Identification of influenza A viruses in ducks and the relation between the prevalence of influenza A viruses in ducks and risk factors in Cipunagara, Java, Indonesia

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November 2010 - January 2011

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Acknowledgements

I would like to thank all the students from the veterinary medicine faculty of Bogor University, especially Arief Ervana and Ni Made Ferawati for helping us surviving in Jati, where our base camp was located and were we lived for two months. Without them it was impossible for us to do this project in Cipunagara. For this, I would also thank Unita Pratiwi, Adrianus Fardin Arut, Haryandhi Eka Putra and field coordinator Kanda Muhamad. Thank you for the good cooperation and the great time we had in Jati.
Also, I would like to thank all the personnel from Dinas Subang. Without this team it was impossible to find the duck flocks and to do the sampling in the early mornings.
Off course, thank you for all the duck keepers who participated in the study for their cooperation and time.
People from West Java Dinas Peternakan Laboratory in Cikole, especially Yoni and Afrida, thank you for processing the 5235 trachea- and cloaca samples and 500 blood samples.
Lots of thank for all the people from Bogor University, especially Ibu Ethi, Yusuf Ridwan, Chaerul Basri and Denny W. Lukman for guidance during the data analyzing and of course for the great excursions. Thank you very much.
Thank you very much, Dr. Patrick Hermans and Dr. Ivo Claassen for your support in Jati and in Jakarta and for supervising the project. Also thank you for the excursions we made in Jakarta and providing a place to sleep if necessary. There was no way we could have done this project without you.
Also thanks to Dr. Annemarie Bouma from Utrecht University for giving us this opportunity to do a research project in Indonesia.
Finally, Sandy van Kruijsdijk, my research partner, thank you very much for our cooperation and for the great time we had during our stay in Indonesia.

Contents

Abstract

Indonesia is one of the countries where Avian Influenza (AI) is endemic. In several parts of Indonesia, nomadic ducks are being used after harvesting the rice for cleaning the rice fields from weed, snails and insects. Nomadic ducks might play an important role in spreading all different types of influenza A viruses, because it is well known that aquatic birds form a reservoir for most influenza A types. In this project, questionnaires were used to obtain information about nomadic duck flocks. Next to this, trachea, cloaca and blood samples have been taken to test for the presence of influenza A virus (including H5-subtype) and antibodies against influenza A (including antibodies against H5-subtype).

The aim of this study is to investigate the types of influenza A that are present in nomadic ducks in Cipunagara subdistrict (Subang district, Java, Indonesia) by taking trachea, cloaca and bloodsamples, and to relate the acquired data to flock size, origin of the ducks, duck breed, keeping reason, type of housing system, location of the ducks at night, transportation methods of the ducks, movements of the flocks and vaccination.

32% of the flocks tested positive with the PCR-test for the presence of influenza A. Influenza A subtype H5 was not found in the flocks. 100% of the flocks showed the presence of antibodies against influenza A. 6% of the flocks have antibodies against H5-subtype. These results were combined with the results of the questionnaire. This showed a relation between flock size and the presence of influenza A. Bigger flocks have a bigger chance of being influenza A positive. Next to this, there is a relation between flocks which are being removed from the rice fields at night and the presence of H5-subtype specific antibodies. Flocks that are being removed from the rice fields at night show more antibodies against H5-subtype.

Introduction

Avian Influenza (AI)

Influenza viruses are placed in the family of Orthomyxoviruses.[1,25] They have segmented, negative-sense RNA genomes and the family consists of five different genera. These five genera are influenza types A, B and C, the Isavirus and Thogotovirus. The influenza type A viruses are the most widespread and are the most important members of the group. They can infect many avian and mammalian species. The influenza types B and C are primarily human pathogens which rarely infect other species.[17,25] The genera of Isavirus is an important fish pathogen[13] , and the genera of Thogotoviruses are tick-borne arboviruses that have been isolated from humans and livestock.[14] Of the family of Orthomyxoviruses, only the influenza A virus is known to infect birds.
Influenza virus type A can be further divided into subtypes. These subtypes are based on the antigenic relationships of the surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). Until now, 16 HA-subtypes (HA1-16) and 9 NA-subtypes NA1-9) have been recognized. Every influenza A virus has one HA and one NA. Every combination is possible, which means that there are 144 possible influenza A viruses. All influenza A subtypes in the majority of possible combinations have been isolated from avian species.
Of the Avian Influenza virus (AI-virus), there are two pathotypes; Low pathogenic (LPAI) and high pathogenic (HPAI). The majority of the AI-viruses is low pathogenic.[3] The LPAI and HPAI designations are derived from inoculation of eight susceptible chickens in the laboratory. HPAI virus causes death in six or more chickens, while LPAI virus does not result in dead of any chicken after inoculation.
As written before, the majority of all possible HA and NA combinations have been isolated from avian species. Thus far, only AI-virus of the subtypes H5 and H7 have been shown to cause HPAI in susceptible species. This does not automatically mean that all H5 and H7 viruses are HPAI.[4] H10 showed to be HPAI once but this could not be repeated.
AI-viruses have been shown to infect both birds and mammals. Even though, birds are infected more readily and efficiently than mammals. Next to this, transmission between birds of the same species and birds of other species occur to a greater extent than the transmission between mammals of the same species and mammals of other species.
A great variety of birds can be infected by AI-virus[21], including free-living birds, captive caged birds and domestic ducks, chickens, turkeys and other domestic poultry. Previous research projects revealed enormous pools of AI-virus in the wild bird population.[16,23] These large pools were mainly found in waterfowl, Family Anatidae, Order Anseriformes. Other studies suggested that waterfowl do not act as a reservoir for all types of AI-viruses.[19,25] A big part of the AI gene pool is in the shorebirds and gulls. The subtypes of these AI-viruses are different from the subtypes found in ducks.[11]

Transmission

Influenza viruses in aquatic birds replicate mainly in the intestinal tract. They are shed by the feces and transmission takes place by fecal oral transmission. This is often through water.[5]
It is likely that transmission between birds occurs as a result of close contact between infected en uninfected hosts. It is believed that for the transmission of AI-virus close contact with infected birds or their feces (or other body fluids) is necessary. This makes the

spreading of the AI-virus by air over large distances very unlikely and until now there is no published evidence that transmission by air occurs.[4]

Avian Influenza in Indonesia

Since 1997 outbreaks of HPAI H5N1 virus among poultry flocks have been reported and since then the infection has spread to many countries all over the world.[9] HPAI outbreaks are often successfully controlled by stamping out the infected flocks, in combination with other measures such as hygienic measures and a stand still. However, these draconic measures cannot always be implemented and in some countries in Asia and Africa H5N1 has become endemic. HPAI can be a real problem for the poultry industry, as it causes high mortality and loss of production. It also has zoonotic features[18], meaning that there is a possibility that HPAI may cause a pandemic in humans. Control of this disease is therefore of major concern for public health authorities.
Indonesia is one of the countries where AI is endemic. In august 2003 the first outbreak was detected and since then the virus spreads rapidly across the country. Human cases have been reported since 2005.[8] Indonesia had in 2009 the highest number of human infections and deaths worldwide.[26] This has prompted the Indonesian government to intensify the current control measures and to focus on the core aspects of the national strategic plan. The Indonesian government is highly committed to combating AI and is supported by a number of national and international agencies in its response. Integrated response plans were developed and are being implemented by various agencies.[27]

Nowadays, 31 out of 33 provinces in Indonesia are considered to be endemically infected. Large outbreaks resulted in high mortality rates among poultry and a great economic loss. In the commercial poultry flocks the disease is now controlled by vaccination programs, reducing the economic losses if virus is introduced in a flock. The programs seem rather effective, but have not prevented further spread of the infection or eradication of the virus from certain areas. There is only a small vaccination program for poultry from small holders and outbreaks in small back yard flocks are still reported. During these outbreaks several humans got infected and more than 100 of these infected humans died from the infection (WHO, 2010). Infections in large commercial flocks are not reported, but it is not clear whether this means that they do not occur or are just not reported to the authorities.

Ducks in Indonesia

In Indonesia, ducks represent 2,77% of the total poultry production.[5] This commodity is less important in comparison with the chicken industry. The duck population consisted of 33,068,244 animals in 2003, 32,572,780 animals in 2004 and 34,275,340 animals in 2005 (Agricultural Statistics Book, 2005). 40% of the ducks of Indonesia are on Java.[5]
Because of the high economic advantages in commercial business and a high nutrition value for humans, ducks are an important poultry commodity, both in small/medium scale poultry and commercial poultry. Ducks provide income and feed to the farmers, but they also help to control insects and weeds in irrigated rice-fields.

In Indonesia, duck flocks that are being herded by one single man usually consist of 90 till 130 ducks. During the day, the flock is held on the rice field while during the night the flocks are being confined. The ducks are only on the rice fields after the rice has been harvested. The food on the rice fields consists of insects, snails, grain, leaf material, crabs and frogs. As written before, during the night the flock is in a confinement, usually not very far from the place where the ducks stay during the day. The confinement is usually a bamboo pen. In this pen the ducks lay their eggs (usually 4-5 per week) and in the morning the herdsman collects these eggs. The eggs are being sold or consumed by the family.
The flock is being removed from one rice field to another when there is not enough food left for the ducks. Thus, as long as there is enough food the ducks stay at one location. When the food is running out the herdsmen moves the flock to another location where more food is available. When there is not enough food on the rice fields additional food will be given to ensure a good egg production. Next to egg production, meat production can be a reason to keep ducks. When the time is there for the flock to remove to an other location there are multiple ways to transport the ducks, for example by car, motorcycle, truck or by foot. Everything the family owns will be taken with them when they move to another location. This means they live nomadic during part of the year. The family usually stays near the ducks on a grassy area with some protection provided by trees.

