

## DETECTION AND ISOLATION OF HIGHLY PATHOGENIC H5N1 AVIAN INFLUENZA A VIRUSES FROM BLOW FLIES COLLECTED IN THE VICINITY OF AN INFECTED POULTRY FARM IN KYOTO, JAPAN, 2004

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**Abstract.** During the outbreak of highly pathogenic avian influenza that occurred in Tamba Town, Kyoto Prefecture in 2004, a total of 926 flies were collected from six sites within a radius of 2.3 km from the poultry farm. The H5 influenza A virus genes were detected from the intestinal organs, crop, and gut of the two blow fly species, *Calliphora nigribarbis* and *Aldrichina grahami*, by reverse transcription-polymerase chain reaction for the matrix protein (*M*) and hemagglutinin (*HA*) genes. The *HA* gene encoding multiple basic amino acids at the *HA* cleavage site indicated that this virus is a highly pathogenic strain. Based on the full-length sequences of the *M*, *HA*, and neuraminidase (*NA*) segments of virus isolates through embryonated chicken eggs, the virus from *C. nigribarbis* (A/blow fly/Kyoto/93/2004) was characterized as H5N1 subtype influenza A virus and shown to have > 99.9% identities in all three RNA segments to a strain from chickens (A/chicken/Kyoto/3/2004) and crows (A/crows/Kyoto/53/2004) derived during this outbreak period in Kyoto in 2004. Our results suggest it is possible that blow flies could become a mechanical transmitter of H5N1 influenza virus.

### INTRODUCTION

The 1997 outbreak of highly pathogenic H5N1 influenza A viruses in Hong Kong<sup>1,2</sup> alerted the medical community that some subtypes of avian influenza viruses involve highly pathogenic strains and have the possibility of causing serious public health problems in animals and humans.<sup>3</sup> From December 2003 to March 2004, outbreaks of acute, highly transmissible, and lethal diseases in chickens occurred at three poultry farms in Japan. The virus isolates from the infected chickens were all identified to be influenza A virus of H5N1 subtype.<sup>4</sup> These highly pathogenic avian influenza epidemics have not occurred for 79 years in Japan.<sup>5</sup>

Tamba Town in Kyoto Prefecture, where the fatal avian influenza outbreak occurred at two poultry farms, the third and the fourth cases during the 2003–2004 outbreaks in Japan, is located on hilly areas of 50 km northwest of Kyoto City. From the beginning of March 2004, some studies that primarily targeted wild birds were undertaken to clarify the transmission route of the H5N1 avian influenza virus in Tamba Town.<sup>5</sup> During and after the outbreak, virus surveillance was carried out for migrating birds around the epidemic areas in Kyoto, and the H5N1 virus was isolated only from dead chickens and crows.<sup>6</sup>

It is well known that the domestic house fly, *Musca domestica* spp., and some other fly species can transmit many kinds of pathogens in mechanically.<sup>7–10</sup> At the early spring season in Japan, some active fly species are there; however, no attempts were made to evaluate the role of flies in transmission of the H5N1 influenza virus. In this study, to know the possibility of flies transmitting the virus, an entomological survey was conducted in March 2004, and flies collected from around the infected poultry farm at Tamba Town were used for virus detection and isolation. We simply believe that some viruses

were transported to contact animals by the contaminated fly surfaces.<sup>8</sup> In our trials, to evaluate a possibility of a virus transmission mechanism at a higher level than by the contaminated fly surface, we targeted the intestinal organs, crop, and gut of the blow flies, and we evaluated the possible role of blow flies in transmission of the avian influenza virus in the affected areas of the virus epidemic.

