exposure of the farmers to this virus may have been limited. However, we recommend sustaining ongoing surveillance and control efforts.

Key words: avian influenza, farmers, poultry, seroprevalence, Indonesia

INTRODUCTION

In 1996, highly pathogenic avian influenza (HPAI) A/H5N1 was isolated from geese at a goose farm in Guangdong Province, China (Xu *et al*, 1999). One year later, an outbreak of the virus among poultry in Hong Kong resulted in the first human case; 18 cases were eventually reported with 6 deaths (Claas *et al*, 1998). After a quiescent period, the virus returned again in 2003. A first outbreak among poultry was reported from the Republic of Korea, followed by infection in tigers and leopards in Thailand and poultry in Vietnam (Keawcharoen *et al*, 2004; WHO, 2004; Lee *et al*, 2005). In subsequent years, infections among poultry and humans were reported from Southeast Asia, the Middle East, Europe and Africa (WHO, n.d.). By the end of June 2006, 31 of 33 provinces in Indonesia had had confirmed outbreaks of H5N1 in poultry, resulting in >16 million poultry deaths from sickness and culling (Sedyaningsih *et al*, 2007).

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The first human cases in Indonesia occurred in July 2005. By then, Indonesia was the worst affected country, with more than 150 reported human cases to date and a case-fatality rate of around 83%. All investigated cases were clade 2, subclade 1 H5N1 viruses, 76% of cases were associated with poultry contact (Sedyaningsih *et al*, 2007). Only a limited number of H5N1 virus seroprevalence studies have been performed among poultry farmers in Asia and none found any seropositivity (Hinjoy *et al*, 2008; Wang and Fu, 2009). Further seroprevalence studies among farmers and other risk groups, especially with clade 2 viruses, are needed.

Fig 1–Sector III farm in Sukabumi District, West-Java, Indonesia.

Studies among poultry and humans

The present study was part of an ongoing bilateral project between the Ministries of Agriculture of Indonesia and The Netherlands regarding the development and implementation of a HPAI control program. One of the goals of the collaboration was to investigate the animal/human interface in regard to the avian influenza virus A/H5N1.

A field trial to assess avian influenza vaccination effectiveness was carried out among poultry. In order to target areas where virus was circulating, thus constituting a potential risk factor for human infection, three postulated endemic sub-districts of Sukabumi District in the province of West Java were selected as study sites, following recent reports of outbreaks among poultry. These sub-districts are rural areas 100 km south of Jakarta, which have almost 270,000 inhabitants in total. An inventory was made of all sector III farms in the area, which are small commercial holders with low biosecurity measures, as opposed to large industrial sector I and II farms. These sector III farms are often family-run, with residential and poultry housing a close distance from the poultry on the same compound (Fig 1).

Twelve farms agreed to participate in the study. The farms had joined themselves into a treatment group where poultry was vaccinated using a locally produced homologous vaccine. Another group of farmers vaccinated their poultry with their own vaccination scheme. Intentionally unvaccinated flocks were considered unethical in this endemic area. Healthy unvaccinated chickens were placed as sentinels in all farms. Prospective follow-up consisted of serological testing of the sentinel birds and virological testing of sick or dying poultry in the flock or sentinel population. Further details of this field trial among poultry are described elsewhere (Bouma *et al*, 2008).

The aim of the human component of the bilateral program was to investigate the seroprevalence of avian influenza A/H5N1 antibodies among poultry farmers in rural West-Java, Indonesia, and to assess risk factors for infection and knowledge about the H5N1 virus.

MATERIALS AND METHODS

We conducted a cross-sectional serosurvey among all persons working and/or living on these twelve farms from 25 January until 1 February 2007. All participants were provided with verbal information about the study and gave written consent for participation. For their time, they were compensated with a bag of groceries or a small financial reimbursement. The study was approved by the Indonesian Ethics Committee.

Definitions

We used the following definitions in our study: influenza-like illness was defined as fever, cough and shortness of breath; a confirmed H5N1 case was a person living and/or working on a farm with H5N1 seropositivity; a suspect H5N1 case was a person living and/or working on a farm who had an influenza-like illness during the previous six months and exposure to sick and/or dying poultry at the farm or at home.