As said before, the natural host and reservoir for al the influenza A viruses is formed by wild birds, especially waterfowl, gulls and shorebirds. Experimental research indicates that ducks may play a major role in the spreading (and thus the maintaining) of HPAI.[4,21,22,28] Ducks infected with AI may show no clinical signs but they can excrete high concentrations of virus which can be pathogenic for other poultry species. [6,10,12,24] Possible risk factors for the spreading of HPAI in Indonesia include duck movements, contact between ducks and other poultry and animal species, poor husbandry, inadequate handling of sick and dead ducks and poor awareness of control strategies among poultry farmers. [5]

The status of influenza A in commercial ducks is in most countries poorly understood or has not been investigated yet. There have been some surveillance projects in the past and they showed very large pools of virus and many subtype combinations have been detected, especially from meat birds which are usually fattened on open fields. A study in Great Britain reported the isolation from 32 viruses from 60 pools of cloaca samples which were taken from ducks at slaughter.[2] Studies in Hong Kong in the late 1970's and early 1980's showed that about 6% of the ducks (carcasses at duck dressing plants or on duck farms) were infected with various types of influenza viruses.[20]

No analytical studies regarding the relation between risk factors and the spreading of HPAI have been conducted in Indonesia.
In relation with the information above and the fact that the free-ranging system is used a lot in Indonesia, even though ducks do not represent a large percentage of the total poultry production, nomadic ducks might play an important role in the spreading of all different types of influenza A virus.[4,5]

Project outline

In 2005, a partnership program was established between the Ministry of Agriculture in Indonesia and the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) to assist the Indonesian government in the control of HPAI in Indonesia.
As part of the activities of this partnership, a multi-intervention pilot project will be conducted. This pilot project aims to control HPAI outbreaks in one defined area, in all sectors of poultry production, i.e. commercial breeding farms, commercial broiler farms, slaughter and poultry collecting facilities, nomadic duck flocks, and village backyard poultry flocks, using a variety of control measures. The main goal of this project is to assist local veterinarians and farmers to improve control measures and to get information about epidemiological characteristics of the infection, which will also contribute to improvement of the control strategies. An important part of the Indonesian-Dutch partnership is improvement of the poultry health and the associated income of the local villagers.

The project took place in Cipunagara subdistrict, Subang, West Java. Cipunagara subdistrict is an area where multiple large sector 1 poultry farms are established. In the area small outbreaks in backyard poultry are still reported. As said before, infections in large commercial flocks are not reported, but it is not clear whether this means that they do not occur or that they are just not reported to the authorities. The fact that small outbreaks still occur is the main reason why there is a lot of interest in the prevalence of influenza A in this area in all different types of poultry (Sector 4 (backyard poultry) till Sector 1 (large broiler, breeder, hatchery farms)) and nomadic duck flocks.
Previous projects showed that none of the tested ducks in Subang district were infected with H5N1, but lots of ducks were infected with other types of influenza. In other parts of Indonesia, in 2005 lots of AI positive ducks were found.[5] This data could reinforce the assumption that ducks might play an important role in the spreading of HPAI to the chicken livestock in Indonesia.
The population of ducks is mainly distributed on areas with large rice fields. The total population of ducks in Subang district in 2005 was 485,090 animals.[5] It is important to find out which type of influenza virus circulates amongst these duck flocks. Questionnaires will be used to acquire information about the duck flocks and samples (cloaca, trachea and blood) will be taken from the ducks. The samples will be analyzed for presence of AI (and partially subtype identification) and these results will be related to risk factors like origin of the ducks, flock size, duck breed, housing, transport method and rate of movement of the flock. The final results may provide a good image of the prevalence of AI in ducks and the role of nomadic duck flocks in the spreading of AI. The identification of risk factors may be of great value to reduce the spread of AI virus strains within a designated area and thereby contribute to control of AI in Indonesia.

Goal & Hypothesis

The goal of this project is to investigate the type of influenza present in nomadic duck flocks in Cipunagara subdistrict and to relate the acquired data to flock size, origin of the ducks, duck breed, keeping reason, type of housing system, location of the ducks at night, transportation methods of the ducks, movements of the flocks and vaccination.

The null hypothesis is that there is no relation between type of influenza A in the ducks and risk factors as rate of movement of the flock, origin of the ducks, size of the flocks, type of duck breeds kept, transport method and type of housing system.

Materials & Methods

Location

The sampling of the ducks took place in Cipunagara subdistrict, district Subang, province West Java, Java, Indonesia (Appendix I). Cipunagara subdistrict was selected because poultry farms of all four sectors are present in this area. Multiple large sector 1 poultry farms are established here and small outbreaks in backyard poultry are still being reported. Next to this, this subdistrict consists largely of rice fields. After harvesting the rice large numbers of nomadic duck flocks come to the rice fields. All together this means that in this subdistrict a good picture can be obtained of the prevalence of influenza A viruses in ducks and the possible role they play in transmitting AI to other poultry.

Sampling

The project took place from November 2010 until January 2011. With the help of staff of the District Livestock Services (Dinas) of Subang the duck flocks were located and visited as soon as they came into Cipunagara subdistrict, or, in the case of flocks who were already in Cipunagara when the project started, as soon as the flocks were located by the Dinas staff. The team who sampled the ducks consisted of 6 Veterinary Medicine students from Institut Pertanian Bogor, Fakultas Kedokteran Hewan, (Bogor, Java, Indonesia), 2 Veterinary Medicine students from Utrecht University, faculty of Veterinary Medicine (Utrecht, Netherlands), 2 veterinarians and 9 person staff from DINAS. One of the veterinarians was the field coordinator. Motorcycles were used to reach the sampling location from the base camp in subvillage Jati (village Jati, subdistrict Cipunagara, district Subang).
With the help of WinEpiscope, computer software for quantitative veterinary epidemiology, was determined how many trachea- and cloaca samples had to be taken from the different flock sizes with a sensitivity of 95%, a specificity of 100%, a prevalence of 5% and a 95% confidence. (Appendix II)
A flock was considered a flock if they move around together always. So they never split up. This means that a flock can consist for example of 4 owners with ducks, separated on the rice fields but they arrive at the same time at the same location as the other flocks and leave at the same time to go to the same destination as the other flocks.
From each duck flock 10 blood samples were taken and, depending on the flock size, a maximum of 60 trachea samples and 60 cloaca samples. Healthy ducks were sampled as well as sick ducks.
After a duck had been sampled this duck was separated from the ducks that still had to be sampled to prevent sampling the same duck multiple times.

Trachea samples & cloaca samples

Trachea and cloaca samples were taken from the ducks using dry Giant Cotton Buds (non sterile), manufactured by Pt. Charmindo Mitra Raharja, Jakarta 11130, Indonesia. The cotton buds were cut into half after sampling because otherwise they didn't fit in the tube. Every time, the trachea sample and the cloaca sample were taken from the same duck, so it would be possible to compare the results of both samples of the same duck later. Sterile tubes were used to transport the samples from the sampling location back to the base camp and from the base camp to the West Java Dinas Peternakan Laboratory in Cikole. The sterile tubes contained virus transport media (pH 7,4), which consisted of PBS buffer, antibiotic solution and glycerin. The samples were transported in cool boxes containing cooling elements to keep the samples cooled and they were send to the laboratory the same day.
Labels were used for identification of the samples for the laboratory. On the labels was written: research project code, flock number, cloaca sample (C) or trachea sample (T) and sample number. For example BA/01/T/05. BA for the project code, in the case of this research project Bebek Anjor (nomadic duck), 01 for flock number 1 (the first flock that was sampled during the project), T for trachea sample and 05 for the fifth trachea sample of that flock. Tape was used to secure the labels on the tubes with the samples. For transport to the laboratory all the cloaca samples of 1 flock were put together in a sealable bag and the same for the trachea samples of 1 flock. The bags were labeled as well as shown before for the individual samples (for example BA/01/T/01-60). Each 5 samples were put together in the laboratory to reduce the costs of analyzing. The sterile tubes containing the virus transport media were stored at -20 degrees Celsius in the base camp. In the laboratory the samples were stored at - 70 degrees Celsius. In the laboratory the samples were tested at the presence of influenza A virus with a PCR-test. For the PCR-test is a kit from QIAGEN used.
Embryonated eggs were used in the laboratory for virus isolation (Virus is isolated following the protocol of the OIE), and the characterization of influenza A viruses.[4]
If either the PCR test for the trachea samples or the PCR test for the cloaca samples was positive (or if they were both positive) the flock was considered influenza A positive.

Blood samples

Blood samples were taken from the brachial vein using 23-gauge (0,6 x 32 mm) needles with 3 ml syringes. The duck was fixated by a second person and the wing was fixated so that the bottom side of the wing was pointing upwards. A few feathers were plucked to provide a good view of the collection side and cotton wool with 70% ethanol was used to disinfect the blood collection site. From each duck at least 2 ml blood was taken. After collection of the blood, the syringe was emptied in a vacuum tube. The blood samples were transported to the base camp, in the same cool boxes as the trachea- and cloaca samples were transported.
For the serology, serum was needed, so the anticoagulant free tubes were centrifuged at the base camp. The serum was separated from the blood, using a centrifuge for 5 minutes with 2000rpm and put into 1,5 ml eppendorf microtubes (Genuine Axygen Quality microtubes MCT-150-C 1,5 ml clear). The eppendorftubes were closed and sealed using parafilm (M Laboratory film Pechiney Plastic Packaging). Labels were put on the eppendorftubes with the sample code as shown before (S instead of T or C) for identification for the laboratory. The serum samples were sent to the West Java Dinas Peternakan Laboratory in Cikole the same

day. If analysis was not possible the same day the samples were stored at - 20 degrees Celsius. In the laboratory the Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect antibodies against influenza A viruses, as well as the Hemaglutinin Inhibition Test (HI-test) to see if there are H5-subtype specific antibodies present in the serum.

Questionnaires

After the samples had been taken standardized questionnaires (Appendix III) were filled in by the enumerators. The questionnaires were used to obtain information about the owner, size of the duck flocks, breed of the ducks, locations the owner/keeper/respondent has visited (inside Cipunagara and every location one month before they entered Cipunagara), used transportation and vaccination status. The flocks which moved from location inside Cipunagara were visited each time they moved so the movements inside Cipunagara could get mapped. At every location inside Cipunagara the global positioning system (GPS) coordinates were filled in the questionnaire so it would be possible to analyze the movements of the flocks inside Cipunagara and see if there is an relation with the prevalence of influenza A in the duck flocks. Google earth was used to determine the distances that the duck flocks travelled.

