Insecticide susceptibility of the blow fly, *Calliphora nigribarbis* Vollenhoven, collected in Yamaguchi Prefecture, Japan

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(Received: 18 January 2006; Accepted: 20 June 2006)

Abstract: Fenitrothion and permethrin are common insecticides for controlling veterinary insect pests in Japan. The efficacy of these two insecticides to the blow fly, *Calliphora nigribarbis*, was investigated, because the highly pathogenic avian influenza (HPAI) virus was detected and isolated from blow flies collected in the vicinity of an infected poultry farm in Kyoto Prefecture in 2004. The adult blow flies collected in Yamaguchi Prefecture, where the first HPAI outbreak occurred in 2004, were directly used for topical application. The LD₅₀ values were 0.078 µg/female for fenitrothion and 0.014 µg/female for permethrin. The results show that the local population of the blow fly was susceptible to these two insecticides.

Key words: blow fly (*Calliphora nigribarbis*), susceptibility, LD₅₀, fenitrothion, permethrin, topical application

INTRODUCTION

The blow fly, *Calliphora nigribarbis* Vollenhoven, 1863 (= *Calliphora lata* Coquillett, 1898), is distributed in East Asia including Japan. It is known that the larval habitats are dung and carcass (Hayashi and Shinonaga, 1979). The adult emerges in spring once a year; it has a one-year life at the longest, and the female oviposits during the cool season from November to March (Kurahashi et al., 1994). Flying activity of the blow fly is very vigorous during these cool seasons, and the adults disappear from the lowlands of the mainland and southern parts of Japan in summer (Kurahashi, 1991; Kurahashi et al., 1991; Kurahashi et al., 1994; Kurahashi and Suenaga, 1997). For this reason, the blow fly has not been recognized as a target of chemical control in poultry farms, pigpens and cow sheds so far, unlike typical veterinary pest flies such as

the housefly (*Musca domestica* Linnaeus), *Muscina stabulans* Fallen and *Fannia canicularis* Linnaeus which keep their high populations from spring to fall (Gotoh et al., 1991).

Since the outbreak of the highly pathogenic avian influenza (HPAI) in 1997 in Hong Kong, the possibility of an epidemic has been a worldwide concern for not only the poultry industry but also for human health (Subbarao et al., 1998; Yuen et al., 1998). In Japan, its outbreak occurred at four poultry houses in three prefectures in the winter 2004 after the latest domestic record of 79 years ago. *Calliphora nigribarbis*, as well as *Aldrichina grahami* Aldrich, were collected as two predominant fly species in March 2004 within 2.5 km from a poultry farm where an outbreak occurred first in Tanba, Kyoto Prefecture, in February 2004. The collected flies for the two species were highly contaminated with the HPAI virus typed H5N1 (Sawabe et al., 2005a; Sawabe

et al., 2005b). From the beginning of March 2004, the infection route of HPAI virus had been investigated; however, no conclusive evidence from the surveillance of migrating and resident birds was obtained. A potential role of the blowfly should be reminded, especially in the virus transmission among close poultry farms such as in a serial HPAI outbreak at two neighboring farms in Kyoto 2004. Thus, we should be forced to control the flies, which are active and predominant even during cool seasons, in order to eliminate possible HPAI-propagating sources all year round. The Japanese government issued a guideline for action in case of HPAI outbreak (MAFF, 2004). The guideline mentions the necessity of controlling insect pests for prevention of HPAI diffusion. The blow fly is the primary target in winter seasons. However, insecticide susceptibility of *C. nigribarbis* has not been reported so far. In this study, we examined the susceptibility of the blow fly to fenitrothion and permethrin using field-collected flies and evaluated their sensitivity level in contrast with known insecticide-susceptible houseflies. Each insecticide is a representative one in the organophosphate or pyrethroid compounds that are registered for use at livestock farms (JVPA, 2004).

MATERIALS AND METHODS

Chemicals

The technical grades of fenitrothion (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate, purity 97.2%) and permethrin (3-phenoxybenzyl (1*RS*,3*RS*; 1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, 91.2%) were obtained from Sumitomo Chemical Co., Ltd.

Insects

Adults of the blow fly were collected in Yamaguchi City, Yamaguchi Prefecture on November 30, 2004. Adults were given water and sucrose, and kept at 20°C under the light period of 11L : 13D for 24–48 hr

for acclimation to laboratory condition. The SRS strain of the housefly for reference was described before (Yasutomi et al., 1988). Body weight of 250 females of the blow flies collected and that of 11 females of the 5 to 7-days-old SRS strain after emergence and nulliparous were measured. The ratio of body weight between two species was applied to comparison of lethal doses.

Susceptibility Tests

Only female blow flies were used for the susceptibility test. A half microliter of acetone solution containing fenitrothion or permethrin was topically applied to thoraxes with PB600-1 Repeating Dispenser (Hamilton Co.). Only acetone was applied for control. After treatment, ten flies were placed in a plastic container (10 cm in diameter, 5 cm in depth), fed with sugar and water and maintained at 25°C. The numbers of survived flies were counted 24 hr after the treatment. The LD₅₀ was calculated by SPSS (SPSS Inc.), the statistical analytical computer software.

