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Original Article

Dispersal of a Blow Fly, *Calliphora nigribarbis*, in Relation to the Dissemination of Highly Pathogenic Avian Influenza Virus

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SUMMARY: A mark-release-recapture study of the dispersal ability of blow flies, *Calliphora nigribarbis*, was conducted in Ikumo-Naka, Ato Town, Yamaguchi Prefecture, Japan in December 2004. A location where a fatal avian influenza outbreak had occurred 1 year previously was selected for the present study. A total of 3,884 *C. nigribarbis* were collected, 1,915 of which were marked and released from 4 different collection sites during 2 successive days. The recapture rate of the released *C. nigribarbis* ranged from 0.014 to 0.029 among the collection sites, and the overall recapture rate was calculated as 0.022. Based on the distance between the released site and the recaptured site, the dispersal rate of *C. nigribarbis* was estimated as 256 m/h on the 1st day and 179 m/h on the 2nd day of the experiment, and the maximum dispersal rate observed in this study was estimated as 500 m/h. Taking into account the active period of *C. nigribarbis* on a fine day (7 h/day), the distance traveled by *C. nigribarbis* within a day was estimated as 1,789 and 1,250 m/day on average for the 1st and 2nd days, respectively, and the maximum distance was 3,500 m/day. These results suggest that *C. nigribarbis* could play a role in the mechanical dissemination of avian influenza virus and spread of the outbreak in Japan.

INTRODUCTION

In March 2004, a highly pathogenic avian influenza virus was detected and isolated from blow flies, *Calliphora nigribarbis* and *Aldrichina grahami*, collected in the vicinity of an infected poultry farm in Tamba Town, Kyoto Prefecture, Japan. Because of their large body size and good flight capability, the ability of *C. nigribarbis* to mechanically transmit highly pathogenic avian influenza virus was suggested (1). Although the possibility of viral multiplication inside the fly body is quite low (2,3), if the virus can survive for a certain period and if flies with virus having an infectious titer disperse to a nearby poultry farm, *C. nigribarbis* could play a role in the mechanical dissemination of avian influenza virus and spread of an outbreak in Japan.

To evaluate the dissemination ability of blow flies, the following studies were required: field studies of the dispersal of blow flies and laboratory studies regarding the length of time for which the virus can survive inside the fly body. The present study focused on the former subject, and a mark-release-recapture experiment of *C. nigribarbis* was carried out in Ato Town, Yamaguchi Prefecture, Japan where a fatal avian influenza outbreak occurred from December 2003 to January 2004 (4).

Fly movements is channeled or restricted by features of the landscape, and different dispersal patterns and flight distances were observed among different habitats such as rural, city suburb, urban, and pastoral (5). In Japan, mark-release-recapture studies of medically important flies have been conducted for *Fannia canicularis*, *Musca domestica*, and *C. nigribarbis*, and the flight distance of the released flies has been found to vary from 400 m to 1,300 m depending on the environmental conditions of the study area as well as the fly species (6-10). To examine the dispersal of *C. nigribarbis* in relation to their ability to disseminate avian influenza virus, it is important to use a study site where an avian influenza outbreak has previously occurred. We therefore selected a location where a fatal avian influenza outbreak had occurred 1 year previously and carried out a mark-release-recapture study of *C. nigribarbis*.

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MATERIALS AND METHODS

Study site: Ikumo-Naka (32°21′34″N and 131°34′35″E), Ato Town, Yamaguchi Prefecture, where the first cases of a fatal avian influenza outbreak were identified in Japan in the 2003 - 2004 period, was selected for the mark-release-recapture study. The poultry farm was located in a small isolated area (900 m by 250 m) surrounded by hilly areas 250 - 400 m above sea level. Because of the outbreak, the poultry farm has been closed and nobody was working there when we conducted this study in December 2004. The main road runs from northeast to southwest, and a pathway enters the study area from the southeast and continues as far as the neighboring villages (Fig. 1).

Mark-release-recapture: The mark-release-recapture experiment was conducted during 1 - 3 December 2004. The locations of the fly collection sites and the distance from the poultry farm are shown in Fig. 1. We visited the study area on 28 and 29 October 2004, at which time preliminary observations of the density of *C. nigribarbis* were carried out. Based on the results of these preliminary observations, three sites along the main road were selected for the mark-release-recapture sites (A, B, and C) to be used during the study period. Site A was in the center of the study area, nearest to the poultry

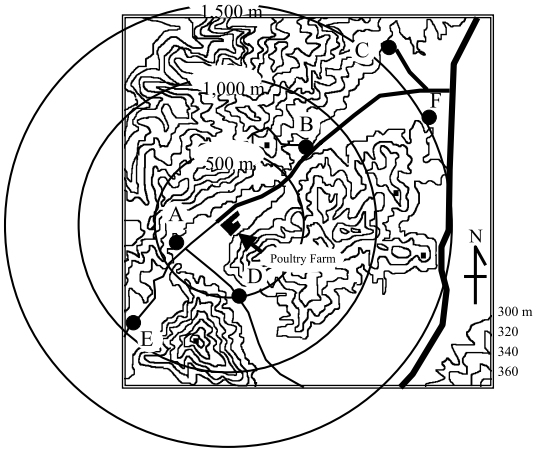

Fig. 1. A map of the study area showing the location of the poultry farm and 6 collection sites, A to F, in Ikumo-Naka, Ato Town, Yamaguchi Prefecture, Japan.