Hygienic measures

Because the ducks were possibly infected with zoönotic agents (including influenza A) certain hygienic measures had to be taken. Protective clothing was used as well as rubber boots, latex gloves (Sensi gloves Latex) and face masks (Standard Tie on Surgical Masks, 3M Health Care). After the sampling was finished the rubber boots were disinfected using ethanol 70% and the facemasks and gloves were discarded in a sealed plastic bag. Hands were cleaned with alcohol and hand disinfectant. Back at base camp the rubber boots were cleaned with water.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 13 was used for the analysis of the data. The Pearson Chi-square test was used for the comparison of frequencies for significance.

Results

Descriptive analysis of the results of the questionnaires

Samples

From November 24th 2010 till December 16th 2010 fifty (50) duck flocks of different sizes were sampled in Cipunagara subdistrict. A total number of 2620 ducks have been sampled in this period. Of the 2620 ducks that have been sampled, 2615 trachea samples were taken (table 1) and 2620 cloaca samples have been collected. A total number of 500 blood samples have been collected from the ducks, with 10 samples per flock.

		Trachea	Cloaca	Blood
N	Valid	50	50	50
	Missing	0	0	0
Mean		52,30	52,40	10,00
Std. Deviation		6,162	6,164	,000
Sum		2615	2620	500

Table 1. Number of samples that have been taken

Flock size

The mean flock size of the flocks that have been visited is 288,20 ducks with a standard deviation 258,137 (table 2). The smallest flock that has been visited consisted of 50 ducks and the largest flock consisted of 1250 ducks. The flock sizes have been sorted into groups. As shown in figure 1, 52% of the flocks have a flock size of 200 ducks or less. 30% of the flocks consisted of between 201-400 ducks and 6% of the flocks we visited consisted of 401-600 ducks. 4% has a flock size between 601-800 ducks and 4% consisted of 801-1000 ducks. 2% of the flocks consisted of 1001-1200 ducks and finally 2% of the flocks that have been sampled consisted of more then 1200 ducks.

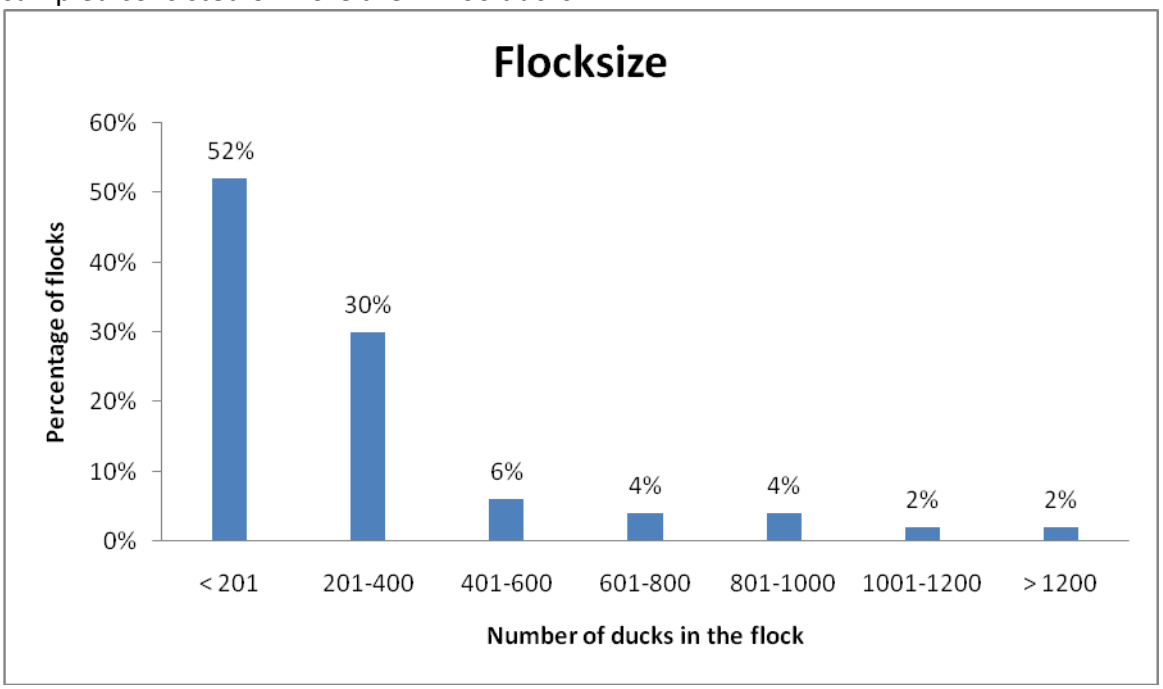


Figure 1. Flock sizes

	Valid	50
	Missing	0
Mean		288,20
Std. Deviation		258,137
Minimum		50
Maximum		1250

Table 2. Flock sizes

Duck breeds kept & reason for keeping the ducks

As shown in figure 2, 86% of the duck flocks we visited consists of Java Duck. 4% consists of mixed breeds and for 8% of the flocks the owner doesn't what breed of ducks he keeps. In 2% of the flocks the answer on the question was left empty.

The reason why the owner keeps the ducks is shown is figure 2. 94% of the owners keeps the ducks only for the egg production. 2% keeps the ducks for the meat production and 2% of the owners keep the ducks for egg- and meat production. 2% of the owners keeps the ducks for egg- and meat production and for economical reasons.

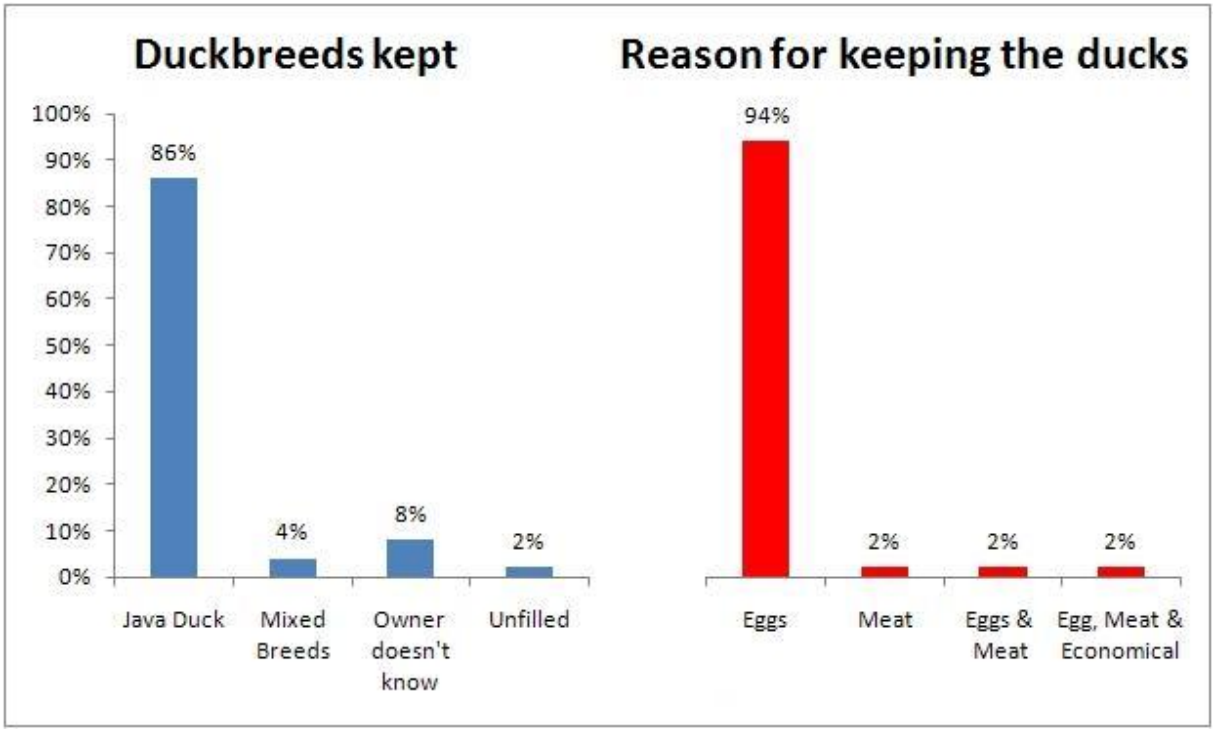


Figure 2. Duck breeds that are kept by the owners and the reason(s) why the ducks are being kept.

Flock location if not moving around

When the flock is not moving around, figure 3 shows that 64% of the duck flocks stay at the place where the owner lives. 36% of the flocks stay somewhere else (n=18).

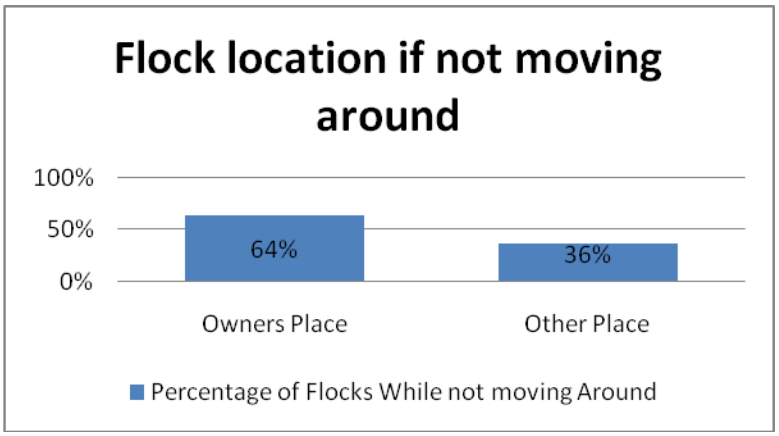


Figure 3. Location of the flock if the flock is not moving around (at the owners place or not).

Of the flocks that stay somewhere else, 66,7% stay in Cipunagara, Subang. 5,6% stays in Binong, Subang and 5,6% stays in Bongas, Indramayu and 5,6% stay in Cibogo, Subang. Of 16,7% of the flocks the location is not known.

