Isolation of salivary gland hypertrophy virus in wild populations of the house fly *Muscadomestica* from Egypt

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ABSTRACT

The MdSGHV was isolated from house flies population around the world. We aimed in this study to isolate this virus from house fly population in Egypt. Screening house fly population using a new method based on homogenizing large number of house fly adults collected from field, clarify and concentrate the filtrate by centrifugation with 1400 g and 28000 g respectively, resuspend the pellet in Tris buffer, sterile by 0.45-µm syringe filter and inject to house fly adults maintained in laboratory colony, leads to the detection and isolation of this virus from Egypt. Injection of filtrate induced SGH symptom in 60% injected flies. The salivary gland symptom was similar to the previously reported symptoms of the MdSGHV. Examination of the SGH suspension with Electron microscopy revealed the presence of rod-shape virus particles with 500-700 nm in length and 50-70 nm in diameter. PCR analysis of SGH homogenate with virus specific primer pair resulted in DNA fragment with expected size as previously mention in literature. These data demonstrate the presence of the salivary gland hypertrophy virus in house fly population in Egypt which provides the opportunity to evaluate the potential use of this virus to control the house fly population in Egypt within the frame of integrated pest management approach.

Key Words: House fly flies, *Musccidae*, salivary gland hypertrophy virus.

INTRODUCTION

The house fly, *Muscadomestica* L. is a well-known cosmopolitan insect pest of both farm and house. This species is always found in association with humans or activities of humans. More than 100 pathogens associated with the house fly may cause diseases in humans and animals, these diseases include typhoid, cholera, tuberculosis, anthrax, mastitis, bacillary dysentery and infantile diarrhea.Recent concerns about food-borne human illnesses have led to increased documentation of the role of flies in spreading disease-causing organisms, especially *Escherichia coli*, *Shigella* spp., and *Salmonella* spp., **(Macovei *et al*., 2008)**.

Until now control of house fly is mainly relied on the insecticides. Organophosphates, arbamates, pyrethroids and Insect Growth Regulators (IGRs) have been used to control house fly**(Kocisova, 2001).**

An alternative control strategy is the joint practice of culture and biological control. Documentation of the natural enemy complex of housefly is extensive and includes a wide range of fungal pathogens, parasitoids and predators**(Geden *et al*., 1998).** The entomopathogenic viruses, as one of the microbial control agents, are specific, efficient and safe to non-target organisms. **(Kurstak, 1991)**.

A group of double-stranded (ds) DNA viruses, referred to as salivary gland hypertrophy viruses (SGHV), has been detected in the house fly *Muscadomestica***(Coler *et al*., 1993)**, the narcissus bulb fly *Merodonequestris***(Amargier *et al*., 1979)** and various tsetse fly *Glossina* species **(Jaenson, 1978; Gouteux, 1987; Minter-Goedbloed and Minter, 1989; Shaw and Moloo, 1993)**.

The hytrosaviruses constitute a unique group of entomopathogenic viruses for which a new family has recently been proposed to the International Committee on Taxonomy of Viruses (ICTV) **(Abd-Alla *et al*., 2009; Lietze *et al*., 2010)**. While viral replication in the salivary glands induces conspicuous hypertrophy, viremia also

reduces reproductive fitness **(Lietze *et al*., 2007)**.

The main target of this study was to get isolation, of this new virus MdSGHV as a first recording from the Egyptian Environment. The potential use of SGHVs as a sterilizing agents for house fly control to avoid the previous diseases and protect human, animals and the environment from the side effect of residual insecticides.

MATERIALS&METHDS

1-Insect strain

Housefly *Muscadomestica*pupae, obtained from a colony maintainedat the Research Institute of Medical Entomology, Dokki, Giza, Egypt, were placed in rearing cages, provided withdeionized water, and maintained under constant conditions (26°C, a photoperiodconsisting of 12 h of light and 12 h of darkness, and 40% relative humidity) untiladults emerged. Adult flies were provided with water and food (a 1:1 mixture [by volume] of powdered milk and sucrose).