farm, and Site C was approximately 2 km northeast of Site A, at a forest fringe of coniferous trees. Site B was situated approximately 1 km from Site A with a pathway branching to the west. The number of *C. nigribarbis* collected at Site A on the 1st day of the mark-release-recapture experiment was not large, so an additional mark-release-recapture site was added on the 2nd day; Site D was situated south of the study area. On the 3rd day (the final day), 2 sites, Sites E and F, were added for recapture of the released blow flies to cover a wider area.

The mark-release-recapture of *C. nigribarbis* was conducted for 1 and 2 days at Site D and Sites A, B, C, respectively. To determine the period of fly collection, hourly changes in fly density were examined preliminarily at the study site for 2 days in late October 2004. Blow flies attracted to bait appeared when the collection site received direct sunshine at 9:00 - 10:00, and the number then increased, peaking at 11:00 - 12:00 and then decreasing until 15:00 in the afternoon. Therefore, in this study fly collection started at 9:00 and ended at 15:00. Rotten fish was used as bait, and *C. nigribarbis* attracted to the bait were captured by a sweep net, 36 cm in diameter. The thorax was marked with correction fluid "MISNON" (white color) (Lion Office Product Corp., Tokyo, Japan). To make 6 different colors of fluid, water-soluble dyes were mixed with the correction fluid and used to distinguish the site and the day that the flies were released. When marked *C. nigribarbis* were recaptured, the time of recapture and the color were recorded, an additional mark was made on the thorax, and they were released again. All captured *C. nigribarbis* were marked and released on the 1st and 2nd days, while no *C. nigribarbis* was marked and released on the 3rd day.

Data analysis: The distances between sites were measured using a map, and this information was used to calculate the distance traveled by a recaptured *C. nigribarbis*. The average distance traveled by recaptured *C. nigribarbis* was calculated for the 1st and 2nd days separately based on the *C. nigribarbis* recaptured on the day of release and on the next day of release. *C. nigribarbis* recaptured at the same site as the released site were excluded from the calculation.

RESULTS

A total of 3,884 *C. nigribarbis* were collected, 1,915 of which were marked and released during the study (Table 1). The recapture rate of the released *C. nigribarbis* ranged from 0.014 to 0.029, but the difference was not significant among the collection sites ($\chi^2 = 2.504$, $P = 0.474$) and the overall recapture rate was calculated as 0.022. The density of *C. nigribarbis* recorded at the 6 collection sites during the study ranged from 23.1 to 109.3 flies/h/site.

The maximum dispersal rate observed in this study was 500 m/h, which was recorded from a *C. nigribarbis* released from Site A and recaptured at Site B, 500 m from Site A at 1-h post-release. The mean distances traveled by the recaptured *C. nigribarbis* are shown in Table 2 based on the distances between the recapture sites and the previous capture sites.

Table 1. Density per hour per site, total number released and recaptured in the mark-release-recapture experiment of *C. nigribarbis* in December 2004, in Ikumo-Naka, Yamaguchi, Japan

Collection site	Total collected	Density (/h)	Total released (M)	Total recaptured (N)	Recapture rate[1)] (N/M)
A	531	23.1	348	10	0.029
B	1,338	39.4	692	16	0.023
C	845	49.7	545	8	0.014
D	710	64.5	330	8	0.024
E	328	109.3	0	–	–
F	132	26.4	0	–	–
Total	3,884	41.8	1,915	42	0.022

[1)]: The recapture rate (total no. of blow flies recaptured/total no. of blow flies released) was not significantly different among collection sites.

Table 2. Maximum and mean distance traveled by recaptured blow flies within the day of release and the day after release estimated from the results on the 1st and 2nd days of the mark-release-recapture experiment of *C. nigribarbis* in December 2004, in Ikumo-Naka, Yamaguchi, Japan

	Within the day of release		Day after release	
	1st day	2nd day	1st day	2nd day
Maximum distance traveled (m)	1,150	1,000	2,000	1,150
Mean distance traveled (m)	1,150	750	1,000	713
Mean hours to recapture (h)	4.5	4.2	–	–
No. of flies recaptured	2	6	17	8

Table 3. Movement of released *C. nigribarbis* among collection sites during 1-3 December 2004, Ikumo-Naka, Yamaguchi, Japan