### MATERIALS AND METHODS

**Study site.** Tamba Town (35°9'42" N and 135°26'31" E) is located on hilly areas 150–300 m above sea level and 50 km northwest of Kyoto City, Japan (Figure 1). The A poultry farm, a commercial layer chicken farm, where the severest epidemic of highly pathogenic avian influenza caused by H5N1 viruses<sup>3</sup> occurred in February 2004 was located at the end of a small valley. There are four villages at the foot of the surrounding hills. The flat basin of the valley was used for rice cultivation and the hillside was used as a plantation for coniferous trees. This outbreak resulted in the economic loss of 225,000 chickens to infection and/or to slaughtering in an effort to control the outbreak. After the outbreak in the A poultry farm, the next epidemic occurred in the B poultry farm, a commercial broiler chicken farm locating 4 km northeast of the A poultry farm. The fourth outbreak occurred in the beginning of March 2004 and resulted in the loss of 15,000 chickens. Our fly collection was carried out just after the fourth outbreak occurred at the B poultry farm.

**Fly collection.** Fly collection was conducted on 10 and 11 March 2004. The maximum and minimum temperatures were 18.7°C and –2.0°C on 10 March and 19.2°C and 4.2°C on 11 March, respectively. Each of the three collection sites were chosen both west (upward of the stream, sites 4, 5, and 6) and east (downward, sites 1, 2, and 3) of the A poultry farm (Figure 1). The distance from the A poultry farm and the elevation of each collection site are shown in Table 1. A sunny place protected with protection from strong wind was selected, and rotten fish bait was placed on the ground. Staff members of our department stayed at each collection site, and

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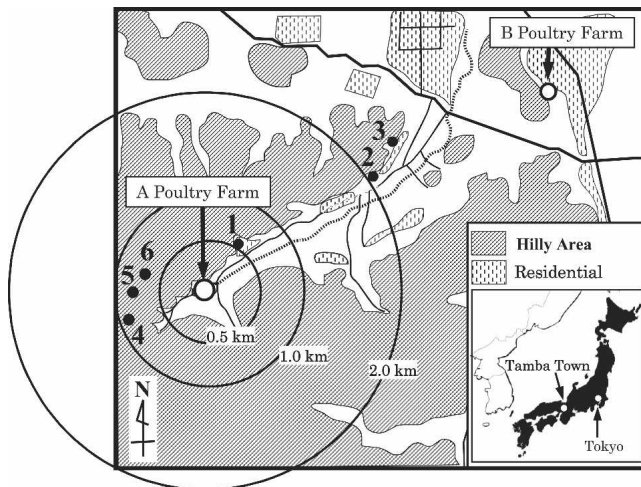


FIGURE 1. Localities in Tamba Town from which flies used in this study were obtained.

female flies coming to the fish bait were collected by sweep netting for 2.3–5.5 hours. All the collected flies were individually put into a 1.8-mL microtube (Eppendorf, Hamburg, Germany) and stored in an icebox during transportation to the National Institute of Infectious Diseases (NIID) in Tokyo. All samples were kept in a freezer at  $-80^{\circ}\text{C}$  in our laboratory at NIID until used in virus detection. All procedures by the step of RNA extraction were performed in a bio-safety level 2 pathogen-contained facility.

#### H5 influenza A virus gene detection from blow flies.

Twenty female flies were dissected on a hole slide glass under a stereomicroscope, and the crop and gut were individually removed from the entire body of each fly using sterilized forceps. Feces and vomited matter adhering to inside of a storage tube were harvested by rinsing with 100  $\mu\text{L}$  of phosphate-buffered saline (PBS; 135 mmol/L NaCl, 3 mmol/L  $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ , and 13 mmol/L  $\text{NaH}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$ , pH 7.2) and collected by centrifugation for a few seconds. The pooled crops and guts from 20 flies were decanted into 2-mL safe-lock microfuge tubes (Eppendorf). The specimens were homogenized using a high-speed mechanical homogenizer (Mixer Mill MM300; Qiagen, Valencia, CA) for 30 seconds at 24 cycles/s. Each homogenate was centrifuged for 10 minutes at 15,000 rpm (Kubota 3740; Kubota, Tokyo, Japan), and the supernatant was decanted into a new microtube. The harvested feces and vomited matter of 20 flies were mixed with this supernatant. Viral RNA was extracted from the supernatants by using a High Pure Viral RNA Kit (Roche, Mannheim, Germany) and from the precipitations by using an RNeasy Mini Kit (Qiagen). To know the virus gene positivity in blow flies, each 10 of blow flies, *C. nigribarbis* and *A. glahami*, were individually dissected, and the intestinal organ, crop, and gut were individually removed from a fly body and homogenized in a stabilization reagent by using MM300. A viral RNA was extracted by using an RNeasy Mini Kit as mentioned above.