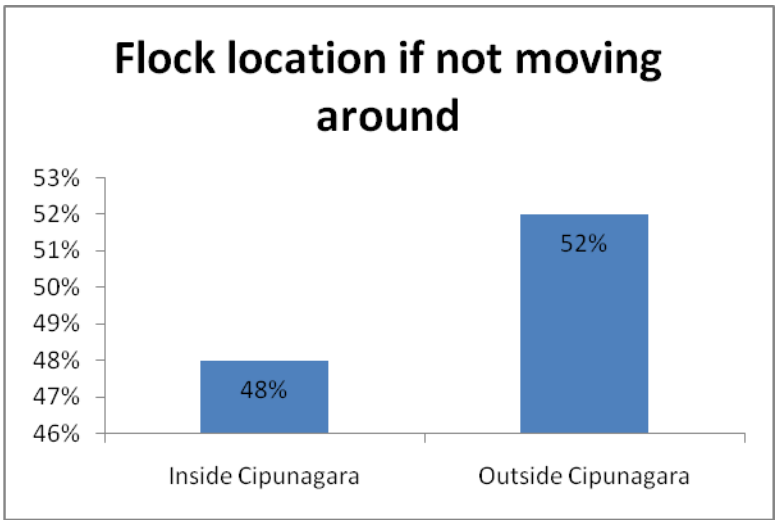
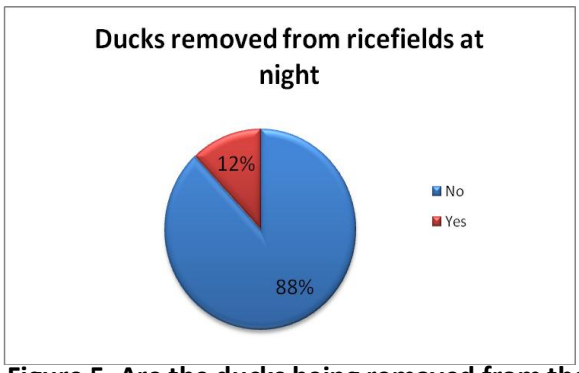


Figure 4. Location of the flock if the flock is not moving around (inside of outside Cipunagara).

Of the owners who live in Cipunagara (n=14), 12 flocks (86%) stay at the owners adress when not moving around. 1 (7%) owner who lives in Cipunagara doesn't keep the ducks at home while not moving around but keeps the ducks at a different location in Cipunagara. The owners who live outside Cipunagara and who keep their ducks not at home (n=36) when they are not moving around, 11 flocks (=31%) stay in Cipunagara when not moving around. All together this means that when the flocks are not moving around 48% of the visited flocks (n=24) stay in Cipunagara.

Location of the flocks at night

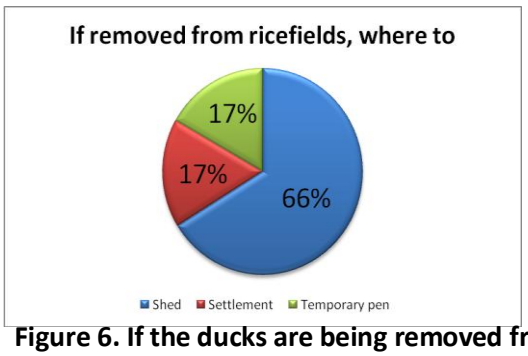


All the duck flocks are being held confined during the night. As shown in figure 5, 88% of the duck flocks are not being removed from the rice fields at night. 12% of the flocks is removed from the rice fields during the night. The flocks that are being removed from the ricefields consist of a maximum of 400 ducks (Table 3).

Figure 5. Are the ducks being removed from the rice fields at night

		Ducks removed at night		
		No	Yes	Total
Flocksize	< 200	22	4	26
	201-400	13	2	15
	401-600	3	0	3
	601-800	2	0	2
	801-1000	2	0	2
	1001-1200	1	0	1
	> 1201	1	0	1
Total		44	6	50

Table 3. Ducks removed at night VS flock size



If the ducks are being removed from the ricefields at night, 66% spends the night in a shed, 17% in a settlement and 17% spends the night in a temporary pen (figure 6).

Figure 6. If the ducks are being removed from the rice fields at night, where to?

Vaccinations

Figure 7 shows that 4% (n=2) of the duck flocks that have been visited have been vaccinated against AI. None of the flocks was vaccinated against ND or any other infectious disease.

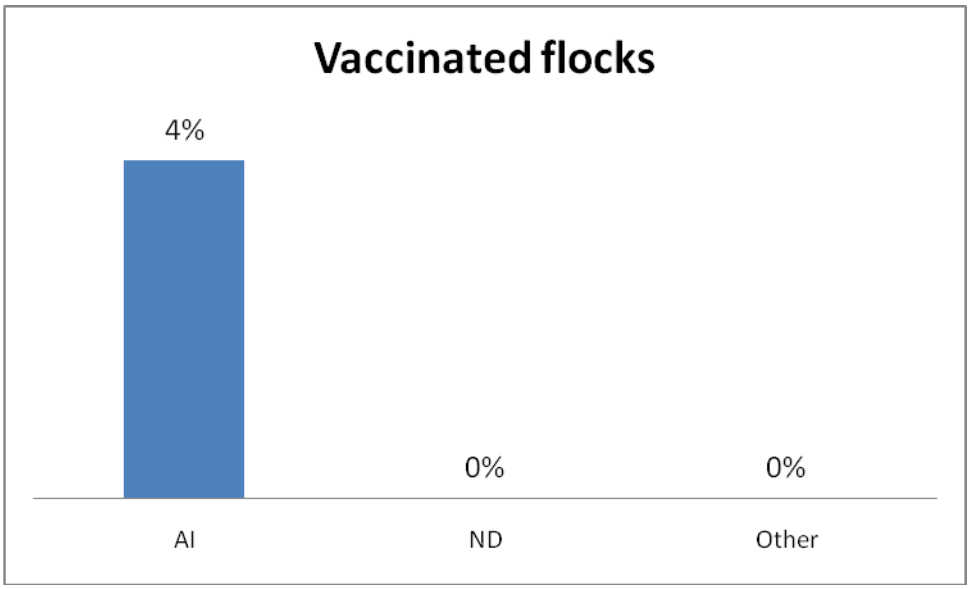


Figure 7. Vaccination status of the flocks

Methods of transport into Cipunagara, in Cipunagara and planned transport out of Cipunagara

As shown in figure 8, 90% of the duck flocks have been transported into Cipunagara with the use of a car. 8% was transported with a motorcycle and for 2% o the flocks transport into Cipunagara was not applicable.

For the transport in Cipunagara (figure 8), 82% of the flocks was moved by car, 2% by motorcycle, 8% was moved on foot and for 8% of the flocks movement in Cipunagara was not applicable.

For 98%of the flocks, the owner has planned to transport the ducks out of Cipunagara with a car (figure 8). For 2% of the flocks transport out of Cipunagara is not applicable.

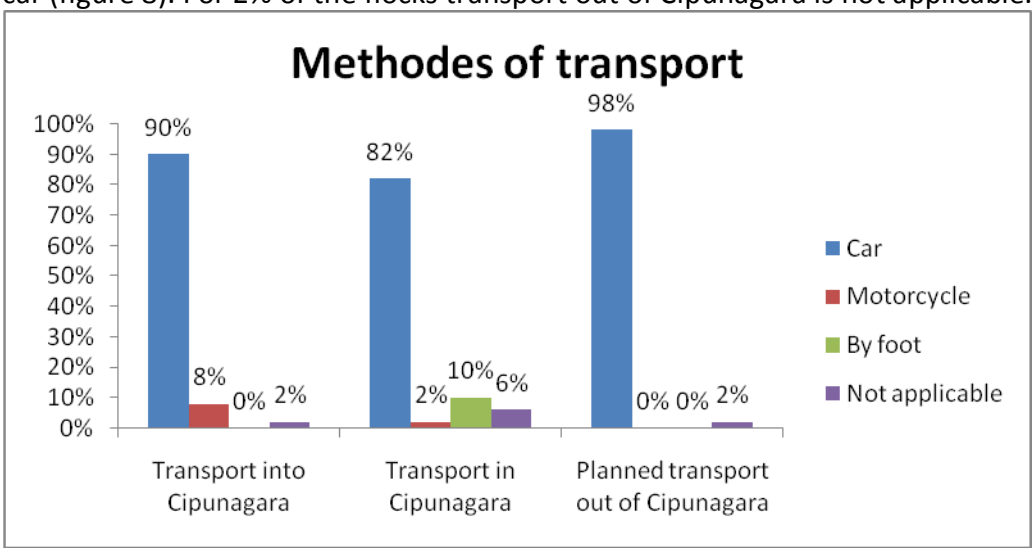


Figure 8. Methods of transport of the ducks, into Cipunagara, in Cipunagara and out of Cipunagara

Movements of the flocks

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Number Of Movements	50	2	3	2,20	,404
Valid N (listwise)	50				

Table 4 Number of movements of the flocks

All flocks move in the time frame we followed the 2 or 3 times. The mean number of movements per flock is 2,20

Traveling distances of the flocks

The mean distance the flocks traveled from the location where they stayed before they reached the location where the sampling took place till the sampling location was 20,054 km with a standard deviation of 17,4298 km. (Table 5)

N	Valid	50
	Missing	0
Mean		20,054
Std. Deviation		17,4298
Minimum		1,5
Maximum		97,7

Table 5. Distance from previous location to sampling location

18% of the flocks traveled a total distance of less than 10 km one month before the sampling took place till the last location in Cipunagara (Table 6). 40% traveled between 10 and 20 km, 32% traveled between 20 and 30 km. 4% of the flocks traveled a distance of between 40 and 50 km and 2% traveled a distance of between 80 and 90 km. 2% traveled a distance between 90 and 100 km and finally 2% traveled a distance of more then 100 km. None of the flocks traveled a distance between 50 and 80 km.