		Previous capture site (Released site)				Total
		A	B	C	D	
Capture site	A	2	2	1	0	5
(Recaptured site)	B	3	10	3	3	19
	C	0	2	3	1	6
	D	3	1	0	2	6
	E	2	0	0	2	4
	F	0	1	1	0	2
	Total	10	16	8	8	42

Two and 6 *C. nigribarbis* were collected on the day of release on the 1st and 2nd days of the experiment, respectively, and their mean distance traveled was calculated as 1,150 m and 750 m, respectively. On average, *C. nigribarbis* recaptured on the day of release were caught 4.5 and 4.2 h after release on the 1st and 2nd days, respectively. The maximum distance traveled by *C. nigribarbis* on the day of release was 1,150 m and 1,000 m on the 1st and 2nd days, respectively. Similar maximum and average distances traveled by recaptured *C. nigribarbis* were observed the day after release (Table 2).

A total of 42 marked *C. nigribarbis* were recaptured during the study, and their capture sites and previous capture sites are summarized in Table 3. *C. nigribarbis* released from Sites A, B, C, and D were recaptured at 4, 5, 4, and 4 collection sites, respectively, and each collection site received *C. nigribarbis* released from more than one collection site.

DISCUSSION

C. nigribarbis has a good flight ability and shows long-distance seasonal migration between low and high ground (9-11) and/or habitats located at low and high latitudes (12,13). Based on the results of previous studies, the life history of *C. nigribarbis* can be summarized as follows. Adults appear in autumn, usually at the end of October in southwestern Japan where the present study was conducted, and reproduce during winter from November to April. Newly emerging adults move to cooler habitats located on high ground or northern areas in May and aestivate until October. Since their breeding season in 2003 and 2004 completely overlapped with the outbreaks of highly pathogenic avian influenza virus in southwestern Japan, it was suspected that there was some relationship between *C. nigribarbis* breeding and the outbreaks.

In March 2004, a highly pathogenic avian influenza virus was detected and isolated from *C. nigribarbis* collected in the vicinity of an infected poultry farm in Tamba Town, Kyoto Prefecture, Japan. *C. nigribarbis* positive for the virus were collected at 3 sites at different distances from the poultry farm (600, 700, and 2,050 m). Because of their large body size and good flight capability, the ability of *C. nigribarbis* to mechanically disseminate highly pathogenic avian influenza virus has previously been suggested (1). Recently, the importance of flies in the transmission of viral diseases has been suggested in relation to both houseflies and blow flies in some studies (2,14-17). Although the dissemination mechanism of the virus differs depending on the disease, the dispersal ability of flies is an important ecological character affecting their dissemination ability.

The dispersal rate of *C. nigribarbis* was estimated as 1,150 m per 4.5 h (= 256 m/h) and 750 m per 4.2 h (= 179 m/h) on average on the 1st and 2nd days of the experiment, respectively, and the maximum dispersal rate observed in this study was estimated as 500 m/h. The activity of *C. nigribarbis* depends largely on direct sunshine. The flies appeared when the collection sites were exposed to direct sunshine, and the number of flies attracted to the bait was largest at noon and then decreased thereafter. Based on the time of direct sunshine in December, the active period of *C. nigribarbis* was assumed to be 7 h (from 9:00 to 16:00) on a fine day. Thus, the distance traveled by *C. nigribarbis* within a day was estimated as 1,789 and 1,250 m/day on average on the 1st and 2nd days of the experiment, respectively, and the maximum distance was 3,500 m/day. As shown in Table 3, movement of the released *C. nigribarbis* was not directional but seemed to be random, and the dispersal rate estimated in this study must have been underestimated, since our estimation was based on the distance between the recapture site and the previous capture site. In Tamba Town, Kyoto Prefecture, Japan, we collected *C. nigribarbis* positive for highly pathogenic avian influenza virus at 3 sites at different distances from the poultry farm, 600, 700, and 2,050 m. Comparing the dispersal rates estimated in this study (2,044 or 1,429 m/day on average, and the maximum rate of 3,500 m/day), it was concluded that virus-positive flies collected in Tamba were able to reach the collected site within a day after taking up the virus from the infected poultry farm.

The results of the present and previous studies clearly show that *C. nigribarbis* feeding at an infected poultry farm are able to take the active virus inside their body, move around the poultry farm, and reach areas at least 3,500 m from the farm within a day. As for methods by which flies can mechanically disseminate pathogens, three possible methods have been suggested (18): (i) contact with the surface of the fly, (ii) regurgitation on food, and (iii) ingestion and defecation of pathogens. In the case of highly pathogenic avian influenza virus, we can also add another possible and plausible mechanism of mechanical dissemination by *C. nigribarbis*: direct feeding by wild birds or chickens on virus-carrying *C. nigribarbis*. The high density of *C. nigribarbis* in winter observed in this study and the previous study (1) suggested the high possibility of direct feeding of *C. nigribarbis* by wild birds as well as chickens. An additional experiment will be required to examine the possibility of infecting chickens through direct feeding on *C. nigribarbis* with an internal virus.

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