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SHORT COMMUNICATION

The housefly, *Musca domestica*, as a possible mechanical vector of Newcastle disease virus in the laboratory and field

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Abstract. Newcastle disease (Paramyxoviridae) is a highly infectious virus shed in the faeces of infected birds. Non-biting Muscid flies characteristically visit manure and decaying organic material to feed and oviposit, and may contribute to disease transmission. The housefly, *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae), has been implicated as a mechanical vector of numerous pathogens. In this study 2000 aerial net-captured houseflies were examined for their ability to harbour Newcastle disease virus (NDV). In an adjacent study, laboratory-reared flies were experimentally exposed to NDV La Sota strain. The virus was detected in the dissected gastrointestinal tract of laboratory-exposed flies for up to 72 h post-exposure, whereas the untreated control flies were negative.

Key words. *Musca domestica*, La Sota, mechanical vector, Newcastle disease virus.

The synanthropic nature of the housefly, *Musca domestica* (Linnaeus, 1758), illustrates its potential for spreading diseases from animals to man and animal to animal. Evidence that muscid flies act as mechanical vectors of gastrointestinal (GI) pathogens has been documented widely (Ugbogu *et al*., 2006). Outbreaks of diarrhoeal diseases in urban and rural areas of countries are closely related to seasonal increases in the abundance of filth flies and fly control is closely related to the decline in cases of enteric diseases (Graczyk *et al*., 2001). The housefly reproduces by depositing eggs in a wide variety of decaying organic materials, including manure, mixtures of manure and bedding, and spoiled food. Upon encountering such materials on poultry farms, the adult fly, using its sponging mouth parts, consumes fluids and frequently defecates. As a result of such feeding behaviour, the housefly has been implicated in the transmission of over 30 bacteria, protozoan, viruses and helminth eggs (Greenberg *et al*., 1970; Kobayashi *et al*., 1999). Houseflies can transmit viruses such as polioviruses (Kettle, 1990) and coxackie viruses, as well as numerous bacteria such as *Campylobacter jejuni*, *Helicobacter pylori* (Grubel *et al*., 1997), *Salmonella* sp. (Greenberg *et al*., 1970), *Listeria* sp., *Yersinia pseudotuberculosis* (Zurek *et al*., 2001), *Shigella* (Levine *et al*., 1991; Ugbogu *et al*., 2006) and *Vibrio* (Kettle, 1990). Flies may be vectors of protozoan parasites such as *Giardia* and *Entameba* (Ugbogu *et al*., 2006) and eggs of several tapeworms (Graczyk *et al*., 2005).

Few reports have described the role of flies in the transmission of Newcastle disease virus (NDV). Rogoff *et al*. (1977) clearly showed that virulent velogenic NDV (VVNDV) can be transmitted to young chickens by *Fannia canicularis* (Linnaeus, 1761), either from a highly infective source or directly from infected birds. The only report of the housefly being involved in the transmission of NDV was published in Russia (Milushev *et al*., 1977). Recently, exotic NDV was isolated from houseflies collected from backyard flocks during an outbreak in the U.S.A. (Chakrabarti *et al*., 2007). Virus concentrations were low, at <1 egg infectious dose$_{[EID50]}$ per fly, in these field-collected flies. Under laboratory conditions the housefly did not carry sufficient quantities of NDV (Roakin strain) to cause disease in chickens (Watson *et al*., 2007). The latter authors speculated that virus virulence factor plays a part in the ability of the housefly to mechanically transmit NDV.

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The purpose of this study was to determine if houseflies collected from the site of a recent ND outbreak carried virus. Secondly, a laboratory study was conducted to determine whether the housefly transmits a vaccine strain (NDV La Sota) to chickens.

In 2006, adult muscid flies were collected from 20 poultry farms near Tehran, Iran. Each farm was positive for ND according to laboratory tests exhibiting high haemagglutination inhibition (HI) titres (Alexander, 2004). Flies were collected with an insect net by making one or two quick sweeps over litter, manure deposits and feed troughs. Collected flies were transferred into plastic bags and transported to the laboratory within 30–45 min of collection. Flies were sorted to species and houseflies were killed at −20°C and stored in a freezer until they were processed as pooled samples of 100 flies per sample.

For surface sterilization, 100 houseflies from each poultry facility were placed in a sterile tube containing 2 mL of phosphate buffer solution (PBS) and vortexed for 10 s. The washed samples were disinfected with sodium hypochlorite 0.05% for 30 s and rinsed twice with sterile PBS for 1 min. Flies were dissected in a sterile Petri dish and dissection instruments were sterilized after each dissection. Digestive tissues including the diverticulum (crop), midgut and hindgut were removed and placed in a sterile tube with 2 mL PBS. Dissected tissues were macerated against the tube wall with sterile forceps. To inhibit bacterial growth, 1000 IU of penicillin, 10 000 μg/mL of streptomycin and 200 IU/mL of ketoconazol were added to gut homogenate. After 1–2 h at room temperature (22°C), the prepared samples were frozen at −20°C until tested for the presence of virus.

Prepared samples were thawed at room temperature and centrifuged for 10 min at 1000 **g**, after which 0.2 mL of supernatant was injected into 9–11-day-old embryonated chicken eggs. Inoculated eggs were placed in an incubator at 37°C and checked daily. After 4 days, all inoculated eggs were transferred to temperature cabinets maintained at 4°C. For virus detection, allantoic fluid was harvested and tested for the presence of virus by haemagglutinating test (HA), as described by the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (Alexander, 2004). Non-haemagglutinating fluids were re-tested at least once to confirm the negative diagnosis for NDV.