Questionnaire

Information was collected by trained public health officers through interviews in Bahasa Indonesian using a standardized questionnaire. Participants were asked their age, sex, residence and work address, occupation, medical history, whether they had symptoms during the previous six months, whether they had used personal protective equipment (PPE) while working with or close to poultry, their influenza vaccination status and their knowledge about and potential risk factors for H5N1. These risk factors included exposure to sick and/or dying poultry on the farm or at home.

Serological testing

All participants were asked to provide a single blood sample. Serum samples were collected, immediately processed and split into three cryotubes in the field and kept cold until transportation to the National Institute for Health Research and Development (NIHRD) in Jakarta where it was stored at -70ºC. One sample was double tested for antibodies to influenza A/H5N1 virus with a modified hemagglutination inhibition (HI) assay using A/Ck/Banten/05-1116/05(H5N1) antigen at NIHRD Jakarta. Another sample set was shipped frozen on dry ice to the National Institute of Infectious Disease neutralization (NIID) Tokyo and double tested for antibodies to influenza A/H5N1 virus with neutralization (NT) assay using A/H5N1/Indo/05/IBCDC-RG virus (Setiawaty *et al*, 2010). According to WHO guidelines, H5N1 seropositivity was defined as having positive results on 2 neutralization (titer ≥80) and hemagglutination Inhibition tests (titer ≥160) as well as two positive independent tests.

Statistical analysis

Data were double-entered into Microsoft Excel (Microsoft, Redmond, WA).

RESULTS

Farms and poultry

The twelve participating farms in Sukabumi District ranged from holding 200 native chicken broilers to 500,000 commercial layer poultry. An outbreak of H5 virus infection occurred on one farm

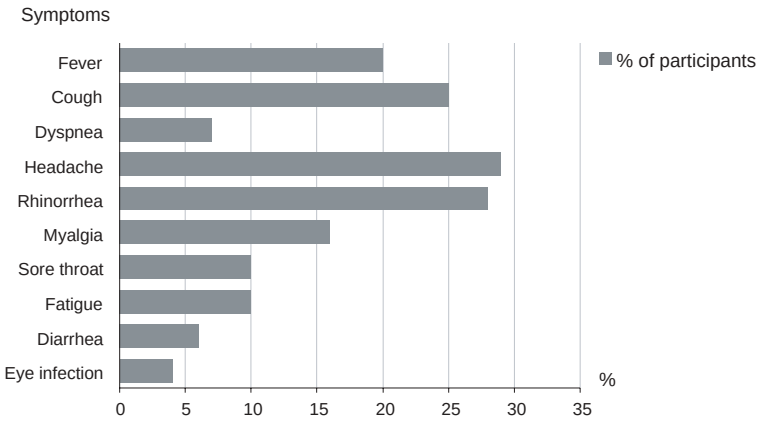

Fig 2–Percentage of participants, living and/or working on poultry farms, reporting symptoms during the previous six months (*N*=493).

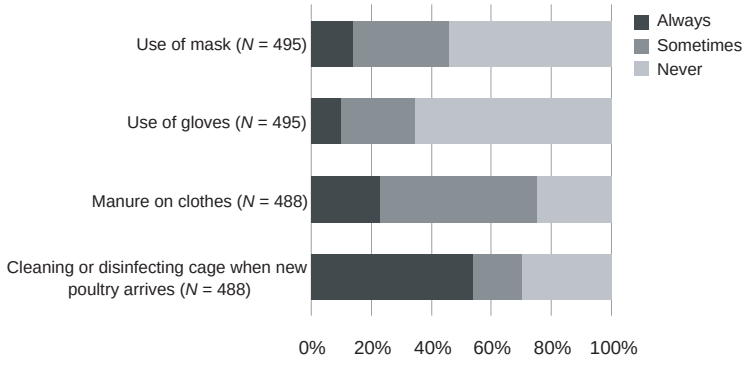

Fig 3–Percentage of persons, living and/or working on poultry farms, reporting use of protective personal equipment and exposure to poultry or manure.

(79.6%) were interviewed and provided serum samples (Table 1). The median age of participants was 29 years (interquartile range 23-36 years), 70.9% were male. Most persons (94.7%) lived on the farm where they work.