**Reverse transcription-polymerase chain reaction and nucleotide sequencing.** Extracted virus RNA was genetically characterized by sequencing reverse transcription-polymerase chain reaction (RT-PCR)-amplified DNA partial fragments of each matrix protein (*M*) and hemagglutinin (*HA*) genes

TABLE 1  
Fly density and location of sample collection sites in Tamba Town, Kyoto, in 2004

| Site  | No. collected | Density of flies (no./h) | Distance from poultry with HPAI outbreak (m) | Elevation (m) |
|-------|---------------|--------------------------|--|---------------|
| 1     | 403           | 134                      | 600  | 240           |
| 2     | 144           | 26                       | 2,050  | 220           |
| 3     | 79            | 20                       | 2,250  | 220           |
| 4     | 125           | 54                       | 900  | 300           |
| 5     | 39            | 17                       | 800  | 300           |
| 6     | 136           | 58                       | 700  | 300           |
| Total | 926           | Average 48               |  |               |

segments. RT-PCR was performed by using the AccessQuick RT-PCR System (Promega, Madison, WI) and Astec PC701 (Astec, Fukuoka, Japan). The first cycle of the amplification program of RT-PCR consisted of a 45-minute period at  $48^{\circ}\text{C}$  and a 2-minute period at  $94^{\circ}\text{C}$ , followed by 45 cycles with the following conditions:  $94^{\circ}\text{C}$  for 1 minute,  $45^{\circ}\text{C}$  for 1 minute, and  $72^{\circ}\text{C}$  for 2 minutes. The program ended with one cycle at  $72^{\circ}\text{C}$  for 10 minutes. Primers used for the *HA* gene (H5 515f/H5 1220r) were recommended by NIID (<http://idsc.nih.go.jp/others/topics/flu/RTpccr.html>). The primer set for the *M* gene (M30f/M264r, T. Saito, unpublished data) was confirmed to detect virus genes for almost all type A strains isolated before. For the *HA* segment, a gene fragment was amplified using a nested PCR design. A 2- $\mu\text{L}$  aliquot of the PCR product was used as the template for a nested reaction with a primer set of H5 529f/H5 1208r (forward, 5'-AAGAGGA-GCTACAATAATAC-3'; reverse, 5'-TTAATGATCGAGTT-GACCTT-3') under  $94^{\circ}\text{C}$  for 2 minutes followed by 35 cycles of  $94^{\circ}\text{C}$  for 30 seconds,  $55^{\circ}\text{C}$  for 30 seconds, and  $72^{\circ}\text{C}$  for 1 minute and then  $72^{\circ}\text{C}$  for 4 minutes. A 5- $\mu\text{L}$  portion of the PCR product was electrophoresed on a 2% agarose gel (NU Sieve 3:1; FMS Bio Product, Rockland, ME), and the remainder was concentrated from agarose gels using the QIAEX II Gel Extraction Kit (Qiagen). Basically, all visible PCR fragments were confirmed by their sequences. Each purified double-stranded PCR product was directly cycle-sequenced from both ends using BigDye Terminator Cycle Sequencing FS ver.1.1 (PE/ABI; Perkin-Elmer; Applied Biosystems, Foster City, CA) and analysis on PE/ABI PRISM 3100-Avant Genetic Analyzer (PE/ABI). The alignment analyses were performed using the program GENETYX-WIN ver. 5 (Software Development Co., Tokyo, Japan). BLAST analyses were conducted on all sequences to identify related reference viruses.

