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Minireview

Muscavirus (MdSGHV) disease dynamics in house fly populations – How is this virus transmitted and has it potential as a biological control agent?

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ABSTRACT

The newly classified family Hytrosaviridae comprises several double-stranded DNA viruses that have been isolated from various dipteran species. These viruses cause characteristic salivary gland hypertrophy and suppress gonad development in their hosts. One member, *Muscavirus* or MdSGHV, exclusively infects adult house flies (*Musca domestica*) and, owing to its massive reproduction in and release from the salivary glands, is believed to be transmitted orally among feeding flies. However, results from recent experiments suggest that additional transmission routes likely are involved in the maintenance of MdSGHV in field populations of its host. Firstly, several hours before newly emerged feral flies begin feeding activities, the fully formed peritrophic matrix (PM) constitutes an effective barrier against oral infection. Secondly, flies are highly susceptible to topical virus treatments and intrahemocoelic injections. Thirdly, disease transmission is higher when flies are maintained in groups with infected conspecifics than when flies have access to virus-contaminated food. We hypothesize that interactions between flies may lead to cuticular damage, thereby providing an avenue to viral particles for direct access to the hemocoel. Based on our current knowledge, two options seem plausible for developing *Muscavirus* as a sterilizing agent to control house fly populations: The virus may either be formulated with PM-disrupting materials to facilitate oral infection from a feeding bait system, or amended with abrasive materials to enhance infection through a damaged cuticle after topical aerosol applications.

Contents

1. Introduction

Natural infections with hytrosaviruses (Hytrosaviridae), a unique, recently classified family of enveloped, non-occluded, dsDNA insect viruses, have been described from three dipteran families, Syrphidae (Amargier et al., 1979), Glossinidae (Jaenson, 1978), and Muscidae (Coler et al., 1993). Within 2–3 days after infection of the adult host, characteristic salivary gland hypertrophy (SGH) is caused by massive viral reproduction in the salivary gland cells and can be diagnosed by dissection of the insect (Lietze et al., 2011a). Furthermore, infection suppresses oogenesis in female flies (Jura et al., 1988; Lietze et al., 2007), but the specific mechanisms underlying this virus-induced sterilization remain to be elucidated. Our current knowledge of Hytrosaviridae is derived from studies of two members of this virus family, the *Muscavirus* (MdSGHV) of

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house flies (*Musca domestica*) and the *Glossinavirus* (GpSGHV) of tsetse flies (*Glossina pallidipes* and closely related species) (Abd-Alla et al., 2009). Various aspects of hytrosavirus biology including host specificity, morphogenesis, pathogenesis, and molecular biology have been reviewed recently by Lietze et al. (2011b). Although MdSGHV and GpSGHV share a number of properties that are distinct from other dsDNA viruses (Garcia-Maruniak et al., 2009), they each express unique traits with regard to their morphology, pathobiology, and genetic and biochemical structure. Furthermore, research initiatives on these two viruses are driven by different motivations: the GpSGHV poses a serious threat to valuable tsetse fly colonies established within the framework of a large-scale sterile insect technique program (Abd-Alla et al., 2011), and research efforts have been dictated by the need to eliminate or suppress symptomatic infections from colonized host populations. By contrast, the research program on MdSGHV has been directed at identifying the potential of MdSGHV as a biological agent in house fly population control. While it has been shown that the hytrosaviruses can be disseminated within fly populations via salivary secretions that are released onto shared food substrates, other horizontal transmission routes likely exist but remain yet to be identified (Lietze et al., 2012). The purpose of this forum article is to initiate a discussion about behavioral mechanisms on the individual and population level that impact the transmission efficiency of insect pathogenic viruses. We expect such a discussion to help evaluate the potential of MdSGHV for use as a biological house fly control agent.

2. MdSGHV infections in house fly populations

Over the past years, intensive field surveys have been conducted in Florida and Denmark to determine the prevalence of MdSGHV-infections in *M. domestica* populations on dairy farms (Geden et al., 2008, 2011). Fig. 1 illustrates typical house fly aggregations sampled at a North Florida dairy operation. Incidental samplings of adult house flies from various sites in the US and from sites in South America, Africa, Southeast Asia, and Australasia have shown that this virus is globally distributed (Prompiboon et al., 2010; D.G.B., unpublished). When assessing infection rates in fly populations two *Muscavirus*-specific properties need to be considered. First, this virus is infectious to only adult flies; larvae do not contract infections (Geden et al., 2008). Second, based on the numerous control assays and screenings conducted in our laboratories, there currently is no evidence for the presence of asymptomatic infections in *M. domestica*. Therefore, MdSGHV infection rates in house fly populations can be determined by sampling and dissecting adults and recording the presence of symptomatic SGH. Average field prevalence of MdSGHV varies between 0.5% and 10%, although occasional peaks (34%) are observed during the summer and are correlated positively with fly density (Geden et al., 2008, 2011; Prompiboon et al., 2010). Interestingly, by introducing a high proportion (40%) of virus-infected flies into confined laboratory populations and monitoring infection rates over time, Lietze et al. (2012) demonstrated that MdSGHV does not cause population collapses; instead, infection rates in these experiments declined to 10% and persisted during the observation period of 12 weeks that spanned ten progeny generations. However, the following question remains: How is MdSGHV maintained in natural populations of its host?