Eleven persons (2.2%) had obtained influenza vaccination, only one since 2002. Many participants (44.2%) reported current or past smoking. Some persons reported underlying conditions in their medical history: 7.3% had allergies, 3.8% had chronic lung diseases (of which 53.6% had asthma), 0.4% had chronic heart disease and 0.2% had diabetes.

Besides the poultry on the farm, 377 of 493 (76.2%) participants kept poultry in- or outside their households as pets (church birds 48.9%, wild birds 22.2%, chickens 5.7%, ducks and geese 3.2%). Most households kept cats (83.8%) and dogs (64.2%), 11.1% kept no pets at all.

within several weeks after vaccination of flocks and placement of sentinel birds in June 2006, as demonstrated by a positive PCR test and virus isolation in embryonated eggs (Bouma *et al*, 2008). No other farms tested positive for H5N1 virus in sentinels or farmed poultry during that time period.

Study participants

Of 622 eligible persons living and/or working at the twelve poultry farms, 495

Occupation and activities on the farm

Table 2 summarizes exposure factors, such as work activities on the farm and animals around the home. Most participants were responsible for feeding poultry and handling eggs on the farm. Keeping pets was common, and cats, dogs, and different type of birds were reported as animals residing around the home.

Symptoms and serological results

Of the 493 participants reporting

Table 1
Demographic and exposure characteristics of study participants (*N* = 495).

	Characteristic	*n*	%
Age group (years)	0-18	50	10
	19-30	237	48
	31-45	180	36
	46-95	28	6
Sex	Male	351	71
	Female	144	29
Residence on farm	Yes	469	95
	No	25	5
Education	Primary school or less	83	17
	At least secondary preschool	412	83
Smoking	Yes, current or past	276	56
	No	219	44
Chronic medical condition	Total	58	12
	Any lung disease	19	4
Influenza vaccination	Yes	11	2
Type of poultry	Layer	409	83
	Native chickens	64	13
	Broiler	2	0

Table 2
Exposure factors reported by persons, living and/or working on poultry farms (*N* = 495).

		n	%
Work activities on the farm (multiple activities possible)	Feeding poultry	192	39
	Handling eggs	134	27
	Cleaning cages, collecting feces	66	13
	Collecting poultry	50	10
	Maintenance of cages	29	6
	Slaughtering	28	6
	Security	28	6
	Administration	21	4
	Selling poultry	17	3
	Animal health / sanitation	11	2
	Driver	10	2
Animals around the home	Cats	415	84
	Dogs	318	64
	Church birds	244	49
	Wild birds	110	22
	Fish	14	3
	No animals	55	11

Table 3
Knowledge of persons living and/or working on poultry farms about symptoms of avian influenza A/H5N1 infection.

	Symptom present in AI		Symptom absent in AI		Don't know	
Symptom	Number	%	Number	%	Number	%
Fever (*N*=495)	382	77	43	9	70	14
Cough (*N*=491)	343	70	62	13	86	18
Shortness of breath (*N*=491)	327	67	74	15	90	18
ILI [a] (*N*=490)	308	63	114	23	68	14
Runny nose (*N*=494)	269	54	104	21	121	24
Headache (*N*=490)	186	38	148	30	156	32
Fatigue (*N*=479)	177	37	143	30	159	33
Muscle aches (*N*=487)	145	30	165	34	177	36
Diarrhea (*N*=488)	107	22	196	40	185	38
Eye infection (*N*=486)	90	19	202	42	194	40

[a] ILI = Influenza- like illness

symptoms (Fig 2), 55 (11%) complained of fever and cough during the previous 6 months. Seventeen (3%) reported an influenza-like illness, of which one person had been exposed to unusual deaths in poultry on the farm and therefore fulfilled the case definition for a suspected case. A 26-year old male had a titer of 20 on the hemagglutination test, which was regarded as an inconclusive titer. According to our case definition it was not viewed as a confirmed case of avian influenza. This person reported no symptoms during the previous six months and had not been exposed to unusual deaths among poultry or H5N1-positive sentinel chickens on the farm. He did report always having poultry manure on his clothes after a day of working as a security officer. Fourteen participants (3%) reported unusual deaths among poultry on their farm during the previous six months.