**Virus isolation and characterization.** For the virus isolation, the virus gene-positive fly was selected from 180 *C. nigribarbis* using RT-PCR and the following nested PCR of the *M* and *HA* fragments. Both intestinal organs, crop and gut, were individually removed from a fly body and homogenized in 400  $\mu\text{L}$  of MEM-diluents (MEM; Eagle's minimum essential medium supplemented with 200  $\mu\text{g}/\text{mL}$  of streptomycin, 200 U/mL of penicillin, 50  $\mu\text{L}$  of gentamicin/mL, 0.5  $\mu\text{g}$  of fungizone/mL; Invitrogen, Carlsbad, CA) using MM300, and centrifuged at 3,000 rpm for 5 minutes. The supernatants were decanted into new tubes (Eppendorf). An additional 400  $\mu\text{L}$  of MEM diluents was added to the precipitations and homogenized again using MM300. The supernatants were pooled, and finally, 800  $\mu\text{L}$  of virus supernatant was obtained from a fly. Supernatant was divided in two: 200  $\mu\text{L}$  for detecting the

virus gene by RT-PCR and 600  $\mu$ L for virus isolation using embryonated chicken eggs. Viral RNA was extracted by using an RNeasy Mini Kit, and the virus gene was detected at the *M* and *HA* gene segments using the specific primers mentioned above.

Each 200  $\mu$ L of intestinal homogenate from a fly was inoculated into the allantoic cavity of three of 10-day-old embryonated chicken eggs. Inoculated eggs were incubated at 37°C for 2–3 days. The supernatant derived from egg amnion and allantoides was tested for the identification of Influenza virus by hemagglutination (HA)-test using 0.5% chicken red blood cells and by Directigen FluA+B kit (Becton, Dickinson and Company, Franklin Lakes, NJ). Among the three eggs tested, the virus-negative supernatants of egg amnion and allantoides were pooled and used for the second culturing using three new embryonated chicken eggs each. The virus isolates were characterized genetically by sequencing RT-PCR amplification and by cycle sequencing with influenza virus universal primers<sup>11</sup> for the full-length of the *M*, *HA*, and neuraminidase (*NA*) gene segments.

The nucleotide sequences obtained from viruses isolated from *C. nigribarbis* in this study are available from GenBank under accession nos. AB212651, AB212649, and AB212650.

## RESULTS

**Fly collection.** Despite it still being the cold spring season in Japan, a total of 926 flies were collected within a radius of 2.3 km of the A poultry farm at Tamba Town (Table 1). Fly densities ranging from 17 to 134 flies/h were observed in the collection, although there was a large difference in fly density among the six collection sites. Fly density was the highest at site 1 (134 flies/h), where is the nearest to the A poultry farm, and the lowest at sites 2 and 3 (26 and 20 flies/h, respectively), located > 2 km from this poultry farm. A total of eight fly species were recorded, and > 80% of the collected flies were identified as either *C. nigribarbis* or *A. grahami* (Table 2).

**H5 influenza A virus gene detection in blow flies by RT-PCR.** Four species of female blow flies, *C. nigribarbis* Vollenhoven, *A. grahami* (Aldrich), *Muscina stabulans* (Falle'n), and *M. angustifrons* (Loew), collected at site 1 of Tamba Town (Figure 1) were used in this study. The H5 influenza A virus gene was detected from *C. nigribarbis* and *A. grahami* (Figure 2). PCR fragments of 231 bp were visible from all pools of *C. nigribarbis* and *A. grahami* at the *M* gene by RT-PCR. At the *HA* gene, each pool of these two species showed visible PCR fragments (of 708 bp) by RT-PCR; however, the following nested PCR detected gene segments for all