The mean total distance of the flocks during the period one month before sampling until the location where the sampling took place is 23,328 km with a standard deviation of 23,1746 km.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	< 10 km	9	18,0	18,0	18,0
	10-20 km	20	40,0	40,0	58,0
	20-30 km	16	32,0	32,0	90,0
	40-50km	2	4,0	4,0	94,0
	80-90 km	1	2,0	2,0	96,0
	90-100 km	1	2,0	2,0	98,0
	> 100 km	1	2,0	2,0	100,0
	Total	50	100,0	100,0	

Table 6. Total Distance traveled by the flocks

N	Valid	50
	Missing	0
Mean		23,328
Std. Deviation		23,1746
Minimum		2,0
Maximum		132,1

Table 7. Total distance traveled by the flocks

Results laboratory tests

The trachea en cloaca samples were tested in the laboratory for the presence of influenza A using a M-PCR test. The results of these tests showed that for the trachea samples, in 26% (n=13) of the flocks at least one duck was tested positive for the presence of influenza A (table 8). In 22% (n=11) of the flocks at least one duck tested positive for the cloaca-samples (table 9). Totally, this means that in 32% (n=16) of the flocks, at least one duck was tested positive for the presence of influenza A (positive trachea and/or cloaca-sample) (Table 10)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	13	26,0	26,0	26,0
	Negative	37	74,0	74,0	100,0
	Total	50	100,0	100,0	

Table 8. Results of the PCR-test of the trachea-samples

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	11	22,0	22,0	22,0
	Negative	39	78,0	78,0	100,0
	Total	50	100,0	100,0	

Table 9. Results of the PCR-test of the cloaca-samples

Flock Status

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	16	32.0	32.0	32.0
	Negative	34	68.0	68.0	100.0
	Total	50	100.0	100.0	

Table 10. PCR-results flock (trachea and cloaca results together)

Next to showing the presence of influenza A in the samples, the positive trachea and cloaca samples were tested for the presence of H5 subtype of influenza A. The results of these tests are that none of the positive influenza A samples tested positive for the presence of H5 subtype of influenza A. So, H5-subtype was not found in the trachea- and cloaca-samples.

As shown in "Materials and Methods", an ELISA test was used to show the presence of antibodies against influenza A in blood-samples. The tests show that 100% of the flocks have antibodies against influenza A.

Also, a HI test was performed to show the presence of antibodies against H5 subtype of influenza A. The results show that 6% of the flocks (n=3) have antibodies against H5 subtype.

Relation between data acquired from questionnaires and results from the laboratory

As written before, the Pearson Chi-square test was used to indicate any possible relations between the questionnaire results and the results from the tests from the laboratory. First, the relation between the results from the questionnaire and the PCR-test-results (of the flock) are shown. Then the relation between the questionnaire results and the H5-PCR tests are shown. After that the relation between the questionnaire results and the results of the ELISA-test are shown and finally the relation between the questionnaire results and the results of the HI-test are shown. There is a relation between two variables when the P-value is 0,05 or less.

Questionnaire results VS Flock status PCR

	Flock status PCR (M-PCR)	
	Relation (y/n)	P-value
Flock size	Yes	0,023
Ducks from Cipunagara	No	0,181
Duck breed	No	0,377
Keeping reason	No	0,089
Ducks removed from rice fields at night	No	0,65
Transport into Cipunagara (car y/n)	No	0,65
Transport into Cipunagara (motorcycle y/n)	No	1
Transport in Cipunagara (car y/n)	No	1
Transport in Cipunagara (motorcycle y/n)	No	1
Transport in Cipunagara (foot y/n)	No	1
Vaccinatie AI (y/n)	No	0,542
Total traveled distance	No	0,707
Number of flock movements	No	0,256

Table 11 Relation between questionnaire results and PCR-results

Table 11 shows there is a relation between the flock size and the chance of a flock being positive or negative for the M-PCR. Flocks which consist of more then 288 ducks have a bigger chance of being influenza A-positive then flocks which consist of 288 ducks or less (p=0,023)

Questionnaire results VS H5-PCR results

Because H5-subtype of influenza A was not found in any of the influenza A positive flocks it is not possible to show any relation between the results of the questionnaire and the results of the H5-PCRtest. This means there is no relation between the questionnaire results and the H5-PCRresults.

Questionnaire results VS ELISA-test results

Because the ELISA-test was positive in all tested flocks it is not possible to show any relation between the results of the questionnaire and the results of the ELISA-test. This means there is no relation between the questionnaire results and the ELISA-test results.

Questionnaire results VS HI-results

	HI-results	
	Relation (y/n)	P-value
Flock size	No	1
Duck breed	No	1
Keeping reason	No	1
Ducks from Cipunagara	No	1
Ducks removed from rice fields at night	Yes	0,035
Transport into Cipunagara (car y/n)	No	0,276
Transport into Cipunagara (motorcycle y/n)	No	1
Transport in Cipunagara (car y/n)	No	1
Transport in Cipunagara (motorcycle y/n)	No	1
Transport in Cipunagara (foot y/n)	No	1
Vaccinatie AI (y/n)	No	1
Total traveled distance	No	0,496
Number of flock movements	No	1

Table 12 Relation between questionnaire results and HI-test results

Table 12 shows there is a relation between the duck flocks that are being removed from the rice fields or not and the chance of a flock being positive or negative for the HI-test. Flocks that are being removed from the rice fields at night have a bigger chance of having H5-specific antibodies. (p=0,035)

Discussion

Results of the questionnaires

The plan was to visit the duck flocks as soon as they arrived in Cipunagara. Even at the end of the project still flocks have been visited that were already in Cipunagara for a couple of weeks. For example, flock 43 was already 4 months in Cipunagara before sampling the ducks took place. If the samples are taken long after the arrival in Cipunagara it is impossible to say whether the virus that was collected from the ducks was picked up in Cipunagara or before. To make sure that the virus is already present in the ducks when to arrive in Cipunagara the flocks should be sampled within 24 hours after arrival. Due to logistic problems this was not always possible. Next to this, the staff from Dinas didn't always know which flocks were in the area which means that they spend a lot of time searching for flocks. When a flock was found it was possible that this flock was already at that location for some time.

As shown in Table 1 the number of trachea samples taken is not the same as the number of cloaca samples that have been taken. In one flock (flock No3) 55 cloaca swabs have been taken and 50 trachea swabs. This should be 55 cloaca samples and 55 trachea samples because the number of cloaca samples and trachea samples should be equal. For taking the swabs dry cotton buds were used. Swabs moistened with virus transport media should be used to obtain samples from living birds.[4] Moistened cotton buds should be used to minimize the trauma in the animal while taking the samples. Dry cotton buds can be used for sampling dead animals.

It was very hard to obtain 2 ml of blood from the ducks. For the ELISA and HI tests, at least 2 ml of blood was needed but sometimes the ducks were very young and/or the vena brachialis was very small. Hereby, the possibility exists that the lab results are not fully reliable because of sample sizes which were to small.

Because of budget technical reasons, only 30 samples per flock have been analyzed (in stead of maximum 60 samples per flock). This decrease in sample size means that the confidence of the test changes as well. With the help of WinEpiscope, the sample sizes have been chosen to obtain a result with 95% sensitivity, 100% specificity, a prevalence of 5% and a confidence of 95%. Because less samples have been analyzed this means that there is only a confidence of between 94,22% and 78,92%. This means the chance of missing influenza A positive ducks will increase.

The biggest part of the flocks that have been visited consisted of 400 ducks or less. The mean flock size was 288,2 ducks. This is not in line with the findings of CIVAS (LIT 5), which says that herded flocks under the care of a single herdsman are usually in the size range of 90 to 130 ducks. In this project a duck flock is considered a duck flock when, as said before, the ducks move around from one location to another location together. This means that one flock can consist of multiple herdsmen with their own group of ducks and that might be an explanation for these different findings.

Many flocks are moving around together during the time-frame we followed the flocks, for example flocks 27 & 28 and flocks 29 & 30. They should be considered as 1 flock if they move around together always. It is possible that they only traveled together in the period that we followed them en they split up after that.

There are some differences between the duck breeds that are kept by the herdsman. Of the flocks that have been sampled, flock 19 until flock 50 all consist of Java Duck. This means that of the first 18 flocks only 61% consists of Java duck (figure 9) next to 86% Java Duck in all the flocks together. 11% of the first 18 flocks consists of mixed breeds (4% of the total number of flocks is mixed breeds). 22% of the first 18 flocks, the owner does not know what duck breed he keeps (8% of the total number of flocks the owner does not know what breeds he keeps. Finally, in 6% of the first 18 flocks that have been visited, the answer on the question was left empty (2% of the total number of flocks).

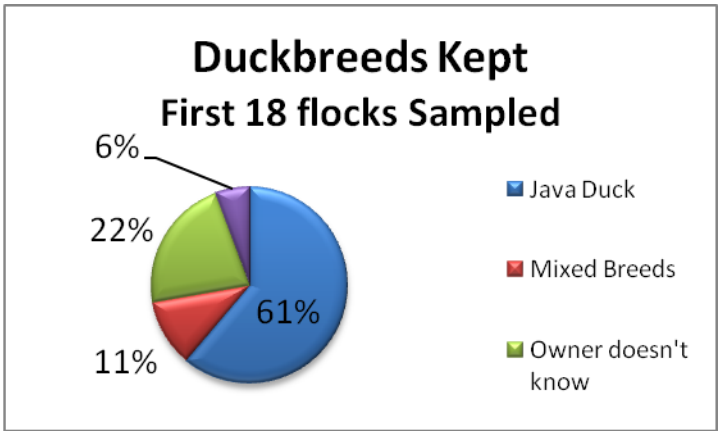


Figure 9. Duck breeds that are being kept of the first 18 flocks that have been sampled.

These results are remarkable, but there is not a good explanation for it. One possibility is that the enumerators filled in the answer themselves without asking the owner. Off course, it also can be just a coincidence and that flock 19 until flock 50 really all consisted of Java Duck. According to experts from Fakultas Kedokteran Hewan from Institut Pertanian Bogor (faculty of veterinary medicine, Boger), Java, almost all duck flocks consist of Java duck in this area. Also the flocks from which the owners filled “mixed breeds” consists mainly (95-99%) of Java duck.
If the answer was “mixed breeds” maybe it would be useful for the next time to ask which species are mixed. The answer “mixed breeds” only does not give any information about the duck breeds the owner keeps.