2- Collection of houseflies from field sites

Adults house fly *Muscadoenstica*were collected from different sites from Giza, Egypt nearby garbage collection and processing sites. Flies were collected with sweep nets and placedimmediately in cages provided with water and food (a1:1 mixture [by volume] of powdered milk and sucrose). Cages were returned to the laboratory and held at 26°C for up to 4 days before being examinedfor infection. These holding conditions prevented anypossible new infections arising from horizontal transmissionin the cages from contributing to survey results**(Geden *et al*., 2011)**.

3-Isolation of MdSGHV from Egyptian house fly population.

The isolation of MdSGHV from house fly population in Egypt was conducted following a methods suggested by prof. DrionBoucias and Dr. VerenaLietze, University of Florida, Fl, USA.

(Bocias, D. and Lietze, V., Personal communication).In brief, more than 1000 Adult houseflies were collected with a sweep net from different sites in Giza. The flies were kept in plastic jars groups until returned to the laboratory then collected flies were kept at -20°C until processed. Frozen flies were homogenized in buffer (50mMTris, 2mM SDS, pH 7.8), The homogenate was filtered through several layers of cotton and muslin **(Tompkins, 1990)**. Clarification of the filtrate suspension took place by a low centrifugation at 1400 g for 5 min using J2-21MIE centrifuge, rotor JA. Potential viral particles were pelleted from the supernatant by centrifugation at 28,000 g for 30 min using the same rotor. Pellet was re suspended in minimum volume of Tris buffer (50 mM, pH 7.8) and filtered with a 0.45-µm syringe filter into sterile microtubes and store at 4^{o}C until used for fly injection. Potential virus particles in filtrate suspension were amplified by injecting the filtered suspension (2 µl per fly) into the thoracic cavity of disease-free adult flies maintained in the colony. Flies were cold-immobilized and injected with filtered suspension and Tris buffer as mock treatment using micro-applicator, injected flies were placed in placed in rearing cages, provided with deionized water, and food as mentioned above and maintained under constant conditions (26°C, a photoperiod consisting of 12 h of light and 12 h of darkness, and 40% relative humidity) for 7 days. Injected flies were cold-immobilized at -20^{o}C for 4 min and dissected, and the condition of the salivary glands andovaries was recorded. Hypertrophied salivary gland pairs were collected andprepared for Electron microscopic examination.

Transmission Electron Microscope (TEM)

Samples of hypertrophied salivary glands and asymptomaticglands were dissected from cold-immobilized flies, homogenized in 500ml of Tris buffer, and filtered through a 0.45-µm syringe filter into sterile micro tube, Gland suspension was examined under Electron microscope after staining with Phosphotungstic

acid (PTA) at TEM lab FARP, Facultyof Agriculture Research park- Cairo University, Egypt).

4- Detection of SHGV by polymerase chain reaction (PCR)

Total DNA was extracted from 5 hypertrophied salivary gland using a Mini elut DNA purification kit (Qiagen). Extracted DNA samples were suspended in TE buffer (10 mMTris-HCl [pH 7.5], 1 mMEDTA), DNA Conventional PCR was conducted using the MdSGHV-specific primers SGHVenvF1 (50-ACCTACGGTCTGGATATTC GTG) and SGHVenvR1 (50-GATGTACGGCAATAGTGTA CGG), which target a 487 bp region of the open reading frame (ORF) MdSGHV047, a gene coding for the envelope protein **(Garcia-Maruniak*et al*., 2008; Salem *et al*., 2009).** Each 25 µl reaction contained 1 µl of ample, 10 pmole of each primer, 12 µl of PCR master mix (Fermentas) The thermocycler (iCycler, Biorad, Hercules, CA) was programmed for initial denaturation at 95 ^{o}C for 10 min, followed by 35 cycles at 95 ^{o}C for 30 s, 55 ^{o}C for 30 s, and 72 ^{o}C for 30 s, and final extension at 72 ^{o}C for 10 min. PCR products were run on a 1.5% agarose gel and stained with ethidium bromide**(Lietze*et al*., 2009)**.