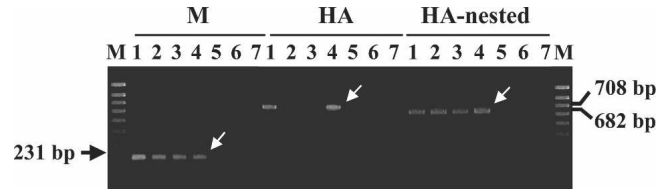


FIGURE 2. Amplification of the *M* and *HA* segments for H5 influenza A viruses by using RT-PCR and nested PCR. Lanes 1 and 2, *C. nigribarbis* (pool-1 and -2); lanes 3 and 4, *A. grahami* (pool-1 and -2); lanes 5 and 6, *M. stabulans* (pool-1 and -2); lane 7, *M. angustifrons*; M, 100-bp ladder.

fly pools of them. The RT-PCR assay using RNA extracted from the supernatants of intestinal homogenates and from the precipitations showed the same results (data not shown). BLAST analyses were conducted on the partial sequences of the *M* and *HA* genes from all visible PCR fragments to identify the related reference viruses. The virus gene detected from blow flies differed at only one site of 663 bp of partial nucleotide on the *HA* gene of A/chicken/Kyoto/3/2004 and A/crow/Kyoto/53/2004 (GenBank accession nos. AB188824 and AB189053, respectively). At the *M* gene, 100% similarity was shown between the virus from blow flies and the infected chickens (AB189048). As shown in Figure 3, no substitutions were found at 220 sites in the inferred amino acid sequences corresponding to the *HA* gene between these two blow flies, *C. nigribarbis* and *A. grahami*. In addition, their sequences coincided with those from the chickens (A/chicken/Kyoto/3/2004) and crows (A/crow/Kyoto/53/2004) in Kyoto. The virus from blow flies was confirmed as H5 subtype influenza A virus. Based on the *HA* gene encoding multiple basic amino acids at the *HAI-HA2* connecting peptide (RERRRKKR↓G), this virus was confirmed as a highly pathogenic strain.

Virus gene positivity of blow flies is shown in Table 3. Ten percent to 30% of *C. nigribarbis* and *A. grahami* were positive for H5 subtype virus at three collection sites (1, 2, and 6). High positivities of 20–30% were obtained from these two fly species collected at located 600–700 m from the A poultry farm (sites 1 and 6), and 10% positivity was shown even at a distance of > 2 km from the A poultry farm, site 2. The virus gene positivities obtained from *C. nigribarbis* were higher than that from *A. grahami* at all collection sites.

**Virus isolation and characterization.** One hundred eighty *C. nigribarbis* were tested for the detection of the *M* and *HA* fragments using RT-PCR and the following nested PCR. A total of 44 of virus gene positive blow flies were identified (positivity was 24.4%). The influenza virus was isolated from the intestinal organs of 2 of 10 blow flies through embryonated chicken eggs. All PCR fragments were amplified using universal primers, and the full lengths of sequences were obtained from *M*, *HA*, and *NA* gene segments sized 991, 1,707, and 1,362 bp, respectively, except for sequences of each primer sets. This virus showed high similarity to A/chicken/Kyoto/3/2004, differing at 1 of 986 sites in the *M*, 1 of 1,004 sites in the *HA*, and 0 of 1,350 in the *NA* genes (> 99.9% identity at the nucleotide level in all three RNA segments). This virus was characterized as subtype N1 based on the *NA* sequences. In addition, the *HAI-HA2* connecting peptide sequence at the *HA* gene segment was RERRRKKR↓G. Finally, this virus isolates from *C. nigribarbis* was characterized as highly pathogenic H5N1 subtype influenza A virus.