The majority of the flock owners keep the ducks for egg production. One of the owners of the flocks that have been visited keeps the ducks for egg and meat production and one owner keeps the ducks for eggs and meat and next to this for economical reasons as well. It is unknown what kind of economical reasons the owner means.

48% of the flocks are in Cipunagara when they are not moving around. This means it is hard to obtain a good view of the ducks that come into Cipunagara and the flocks who leave Cipunagara. All the flocks which are in Cipunagara when not moving around left Cipunagara before the flock was sampled or after the sampling took place. It is unknown what part of

the year the flocks are moving around. Is this the whole year or only a couple weeks per year?

88% of the flocks is not being removed from the rice fields during the night. This means 12% is removed from the rice fields. If the ducks are being removed from the ricefields at night, 66% spends the night in a shed, 17% in a settlement and 17% spends the night in a temporary pen. It is not clear what the difference is between a settlement and a shed. A temporary shed is the same as the sheds that is used when the flocks are not being removed from the ricefields.

Only two flocks of the 50 flocks that have been visited have been vaccinated against AI. One of these flocks does not know when the last vaccination took place and in the other flock that was vaccinated against AI, the vaccination took place less then three months ago. None of the flocks have been vaccinated against other infectious diseases.
For a good protection the vaccination should be repeated. There are various types of vaccines available for vaccination against influenza A.[25] It is not clear which type of vaccine has been used. There are at this moment different vaccine types available; inactivated vaccines and live recombinant vaccines. The live recombinant vaccines are only efficacious in chicken species. It is essential to use a vaccine against the virus hemagglutinin-type that is circulating at that time or against a the virus that is expected to be introduced.[15]

Appendix IV shows the locations where the flocks stayed in Cipunagara and the period one before they entered Cipunagara until they entered Cipunagara.

90% of the duck flocks come into Cipunagara by car. Because the duck flocks consist of an average of 288 ducks this is the only way to transport the ducks all at once. Smaller duck flocks can move into Cipunagara with a motorcycle. None of the flocks came into Cipunagara by foot. This means they travel distances that are too big to walk. It is not very clear what the difference is between a car and a truck. For example, some people consider a pick-up truck as a car.
Transport into Cipunagara is for 2% is not applicable (1 flock). The owner of this flock (flock 4) lives in Padamulya. The month before the sampling took place this flock was in Pagaden (which is not Cipunagara so he leaves Cipunagara). This means that he also has to come back to Cipunagara once.
The biggest part of the duck flocks don't change location in Cipunagara but they have filled in the questionnaire about movement inside Cipunagara. Only 4 flocks visited 2 locations in Cipunagara. The other 46 flocks visited only 1 location in Cipunagara and then left Cipunagara so why did they fill in the questionnaire that they travel inside Cipunagara by car?
Flock 22 & 25 have more movements inside Cipunagara but it is not known where to. In one flock (flock 34) we saw that the locations where they planned to go sometimes differs from the destination where they really go. This means information is missing so the results of this study might not be accurate.
There is one flock where the method of transport out of Cipunagara is not applicable. These ducks stay in Cipunagara when not moving around and apparently the ducks never leave Cipunagara. There are other duck flocks that stay in Cipunagara when not moving around but they come outside Cipunagara as well.

All the flocks have planned to leave Cipunagara with a car, even though they didn't arrive in Cipunagara with a car.

The majority of the flocks traveled a distance of between 10 and 30 km in the timeframe we followed them. There were some exceptions; 2 flocks for example came from far (Bekasi, near Jakarta, and 1 flock came from Sukamaju (south of Bogor)). This means that 47 flocks travel 50 km or less. Given the fact that travelling is very time consuming and there are plenty of rice fields in this area of Java it is not necessary to travel far.

Results from the laboratory tests

In this project, there was no interest in individual cases (ducks) but only in the flock status. This means a positive flock consists of at least one positive animal and this is also the reason why the results of the PCR-test of the trachea and the cloaca samples are taken together. 32% of the flocks tested positive for the presence of influenza A. This does not mean that 68% of the flocks is negative. Not all the samples that have been taken have been analyzed so there is a possibility that flocks that are considered negative are in fact positive.

H5 subtype of influenza A was not found in the flocks in the trachea and cloaca samples of the sampled ducks. This does not automatically mean that there is no H5 subtype present in the flocks. Samples have only been taken from a small number of ducks from each flock so the possibility exists that positive animals have been missed. Next to this, due to budget-technical reasons not all the samples that have been taken have been analyzed. So it is possible that there is H5 subtype of influenza A present in the flocks.

Blood samples were taken to analyze for the presence of antibodies against influenza A. Not every sampled duck is positive but every flock is positive. So in every duck flock that was sampled there is at least one duck with antibodies against influenza A. This means that every flock has been in contact with influenza A.

6% of the flocks (n=3) have antibodies against H5 subtype influenza A. This means that only 6% of the flocks have been in close contact for a certain amount of time so the duck's body was able to respond and could make protective antibodies against H5 subtype. A test result with a value of 4 was considered positive. Values 1, 2 and 3 indicate that there has been contact with H5 subtype but not enough to ensure the formation of protective antibodies (by for example an early or late field infection). 3 flocks have a value of 4, 11 flocks have a value of 1, 2 or 3. This study concerns about a value of 4.

Relation between data acquired from questionnaires and results from the laboratory

Questionnaire data VS PCR-test results

As said before, flocks of 288 ducks or more have a bigger chance of being influenza A positive than flocks of 288 ducks or less (p=0,023). Bigger flocks cover a larger area than smaller flocks so the chance that a duck picks up influenza A in a certain area is bigger than the chance that a duck in a smaller flock picks up influenza A. Influenza A is simply

transmitted to other ducks of the flock. This can be an explanation why larger flocks have a bigger chance of being influenza A positive.
There is no relation between ducks that come from Cipunagara or not and the PCR results. This means that ducks that come from Cipunagara do not have a bigger (or smaller) chance of being positive than flocks who come from outside Cipunagara.

There is also no relation between duck breed and the PCR results. Most of the flocks consist of Java duck only, and some mixed flocks consist of mainly Java duck. The duck breed is almost constant so the chance of finding a relation between PCR-results and duck flock is very small. This can also be the reason why there is no relation between "reason for keeping the ducks" and the PCR-results. Egg-production is the main reason for keeping the ducks. 98% of the owners keeps the ducks for egg-production (some of them in combination with (an) other reason(s)). Keeping reason is almost a constant (egg-production) so the chance of finding a relation between keeping reason and PCR-results is very small.

Removing the ducks from the rice fields at night does not affect the prevalence of influenza A. All the ducks are being held confined during the night. The difference between confining the ducks on the rice field or somewhere else (for example in a village) does not affect the presence of influenza A in the flocks. This makes it probable that the ducks get infected with influenza A during the day when they are on the rice fields.
Also, there is no relation between the way the ducks got transported into Cipunagara and the PCR-results. Probably, the ducks got infected with influenza A on the rice fields (or not) and the way of transportation into Cipunagara doesn't make the chance bigger to get infected with influenza A. The same applies to the way of transportation in Cipunagara.

There is also no relation between flocks that have been vaccinated against influenza A and the PCR-results. The goal of vaccination is that ducks don't get ill from infection with a certain type of influenza A. This does not mean that virus will not be found in vaccinated ducks. Ducks that have been vaccinated can also pick up the virus in the rice field but the duck can handle it. This means that vaccinated ducks will not play a big role in spreading the virus but they still can pick up the virus and this means that it can be found in cloaca and/trachea.

Duck flocks that frequently chance location or duck flocks which have been moved over longer distances do not have a bigger chance of a positive PCR-test result. Apparently, duck flocks which move from one location to another more often, do not have a bigger chance of positive PCR-test results even though they visit more rice fields (and thus come in theory more in contact with influenza A) than flocks which do not move frequently from one location to another location. It is not likely that duck flocks get infected with influenza A while they are being transported. This might be an explanation why duck flocks that have been moved over longer distances do not have a bigger chance on a positive PCR-test result.

There is no relation between the data from the questionnaire and the H5-PCR results because one of the variables (H5-PCR results) is constant. As shown in the results, the H5-PCR test was negative in every flock. This means it is impossible to see if there is a relation between the H5-PCR results and the questionnaire data.

Also, there is no relation between the data from the questionnaire and the ELISA-test results because the ELISA-test results are positive in every flock. As shown in the results, the ELISA-test was positive in every flock so it is impossible to see if there is a relation between the ELISA-test results and the questionnaire data.

Questionnaire data VS HI-test results

There is no relation between de flock size and de HI-test results. Even though bigger flocks come into more contact with influenza A, apparently they do not come into more contact with influenza A subtype H5. A reason for this can be that H5 subtype is less common. There are 144 different subtypes of influenza A so the chance of picking up H5 subtype is small and with this the formation of protective antibodies against H5-subtype.

Also, there is no relation between duck breed and the HI-test results. Most of the flocks consist, as said before, of Java duck only and some mixed flocks consist of mainly Java duck. The duck breed is almost constant so the chance of finding a relation between HI-test results and duck flock is very small. This can also be the reason why there is no relation between "reason for keeping the ducks" and the HI-test results. 98% of the owners keeps the ducks for egg-production (some of them in combination with (an) other reason(s)). Keeping reason is almost a constant (egg-production) so the chance of finding a relation between keeping reason and HI-test results is very small.

There is no relation between HI-test results and flocks which come from Cipunagara or not. This means that there is no bigger risk (or smaller) to get infected with H5-subtype. So, the prevalence of H5-subtype is not bigger inside Cipunagara than outside Cipunagara.