Results and Discussion

The aim of this study was to isolate the salivary gland hypertrophy virus (SGHV) from house fly population in Egypt to provide an opportunity to use this virus as a biocontrol agent against this pest. The fact that this virus is widely distributed in the house fly population around the world make this study feasible with high expectation of success **(Prompiboon *et al*., 2010)**. A new method was implemented to enhance the efficiency of screening wild house fly population to detect and isolate the virus infection. The current regular methods for isolating the salivary gland hypertrophy virus was so far depending on collecting the wild house fly adults, cold-immobilized it and dissect to watch the salivary gland status

and collect the enlarged glands. This method requires dissecting large number of flies to pick-up the infected glands due to the low prevalence of the virus infection in wild population known about this group of viruses **(Geden *et al.*, 2008; Lietze *et al.*, 2011; Prompiboon *et al.*, 2010)**. This method is laborious and time consuming. Due to the available information on the virulence of this virus which seem very high as injecting the suspension of salivary gland hypertrophied diluted more than 10,000 time still can induce virus infection and cause salivary gland hypertrophy symptom (unpublished data), it was suggested that a suspension of around 1000 flies with only one fly carry the symptom can induce the virus infection.

Implementing this new method reduce significantly the time needed to isolate the virus. Injecting the filtrate suspension from thousands wild flies into 25 healthy adults of house flies resulted in the observation of few (15) flies with salivary gland hypertrophied (60%). The isolation process was repeated three times with flies collected from Giza governorate and one time with flies collected from Qalyubia governorate. Although some technical challenges were faced with the micro-injection process with high rate of mortality due to injection process and mechanical damage, enough flies were survived during the experimental period **(Table 1)**. No salivary gland hypertrophied observed in the mock injection with Tris buffer one week post injection. Flies with salivary gland hypertrophied showed a sever reduction in the ovary size **(Figure 1)** which agrees with previous observation made on the pathology of this virus **(Geden *et al.*, 2008; Lietze *et al.*, 2007; Lietze *et al.*, 2010; Lietze *et al.*, 2011; Prompiboon *et al.*, 2010).**

Electronmicroscopy observation of PTA-stained (phosphor tungsticacid) of hypertrophied salivary gland suspension revealed rod-like particles 500-700 nm in length and 50 nm in diameter **(Figure 2)**. This observation occurs with the virus previously described in *Muscademostica***(Abd-Alla *et***

***al*., 2010; Geden *et al*., 2008; Lietze *et al*., 2007; Lietze *et al*., 2010; Lietze *et al*., 2011; Prompiboon *et al*., 2010)**.

The detection of SGHV in the DNA extracted from hypertrophied salivary gland with the virus SGHV-specific primers confirms the presence of MdSGHV in the infected gland. The achievement of equivalent DNA PCR product (487 bp) **(Figure 3)** to the previously reported result indicate that the gene is conserved in all SGHV strains. The absence of any PCR product if the non-hypertrophied glands indicate that the primers are virus specific and the virus is associated with the SGH symptoms as previously described **(Lietze*et al*., 2009)**.

This result demonstrates the presence of salivary gland hypertrophy virus in house fly population in Egypt and confirms previous observation that this virus is globally distributed **(Prompiboon *et al*., 2010)**. This achievement is expected to open the door for further studies which aim to gather required information to consider this virus as a potential use to control house flies population in Egypt. The studies need to be done concern the following aspects: (i) a complete characterization of this virus isolate using molecular techniques (i.e. protein analysis and nucleic acid analysis including genome sequencing) to determine its relation with other isolated around the world, (ii) determine the distribution of this virus in different geographical locations in Egypt (i.e. north, middle and south Egypt), (ii) determine the genetic variability of this virus in house flies populations in different locations in Egypt, do they have one virus strain or more, (iii) determine the virulence of the virus isolate from Egypt and its impact on the flies using bioassay, (iv) compare the virus virulence of the Egyptian strain(s) with other strains isolated around the world, (v) estimate the feasibility of using this virus to control the wild population in flied by testing application methods (baits or areal spay, additives, stability of the virus treatment, virus concentration

and treatment frequency, producing the virus in large scale etc..) (vi) analyze the impact of this virus on other

non-targeted organism (i.e. bees). Although some of these

studies were conducted and published on the virus isolated from Florida, US, it is recommended to conduct these studies on the Egyptian isolate, first because it still early to know whether the Egyptian isolate is similar or different from the Florida isolate and secondly because of the different ecosystem inEgypt that the once in Florida.