TABLE 2

Species composition of flies collected at site 1 of Tamba Town

| Species  | No. collected | Proportion (%) |
|--|---------------|----------------|
| <i>Aldrichina grahami</i> (Aldrich)            | 73            | 40.3           |
| <i>Calliphora nigribarbis</i> Vollenhoven      | 72            | 39.8           |
| <i>Muscina stabulans</i> (Falle'n)             | 29            | 16.0           |
| <i>Triceratopyga calliphoroides</i> Rohdendorf | 2             | 1.1            |
| <i>Muscina angustifrons</i> (Loew)             | 2             | 1.1            |
| <i>Muscina pascuorum</i> (Falle'n)             | 1             | 0.6            |
| <i>Lucilia porphyrina</i> (Walker)             | 1             | 0.6            |
| <i>Phaenicia sericata</i> Meigen               | 1             | 0.5            |
| Total  | 181           | 100            |



FIGURE 3. Amino acid sequences converted from a partial nucleotide sequence of the HA segments isolated from *C. nigribarbis* and *A. grahami*. Sequences of H5N1 influenza viruses isolated from infected chickens (A/chicken/ Kyoto/3/2004) and crows (A/crow/Kyoto/53/2004) derived during this outbreak period in Kyoto were used as references. Enclosed alignments indicate the cleavage site.

DISCUSSION

The virus from *C. nigribarbis*, namely A/blow fly/Kyoto/93/2004, was recognized as the same strain of the virus from infected chickens (A/chicken/Kyoto/3/2004) and crows (A/crow/Kyoto/53/2004) in Kyoto. Our results are the first indications that highly pathogenic H5N1 influenza A viruses were isolated from blow flies. The blow fly actively ingested the virus through droppings and secretions of infected chickens. A large amount of viruses must be ingested and survive in the larger-sized blow fly species. Chickens and many wild birds eat flies, even when they are flying. It is possible that chickens can take in the virus with fresh flies orally and/or contact with some contaminated feces excreted and vomited matter from infected flies. Reason why, it is no wonder that the blow fly was regarded as the best transmitters of influenza viruses, at least in a 2004 outbreak of H5N1 occurred in Kyoto.

During and after the H5N1 avian influenza outbreak period in Kyoto in 2004, a virus survey of migratory birds was carried out around epidemic areas. Some dead birds were found within a 30-km radius of the infected A poultry farm of Kyoto. Although H5N1 viruses were isolated from a total of nine large-billed crows, *Corvus macrorhynchos*, seven crows in Kyoto (A/crow/Kyoto/53/2004) and two in Osaka (A/crow/Osaka/102/2004), no virus was harbored in any other species of 102 dead birds in 11 families and 21 species examined.<sup>5</sup> Unfortunately, the exact transmission route has not yet been clarified. Because small sample trials were performed here, it might be supported that nearly 5% of *C. nigribarbis* contained active virus inside their bodies around the epidemic areas in Kyoto, based on the calculation that ~20% of the

virus isolation rate (2 positives among 10 eggs examined) were obtained from 24.4% of the virus gene-positive *C. nigribarbis* (44 positives among 180 flies examined).

A total of 926 flies were collected at six sites at distances of 600–2,250 m from the A poultry farm over 2 days. The fly density (numbers of flies collected per hour) was clearly higher closer to the poultry farm, and the highest density was 134/h at collection site 1. Two blow fly species, *C. nigribarbis* and *A. grahami*, comprised > 80% of the species composition. They are known to be active from the winter to spring seasons and reproduce from late autumn to the next spring in Japan.<sup>12–16</sup> An accumulated dropping of poultry farm was suspected to be a good breeding site of those flies. If they reproduced at the poultry farm, they were expected to have great opportunities of contacting with the viruses through the feces of infected chickens and/or their dead bodies.

It has been known that *M. domestica* spp. are the most important fly species at poultry farms<sup>17</sup> with regard to mechanical transmission of > 30 various pathogens<sup>18</sup> such as bacteria, protozoan, virus, and parasite oocysts and eggs. *M. domestica vicina* shows high activity generally in the summer season in Japan. In fact, no house fly was found around any poultry farms and pigpens of Tamba Town during our survey in March. It seems reasonable that the winter blow flies show some relationships to the winter pathogens, such as an influenza virus. In the case of house fly, it was suggested that the rotavirus is mechanically transported by contaminated fly surfaces.<sup>8</sup> House flies frequently defecate while feeding and resting on surfaces of the foods.<sup>19</sup> However, in *C. nigribarbis*, neither defecation nor vomiting was observed within 24 hours after feeding (data not shown). The body surface of the house fly must be contaminated by viruses easier than blow flies. This suggests that the mechanisms of virus transmissions of blow flies are possibly different from those of the house fly.