There is a relation between ducks that are being removed from the rice fields at night and de HI-test results. This means that ducks who spend the night outside the rice fields are more exposed to H5-subtype, because protective antibodies against H5-subtype were found. It can be concluded that H5-subtype is mainly outside the rice fields. This is only about H5 subtype because, as shown before, there is no relation between the PCR-results and ducks that are being removed from the rice fields at night. 12% (n=6) of the flocks that have been visited is being removed from the rice fields at night. 3 flocks were HI-test positive and of these 3 flocks, 2 flocks are being removed from the rice fields. For a reliable answer on the question if there is a relation between ducks that are being removed from the rice fields at night and the HI-test results more flocks are needed. It can be a coincidence that 2 of the 3 flocks that are HI-test positive are being removed from the rice fields at night. Of course is it also possible that ducks that are being removed from the rice fields at night really have a bigger chance of testing positive on the HI-test (like this project shows). More research is necessary.

There is no relation between the HI-test results and the way ducks are being transported (into and in Cipunagara). This means that for the exposure to H5 subtype, it doesn't matter how the ducks are being transported. The risk of being exposed to H5-subtype is not bigger when transportation takes place in a car, on a motorcycle or by foot. It can be concluded that transportation does not influence exposure to H5-subtype so exposure is equal in every transportation method. The virus is picked up when the ducks are foraging and not while they are being transported so this explains why the way of transportation does not influence the exposure to H5-subtype.

Remarkable is that the flocks that have been vaccinated all have a HI test value of 0. It is unknown why this is. Especially the vaccinated flocks should test positive (or at least a value which of 1, 2 or 3). Maybe the flocks have been vaccinated with a vaccine containing a different type of influenza A. Other reasons can be that the way of administering the vaccine was not right or maybe not all the ducks of a flock have been vaccinated so maybe only unvaccinated ducks have been sampled. Finally, it is possible that the results from the are not reliable.

Finally, there is no relation between the HI-test results and the total traveled distance and the total number of movements. Flocks that travel a lot (long distances or change location often) do not have a bigger chance of a positive HI-test result. This means they do not come into more contact with H5-subtype than duck flocks which travel smaller distances or change location less often. Flocks that change location more often visit more locations, so it is expectable that these ducks come in contact with more virus so they have a bigger change of having protective antibodies against H5-subtype. This is not in agreement with the results of this study

Recommendations for further studies

- Next time, a better system to find the duck flocks is necessary. If there is a better system the flocks can really be sampled at the moment they enter Cipunagara subdistrict. It is important to sample a flock as soon as possible after arriving in Cipunagara.

- Wet cottons should be used for taking cloaca and trachea samples in life animals. Also, sterile cotton buds should be used to prevent contamination.

- In the questionnaires, some things should be further specified. For example, if the answer to the question "what breed of ducks do you keep?" was mixed breeds, next time ask which breeds are mixed and in what percentage. The answer "mixed breeds" only does not give much information.
  This also applies for the answer on the question "what is the reason for keeping the ducks" and the answer was "economical reasons". These economical reasons should be further specified. Same for the question: are the ducks being removed from the rice fields at night? If yes, where do they stay at night. With the results of this additional question, the information about the flock is more specific.

- It is important to know for how long the duck flocks move around each year. If a flock stays on the same location for the biggest part of the year are these ducks less exposed to influenza A than flocks that move around all year long.

- Every questionnaire should be checked when still on the sampling location for missing information or answers that do not make any sense. It is important to get clear information and a fully answered questionnaire. For example, in flock number 35, there is no information about where this flock was from November 27th until December 5th. If the questionnaires are being checked for missing information this kind of gaps can be prevented.

- For more reliable results, more samples from more flocks are needed. Also, it is important to test all the samples that have been taken. Due to budget technical reasons, not all the samples have been tested in the laboratory, so the confidence of results is lower than was intended.

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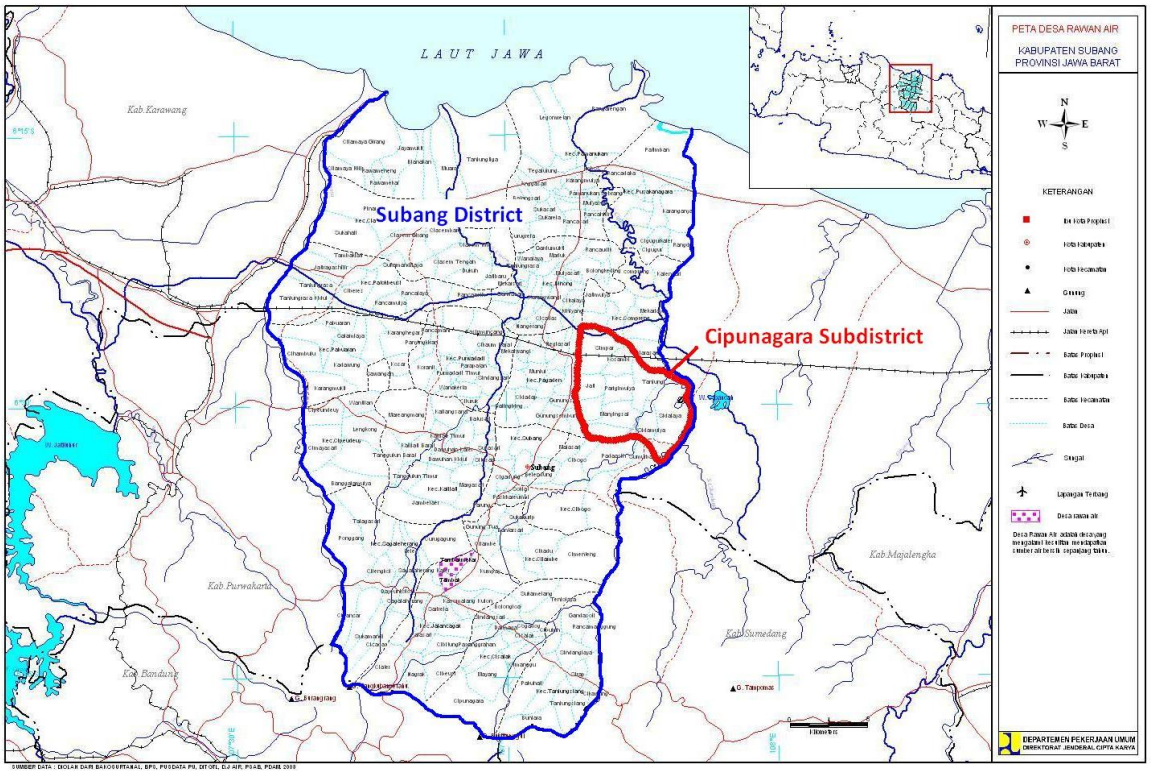
Appendix I

Research location


LAUT JAWA
Subang District
Cipunagara Subdistrict
Kab Karawang
Kab Purwakarta
Kab Bandung
Kab Sumedang
Kab Majalengka
PETA DESA RAWAN AIR
KABUPATEN SUBANG
PROVINSI JAWA BARAT
KETERANGAN
DEPARTEMEN PEKERJAAN UMUM
DIREKTORAT JENDERAL CIPTA KARYA

Appendix II

Flock size	Required sample size
10	10
20	20
30	25
40	30
50	35
60	40
70	40
80	45
90	45
100	45
110	45
120	50
130	50
140	50
150	50
160	50
170	50
180	55
190	55
200	55
210	55
220	55
230	55
240	55
250	55
260	55
270	55
280	55
290	55
300	55

Flock size	Required sample size
310	55
320	55
330	55
340	55
350	55
360	55
370	55
380	55
390	55
400	60
410	60
420	60
430	60
440	60
450	60
460	60
470	60
480	60
490	60
500	60
>500	60

Sensitivity: 95%
Specificity: 100%
Prevalence: 5%
Confidence: 95%

Appendix III

Questionnaire Nomadic Duck

Collaboration of faculty of Veterinary Medicine – IPB
Indonesian – Dutch partnership on HPAI control

Flock code:

Date:

Enumerator:
No Samples →
Trachea:

Cloaca :

Blood :

General flock information

1. Name of owner:

2. Phone No.

3. Name of keeper:

4. Name of respondent:

5. Address of the owner:
Village:

Sub District:

District:

Province:

6. Do the ducks stay at the owner's address when they are not moving around?
Yes
No. Where do they stay:
Village:

Sub District:

District:

Province:

7. Location of the flock at the time of the interview:
Village:

Sub District:

District:

Province:

GPS Coordinates (filled in by enumerator): Long (S): Lat(E):

When did the flock arrive at the present location (ddmmyyyy): _____

Farm characteristics

8. How many ducks are in this flock at this time? _____

9. What breed of duck do you keep in this flock?
Alabio
Javanese Duck
Balinese Duck
Mixed species
Other (specify): _____
Don't know

10. What is the reason for keeping ducks?
Egg production
Meat production
Egg and meat production
Other (specify): _____

11. How do you keep the ducks at night?
Confined (i.e. in a cage, fenced in, etc)
Free range
Other: _____

12. Are your ducks moved from the ricefields at night?
Yes, where do they go at night? _____
No

13. How did you transport the ducks into Cipunagara?
Truck
Car
Motorcycle
On foot
Other (specify): _____
Not applicable

14. How do you transport the ducks within Cipunagara?
Truck
Car
Motorcycle
On foot
Other (specify): _____
Not applicable

15. How do you plan to transport the ducks out of Cipunagara?
Truck
Car
Motorcycle
On foot
Other (specify): _____
Not applicable

16. Have the ducks been vaccinated?
No
Yes, (please fill in table):

Disease	Have the ducks been vaccinated?	When was the last vaccination done?
Avian Influenza	☐ Yes ☐ No	☐ Less than 3 months ago ☐ Less than 1 year ago ☐ More than 1 year ago
Newcastle Disease	☐ Yes ☐ No	☐ Less than 3 months ago ☐ Less than 1 year ago ☐ More than 1 year ago
Other, Specify		

Flock movements during the previous month

17. Please list all the locations where this duck flock has been during the previous month?

Location (village, subdistrict, district, province)	Date of arrival	Date of departure

Flock movement in Cipunagara

Flock code:
Owner's name:

Date (dd/mm/yyyy)	Former location (village/ subdistrict/ district)	Current location (village)	GPS of Current Location	Date of arrival (dd/mm/yyyy)	Method of transportation to current location	Anticipated date of departure (dd/mm/yyyy)	Anticipated destination (village/ subdistrict/ district)
			Lat:……. Long:…….		☐ Truck ☐ Car ☐ Motorcycle ☐ On foot ☐ Other ……..		
			Lat:……. Long:…….		☐ Truck ☐ Car ☐ Motorcycle ☐ On foot ☐ Other ……..		