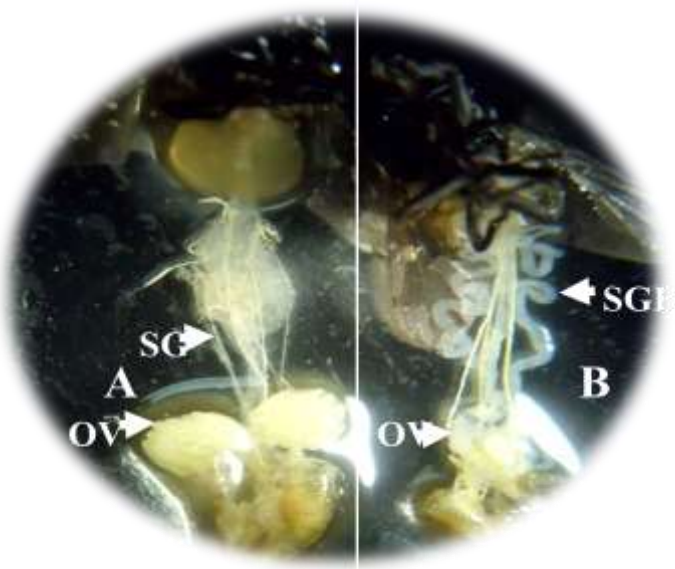

Figure 1. Disease symptoms of salivary gland hypertrophy virus**:** (A) Normal (SG) and(B)hypertrophied salivary glands (SGH) of health and infected house fly *Muscadomestica* (OV: ovaries).

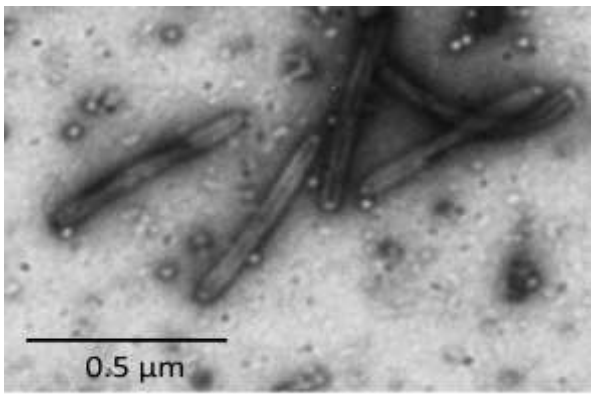

Figure 2.Negatively-stained salivary gland hypertrophy virus (SGHV) suspension examined my electron microscopy.

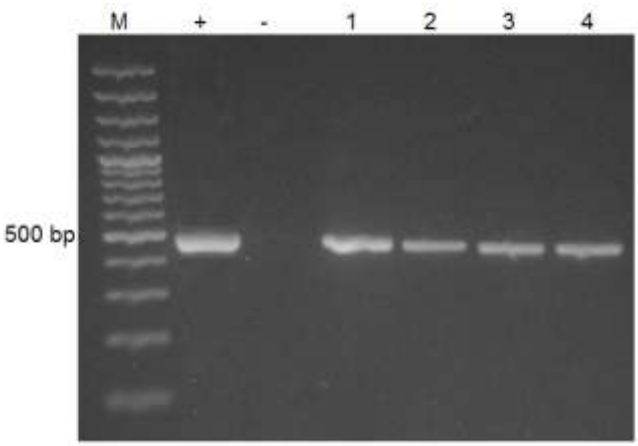

Figure 3.Conventional PCR detection of MdSGHV in total DNA extracted from homogenized salivary gland from house flies adult.Lane 1: 1Kb DNA ladder, lane 2: positive control, lane 3: DNA extracted fron non-hypertrophied glands, lanes 4-7 DNA extracted from hypertrophied salivary gland.

Table 1. Prevalence of salivary gland hypertrophied in filtrate-injected adults of house fly *Muscadomestica.*

Isolation process	location	Treatment	Total number of injected flies	No. of flies died mechanically	No. of flies with SGH	% of SGH
1	Giza 1	Filtrate	30	20	6	60
		Buffer	30	22	0	0
2	Giza 2	Filtrate	30	23	4	57.14
		Buffer	30	25	0	0
3	Giza 3	Filtrate	30	15	8	53.33
		Buffer	30	20	0	0
4	Qalyubia 1*	Filtrate	30	25	3	60
		Buffer	30	23	0	0

*This isolation process was conducted only one time and was drop out of this study at later stage.

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