To determine the role of the housefly as a vector of NDV *in vitro*, *M. domestica* larvae were collected from an ND-negative poultry house. The larvae were kept at room temperature in litter material and transferred to a glass container upon pupation. After the adult flies emerged, they were exposed to virus inoculum composed of the lentogentic vaccine strain La Sota (Razi Vaccine and Serum Research Institute, Tehran, Iran) in suspended glucose containing PBS. The flies were provided with virus-soaked cotton pads and allowed to consume the virus over a 24-h period. Pools of 10–20 flies were removed at 0.5, 1, 2, 4, 24, 48, 72 and 96 h and 8 days post-exposure. Flies were immediately frozen to kill the fly and preserve the virus (Watson *et al*., 2007). Digestive tissues were removed and virus detection performed as described above.

Although NDV was present on the 20 farms sampled, gut homogenates of the 20 pools of 100 flies were all negative for NDV. As the HA test is reliable for the presence of virus in fly tissues (Watson *et al*., 2007), we conclude that the virus titre was below detectible levels or was no longer active at the time of our collections. To test the hypothesis that the housefly is able to acquire the virus and to confirm the reliability of the test, we conducted a laboratory test using the La Sota vaccine strain.

Table 1. Haemagglutination activity in houseflies exposed to Newcastle disease (La Sota) virus.

Sample number	Sampling interval, h	Haemagglutination activity
1	0	−
2	0.5	+
3	1	+
4	2	+
5	4	+
6	24	+
7	48	+
8	72	+
9	96	−
10	192	−

+, antibody inhibition; −, no antibody inhibition.

Newcastle disease virus La Sota was detected by HA tests in dissected digestive tissues from NDV-exposed flies up to 72 h post-exposure (Table 1). These results indicate that houseflies are capable of acquiring and harbouring NDV for extended time periods.

Newcastle disease is a highly contagious disease of poultry which causes significant economic losses to the industry (Alexander & Jones, 2001). Inhalation and ingestion of virus-containing droplets are the main routes of infection. During the course of infection most birds with NDV excrete large amounts of virus in their faeces (Alexander, 2003). Flies are attracted to manure and dead birds and thus have ample opportunity to be exposed to pathogens (Calibeo-Hayes *et al*., 2002).

Filth flies including Sarcophagidae, Calliphoridae and Muscidae are considered to play important roles in the transmission of human and animal pathogens because they are in close association with animals, humans and their food stuffs (Kettle, 1990; Graczyk *et al*., 2001). Although virus was not isolated from field-collected flies in this study, NDV has been previously isolated from field-collected houseflies, the lesser housefly, *F. canicularis*, and the bronze blowfly, *Phaenicia cuprina* (Wiedemann, 1830), in the U.S.A. (Chakrabarti *et al*., 2007). The biology and ecology of *M. domestica* make it an ideal mechanical vector of human and animal pathogens (Graczyk *et al*., 2001). Many viruses have been isolated from the digestive tract of houseflies; Davidson *et al*. (2005) demonstrated the involvement of houseflies in the transmission of reticuloendotheliosis virus (REV). Houseflies in this experiment were able to harbour REV in the digestive tract for up to 72 h and could infect chickens (Davidson *et al*., 2005). During an outbreak of highly pathogenic avian influenza in Kyoto, Japan in 2004, Sawabe *et al*. (2006) collected a total of 926 flies from six sites within a radius of 2.3 km of the infected poultry farm. Reverse transcription polymerase chain reaction (RT-PCR) analysis determined that two blowflies harboured avian influenza virus which had more than 99.9%

identities in all three RNA segments to a strain from chicken (A/chicken/Kyoto/3/2004) and crow (A/crow/Kyoto/53/2004) derived during this outbreak. In a study by Calibeo-Hayes *et al*. (2002), houseflies were examined for their ability to harbour and transmit Turkey coronavirus (TCV). In laboratory studies, TCV was detected in fly crops up to 9 h post-exposure; no virus was detected in either sham-exposed flies or dissected intestinal tissues. The potential of the housefly to directly transmit TCV to live turkey poults was examined by placing 7-day-old turkey poults in contact with TCV-exposed flies at densities as low as one fly per bird. Antigens of TCV were detected at 3 days post-exposure in the tissues of three of 12 turkeys. However, an increasing rate of exposure was observed with higher fly densities.

Rogoff *et al*. (1977) reported an association of VVNDV with wild flies, especially *F. canicularis*. They reported the transmission of VVNDV to chickens via exposed *F. canicularis*. This was the first demonstration of arthropod transmission of ND. It was asserted that *F. canicularis* is capable of retaining the virus for $\geq$6 days, that the virus can be transmitted from a highly infected source to healthy birds or from one bird to another and that further experiments should be conducted with *M. domestica* (Rogoff *et al*., 1977). Milushev *et al*. (1977) reported the involvement of houseflies in the transmission of NDV. The results of this study and others have shown that the housefly is capable of harbouring NDV for up to 72 h post-exposure. No NDV was detected in net-captured houseflies collected from poultry farms; however, NDV has been recovered from field-collected flies and reported in other studies. These results illustrate that houseflies are capable of harbouring NDV and disseminating enteric diseases of poultry.

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