Both blow flies, *C. nigribarbis* and *A. grahami*, are categorized as large-sized fly species, and they prefer to lick animal carcasses and droppings. If food for blow flies is contaminated by pathogens, the blow flies could possibly take in great numbers of pathogens. One possible means for mechanically transmission of pathogens by blow flies is regurgitation on the food source.<sup>18,20</sup> The effectiveness of mechanical transmission through regurgitation may depend on the activity of pathogens in the fly body and amount of pathogens. The consumption rate of both *C. nigribarbis* and *A. grahami* might have been high because of their large body size. In this study, virus

TABLE 3

Detection of H5 influenza A virus from blow flies collected at Tamba Town in 2004

| Site* | Species               | n  | Crop | Gut | No. positive flies (%)† |
|-------|-----------------------|----|------|-----|-------------------------|
| 1     | <i>C. nigribarbis</i> | 10 | 1    | 2   | 2 (20)                  |
| 1     | <i>A. grahami</i>     | 10 | 0    | 2   | 2 (20)                  |
| 6     | <i>C. nigribarbis</i> | 10 | 2    | 3   | 3 (30)                  |
| 6     | <i>A. grahami</i>     | 10 | 0    | 0   | 0                       |
| 2     | <i>C. nigribarbis</i> | 10 | 1    | 0   | 1 (10)                  |
| –     | <i>A. grahami</i>     | 10 | 0    | 0   | 0                       |

\* See Table 1 for site 1, 2, and 6 details.

† Virus gene-positive flies were detected by RT-PCR results from crop and gut specimens.

gene positivity in *C. nigribarbis* was higher than that in *A. grahmi*. The body length of female *C. nigribarbis* is ~1.5 times as large as female *A. grahmi* (11–15 mm in the former and 8–13 mm in the latter). The capacity of the crop of female *C. nigribarbis* is approximately five times as much as female *A. grahmi* (average, 23 and 4.4  $\mu$ L, respectively). In fact, a small number of *M. stabulans* and *M. angustifrons* obtained at same collection sites and at same time of the fly surveillance were much smaller than *C. nigribarbis* and *A. grahmi*, and no virus was detected from these smaller-sized flies. In our study, avian influenza virus was detected and isolated from the intestinal organs of blow flies.

The excellent flight capacity of the blow fly is well known in Japan.<sup>21,22</sup> Blow flies collected from ships located on the East China Sea suggested that they immigrate to Kyushu Island and the northern seaside areas of Chugoku District, mostly from overseas countries.<sup>21</sup> The longevity and high dispersal ability of these blow flies<sup>21,22</sup> may also result in a wide dispersion of viruses. The possibility of viral multiplication inside the fly body must be low<sup>7,23</sup>; however, if viruses can survive for a certain period, and the infected flies migrate to at least the nearer poultry farm with viruses having an infectious titer, *C. nigribarbis* could be the most likely candidate for a transmitter of H5N1 influenza viruses. The B poultry farm was located only 4 km from the A poultry farm.

In Southeast Asian countries, the prevalence of H5N1 avian influenza has become a public health problem for birds and humans. In these countries, various kinds of species and high densities of flies inhabit at all seasons; however, no attempts have been made to clarify their ability to transmit the viruses. Our results strongly suggest that field studies focusing on the mechanical transmission by flies should be performed to control the outbreak of H5N1 avian influenza, especially in these Southeast Asian countries. Additional laboratory studies will be required to evaluate its transmission ability to chickens and other animals. We are continuing these studies to answer several questions: how long and how much viruses survive in the intestinal organs of the blow fly with virus maintaining minimum infectious titer.

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