RESULTS AND DISCUSSION

The susceptibility test was conducted by topical application using females of the blow flies collected in Yamaguchi City. The results are shown in Fig. 1 and Table 1. The LD₅₀ values for fenitrothion and permethrin were estimated to be 0.078 µg/♀ and 0.014 µg/♀. The observed response fitted a probit curve at significance level 5%, although each result included some outliers. Heterogeneity shown in insecticides-sensitivity is probably due to either genetic variations or heterogeneous physiological conditions, or both. Insecticide susceptibility of Calliphoridae flies has been reported only for *Chrysomya megacephala* Fabricius in Japan. Susceptibility of the blow fly to the two insecticides was very similar to that of *C. megacephala* (Mihara and Kurahashi, 1991).

The LD₅₀ values after correction by body weight are shown in Table 2 in order

to compare insecticide susceptibility of the blow fly with that of the housefly. The LD_{50} values corrected per gram body weight for the blow fly were almost equivalent to those for the insecticide-susceptible housefly (SRS strain). Application amounts of common insecticide formulations at livestock barns are 0.1 to 1 g a.i./m² (i.e. 0.1 to 1 μ g a.i./mm²) (JVPA, 2004). These concentrations are regarded as sufficient for control of a blow fly which shows an LD_{50} value below 0.1 μ g a.i./fly, taking the superficial dimension of the blow fly in square millimeters into account.

Although the present examination was based on the fly sampling at a single site, it is speculated that the blow flies distributed in Japan show similar susceptibilities because of their prominent flight and migratory ability (Kurahashi, 1991; Kurahashi et al., 1991; Kurahashi et al., 1994; Kurahashi and Suenaga, 1997) and a lesser extent of insecticide exposure to the

Fig. 1. Dose-response curve of the blow fly to fenitrothion and permethrin.

Table 1. Regression analysis of lethal activity of fenitrothion and permethrin against the blow fly by topical application.

Insecticide	Pearson's Goodness-of-Fit			N	Slope	SE	LD_{50} (μ g/♀)	95% C.L.
	χ^2	df	P					
Fenitrothion	7.274	4	0.122	110	1.30	0.28	0.078	0.040–0.123
Permethrin	0.781	3	0.854	110	1.14	0.25	0.014	0.007–0.025

blow fly whose larvae develop in the cool season. Insecticide resistance is generally the biggest problem in controlling pests such as houseflies at livestock barns in Japan (Yasutomi and Tomioka, 2000). Chemical control depending on a single variety of a compound group such as organophosphates or pyrethroids generally accelerates the development of insecticide resistance. The variation in the insecticides registered as veterinary drugs is limited in Japan, compared with agrochemicals (JVPA, 2004): pyrethroid and organophosphorus insecticides are the major insecticides used (Table 3, summarized from JVPA, 2004), and most of the active ingredients are common to those for human hygiene as well (Shono, 2005). Although the blow fly will not be a resistance-leading fly species, the development of new insecticide ingredients is desired for controlling not only possible resistant blow flies in the future but also the flies whose population have already raised a serious resistance problem, for the preparation of year-round HPAI prevention.

Even if the blow fly is as susceptible to insecticides as demonstrated by the present sampling, we probably face another difficulty that comes from their high flight activity: the blow fly does not remain in

Table 2. Comparison of insecticide-sensitivity between two fly species.

Insect	Body weight (mg)± SD	LD_{50} (μ g/g body weight)	
		Fenitrothion	Permethrin
Blow fly	58.4±12.6	1.3	0.24
House fly ¹⁾	19.9±1.5	2.0 ²⁾	0.35 ²⁾

¹⁾ SRS strain

²⁾ Averaged from three references (Shono et al., 1982; Yasutomi et al., 1988; Lee et al., 1996).

Table 3. Active ingredients list of the insecticides registered for domestic animal use in Japan (2004).

Group	Chemical name
Pyrethroids	Allethrin, Bioresmethrin, Cyfluthrin, Etofenprox, Permethrin, Phenothrin, Prallethrin, Resmethrin, Tetramethrin
Organophosphates	Azamethiphos, Dichlorvos, Fenitrothion, Prothiofos, Trichlorfon
Carbamates	Carbaryl, Fenobucarb, Propoxur
Insect growth regulators	Cyromazine, Diflubenzuron, Pyriproxyfen
Synergists	Piperonyl butoxide

the same place. The housefly is prone to stay and spend a life cycle within livestock barns as long as the barns are sufficiently attractive to the fly, while the blow fly moves back and forth between a single livestock barn and the outside and it does not remain in a livestock barn for a long time. In consideration of the behavior of the blow fly, it is suggested that only direct spraying or residual treatment of insecticides is not sufficient to control them at livestock barns. Some physical control measures should be conducted in combination with chemical control of the blow fly. Quick detection and removal of poultry carcass that the blow fly especially prefers are necessary. When an HPAI outbreak occurs in a poultry house, the feces are possibly contaminated with the pathogen and so it is important to prevent blow flies from feeding on the feces. In the case that the outbreak spreads faster than the removal of feces, the feces surface needs to be promptly covered with some foaming or sheet material. For this purpose, easy-to-use covering materials should be developed for HPAI prevention.

ACKNOWLEDGEMENTS

We are grateful to Drs. Hiromu Kurahashi, Toshihiko Hayashi and Yukiko Higa for collecting field samples. This research was supported in part by a Grant-in-Aid of Ministry of Health, Labour and Welfare (H15-Shinko-Ippan-18 and H17-Shinko-Ippan-017).

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