Protective personal equipment was scarcely used by participants (Fig 3). Masks were always worn by 14.1% and sometimes by 32.1% of participants. A large proportion of participants reported having poultry manure on their clothes after a day at work.

Knowledge about avian influenza

Fever, cough and shortness of breath were reported as symptoms by 77, 70 and 67% of respondents, respectively (Table 3). A combination of all three symptoms was reported by 63%. Replies about symptoms by farm owners were more accurate than those by poultry workers on the farm (93% *vs* 62%, $p<0.05$). Results for men and women were similar (62%). Symptoms reported were less accurate from those 18 years old and younger, than by adults (46% *vs* 65%, $p<0.01$). Those with primary education or lower reported symptoms more accurately, than those with a higher education (74% *vs* 61%, $p<0.05$). Eighty-six percent of participants were afraid of be-

coming infected with avian influenza. An open question asking participants about their opinions about avian influenza showed 22 subjects (3%) replied avian flu is curable, not serious or did not affect humans, while 473 comments (95%) stated it is transmissible to humans and is a frightening and deadly disease in poultry workers.

DISCUSSION

Our field study did not find evidence of antibodies against avian influenza A/H5N1 among West-Java poultry farmers, despite recent reports of H5N1 outbreaks among poultry in the area. This finding is consistent with other Asian studies that found limited seropositivity among poultry farmers exposed to H5N1-virus since 2004 (Hinjoy *et al*, 2008; Wang and Fu, 2009).

Recent reports of outbreaks among poultry in the area, as well as a study of backyard water fowl from the same province showed 4.6% were positive for H5N1 virus, implying active circulation of the virus (Susanti *et al*, 2008). During the study period only one farm had an outbreak of H5 virus infection among poultry as detected by testing of sentinel chickens. This may indicate the absence of circulating virus and/or vaccination of poultry on the other farms that participated in our study. Farms with strict biosecurity measures in place may have been more likely to participate, due to the sensitive nature of the situation. The low seroprevalence in our human study population may be due to limited exposure of our participating farmers to the H5N1 virus. A study among highly exposed poultry workers, which was also performed by our collaboration group, showed different seropositivity results and will be published elsewhere.

Another limitation of our study is that this outbreak occurred in July-August 2006 and participating farmers were only tested in January 2007. Persons exposed to and infected by the circulating virus in 2006 may have had their antibody levels return to undetectable values 6 months later when testing was performed, explaining the lack of seropositivity among farmers. Only one serum sample was obtained from each study participant. Therefore, our study provides no information regarding incident cases, the duration of antibodies and titer levels in the human body. We lack information regarding interpretation of diagnostic test results, since the implications of low titers remain unknown. Further antibody titer studies in humans need to be performed.

Eleven percent of persons reported symptoms of fever and cough during the previous six months, similar to 18% of persons reporting these symptoms after having been exposed to sick or dead poultry in Vietnam (Thorson *et al*, 2006). Similar symptoms, such as fever, cough and rhinorrhea, were reported by confirmed H5N1-patients in Indonesia (Kandun *et al*, 2008). Recall bias may have occurred though, as participants were asked about exposures and symptoms during the previous six months. Risk factors for infection could not be assessed due to the absence of seropositive cases.

Avian influenza was considered a dangerous disease by most respondents. Only two-thirds of participants accurately recognized the symptoms of an H5N1-infection. Comparable studies of knowledge about and concern regarding avian influenza are limited, especially from developing countries.

Our study was performed in early 2007 and the current situation may have

changed. Therefore, we need to remain vigilant and not draw premature conclusions. Further studies investigating the seroprevalence of H5N1 should be carried out, especially among risk groups in a population with frequent exposure to the virus.

In summary, our results suggest a low seroprevalence of H5N1 in poultry farmers in West-Java, which may reduce concern for frequent mild infections in poultry farmers. However, incomplete evidence for infection of poultry with avian influenza sheds doubt on the H5N1-exposure of these farmers. Further studies are needed to investigate the presence of the avian influenza virus in humans, as well as the implications of low titers on diagnostic tests and changing antibody levels in humans. In light of the severity of human HPAI infection and the risk of another pandemic, we recommend ongoing surveillance and education of poultry farmers about HPAI prevention and symptoms.

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