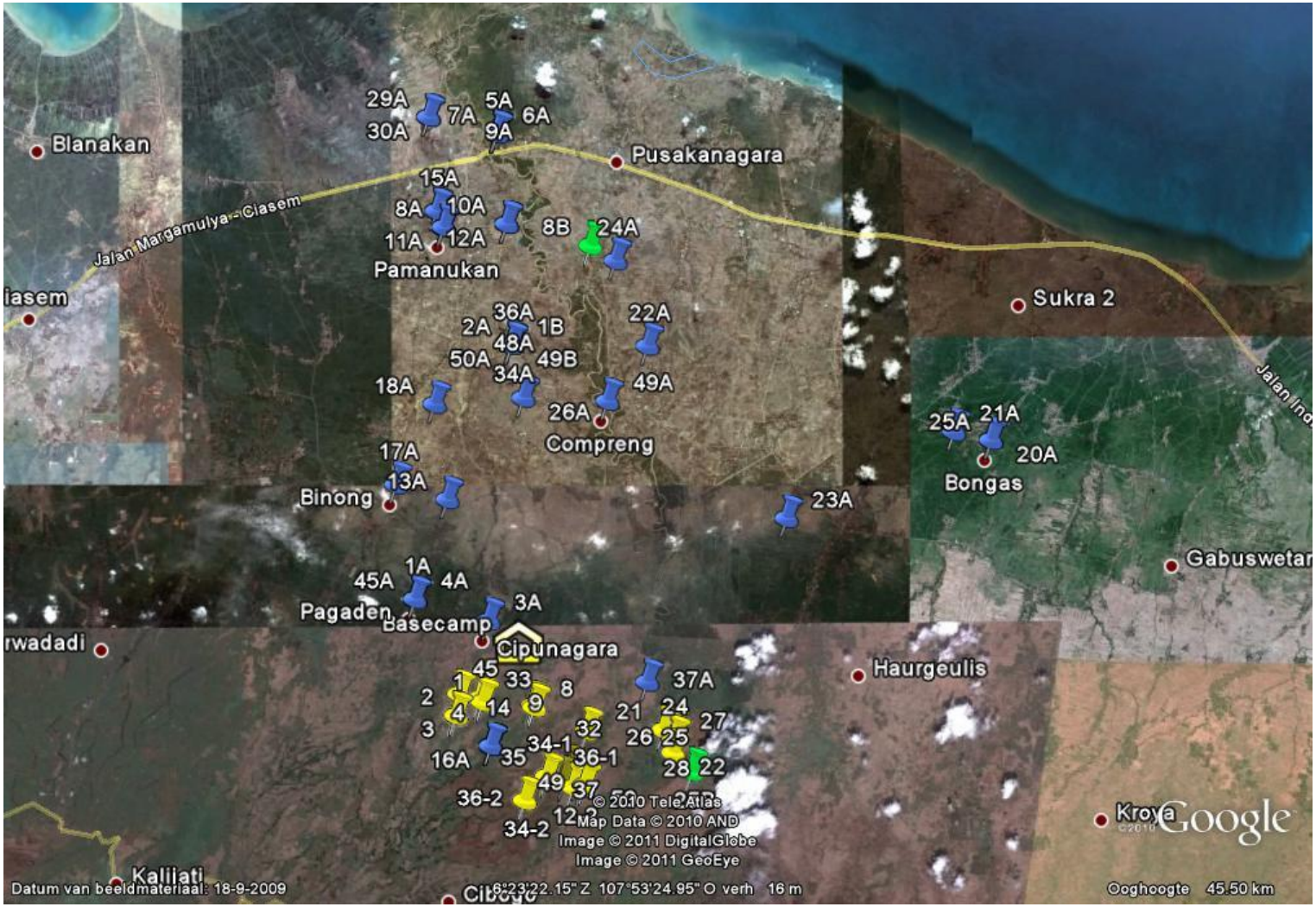
Date (dd/mm/yyyy)	Former location (village/ subdistrict/ district)	Current location (village)	GPS of Current Location	Date of arrival (dd/mm/yyyy)	Method of transportation to current location	Anticipated date of departure (dd/mm/yyyy)	Anticipated destination (village/ subdistrict/ district)
			Lat:……. Long:…….		☐ Truck ☐ Car ☐ Motorcycle ☐ On foot ☐ Other ……..		
			Lat:……. Long:…….		☐ Truck ☐ Car ☐ Motorcycle ☐ On foot ☐ Other ……..		
			Lat:……. Long:…….		☐ Truck ☐ Car ☐ Motorcycle ☐ On foot ☐ Other ……..		
			Lat:……. Long:…….		☐ Truck ☐ Car ☐ Motorcycle ☐ On foot		

Date (dd/mm/yyyy)	Former location (village/ subdistrict/ district)	Current location (village)	GPS of Current Location	Date of arrival (dd/mm/yyyy)	Method of transportation to current location	Anticipated date of departure (dd/mm/yyyy)	Anticipated destination (village/ subdistrict/ district)
					☐ Other ……..		

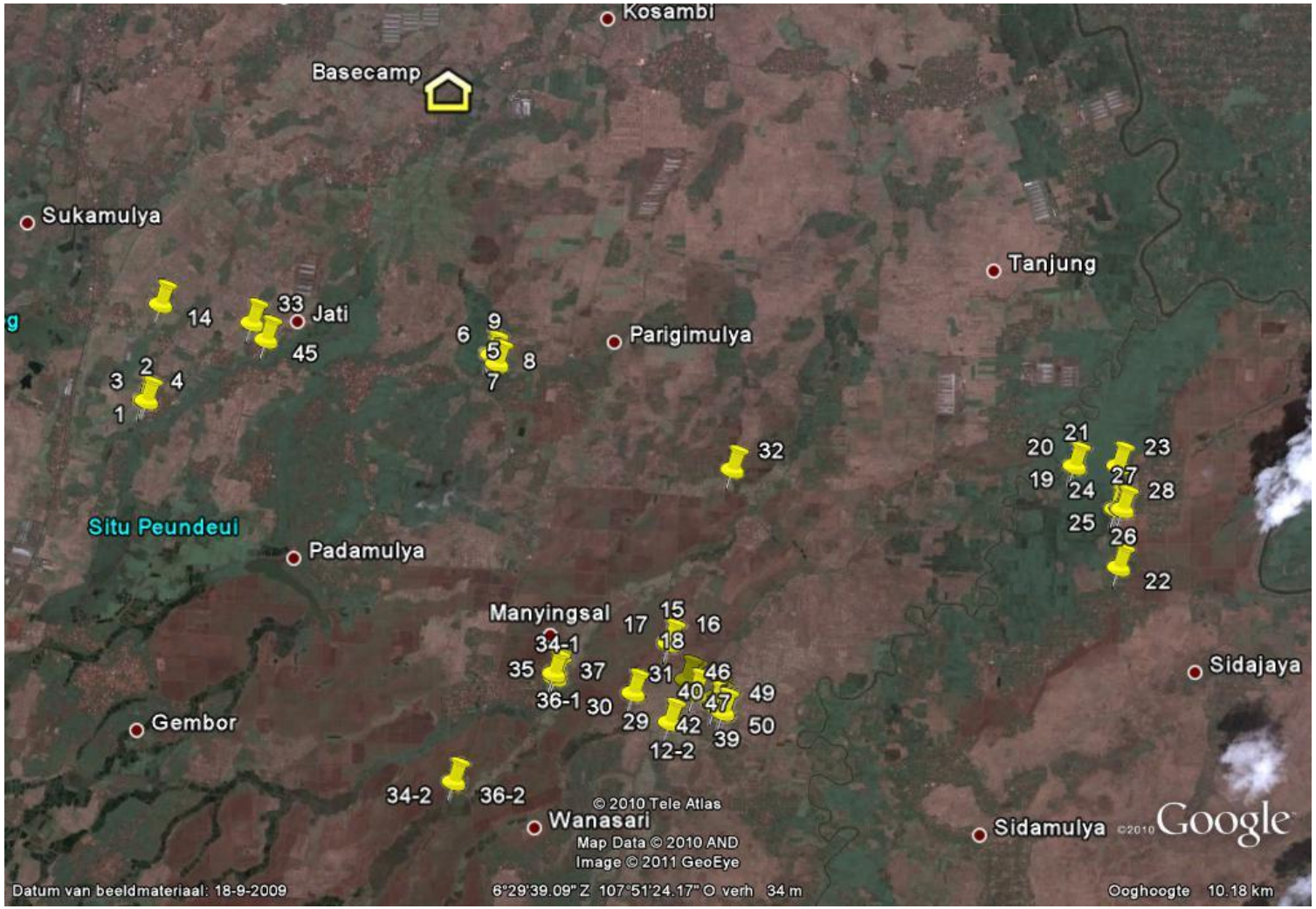
Thank you for your cooperation!

Appendix IV

Blue: 1st location outside Cipunagara; Green: Second location outside Cipunagara; Yellow (x-1): Location where the sampling took place; Yellow (x-2): Second location in Cipunagara. Chronological order: Blue, Green, Yellow (x-1), Yellow (x-2). (x stands for flock number)



Blue: 1st location outside Cipunagara; Green: Second location outside Cipunagara; Yellow (x-1): Location where the sampling took place; Yellow (x-2): Second location in Cipunagara. Chronological order: Blue, Green, Yellow (x-1), Yellow (x-2). (x stands for flock number)



Blue: 1st location outside Cipunagara; Green: Second location outside Cipunagara; Yellow (x-1): Location where the sampling took place; Yellow (x-2): Second location in Cipunagara. Chronological order: Blue, Green, Yellow (x-1), Yellow (x-2). (x stands for flock number)