The massive production and release of virions from the salivary glands suggest that MdSGHV is transmitted orally among flies that share a common food substrate (Lietze et al., 2009). However, results from various experiments indicate that additional transmission routes likely exist. For instance, the peritrophic matrix (PM) is presumed to be an effective barrier against oral infection of *M. domestica*. Within 2–4 h after adult emergence, a continuous, thick PM is secreted, and force-feeding of flies that are older than 6 h produces no or low (8% average) infection; by contrast, force-feeding of newly emerged, less than 2-h-old flies results in an average 53% infection (Prompiboon et al., 2010). Thus, several hours before emerged feral flies would begin feeding, their resistance to oral infection is drastically increased. This resistance can be overcome by pre-treating flies with reducing agents presumed to disrupt the PM (Boucias et al., unpublished). Furthermore, house flies can contract SGH when maintained in cages that previously had been

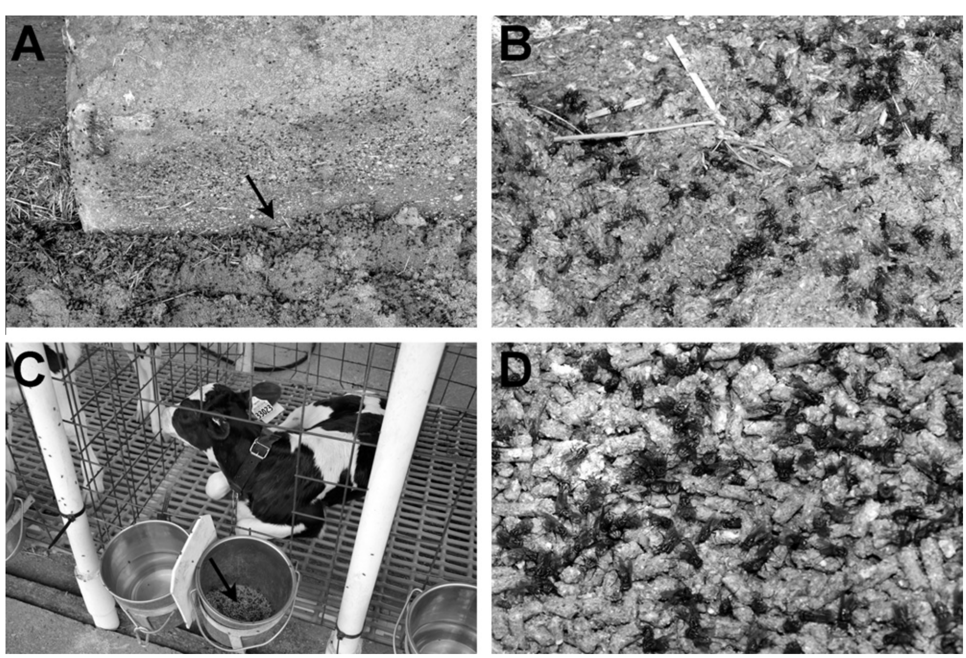

Fig. 1. Aggregations of house flies sampled at a dairy farm in North Florida during surveys of MdSGHV infections. Hot spots of fly activity are observed in (A and B) feed storage barns along the walls and on the various feed substrates as well as on (C and D) the brewer's grain fed to calves. Arrows in panels A and C indicate zoomed-in areas that are displayed in panels B and D, respectively.

occupied by infected flies, demonstrating that environmental contamination provides an additional source for MdSGHV transmission (Geden et al., 2008). Adult flies also are highly susceptible to intrahemocoelic injections (Geden et al., 2011; Lietze et al., 2007) and topical virus treatments (Geden et al., 2011). One may assume that cuticular damage would allow direct ingress by virus particles into the hemocoel, thereby circumventing the PM barrier in the midgut. Lastly, our most recent bioassays have shown that disease transmission is higher (14%) when flies are maintained in mixed-gender groups with infected conspecifics (Lietze et al., 2012) than when flies have access to virus-contaminated food (1%, V.-U.L. unpublished). We therefore hypothesize that hitherto unidentified interactions between infected and healthy house flies can promote MdSGHV transmission and that certain behaviors may result in wounding and allow viral invasion of the hemocoel.

3. House fly behaviors that impact disease transmission

Although there are no gender-specific differences in the susceptibility of house flies to oral and intrahemocoelic infection with MdSGHV (Lietze et al., 2007, 2009; Prompiboon et al., 2010), field surveys have shown that infection rates of sampled flies are up to twofold higher in males than in females (Coler et al., 1993; Geden et al., 2008; C.J.G., unpublished). Plausible explanations could be an increased likelihood to catch infected males than females, a higher mortality of infected females than males, or a higher transmission rate to male than to female flies. While personal observations by all authors do not support the former scenarios, our recent study on MdSGHV disease dynamics in confined fly populations confirmed that male flies contract MdSGHV infections at higher rates than female flies do (Lietze et al., 2012). This result is observed consistently regardless whether experimental groups of exposed and donor flies consist of only one or both genders. As previously discussed, fly behaviors conducive to MdSGHV transmission may include grooming, mounting, any aggressive behavior that leads to wounding, contacting secretions and excretions deposited by conspecifics, and cofeeding on food substrates and water sources (Lietze et al., 2012). Moreover, in field populations, encounters of females with infected conspecifics or a virus-contaminated environment may be lower than those of males. Male flies are known to be more gregarious, they aggregate around so-called resting spots and avidly search for mating partners (Tobin and Stoffolano, 1973). Females do aggregate at oviposition sites (Jiang et al., 2002), but infected females, due to lack of mature ovaries, may not be present at such sites.

In order to identify testable variables that may regulate MdSGHV transmission, we have initiated a program to generate a mathematical model that possibly could describe a stable maintenance of MdSGHV within house fly populations. A system of differential equations has been developed with constant parameters for the transmission rates including feeding, environmental contamination, and male-male interactions as transmission pathways. Preliminary results indicate that male-male interactions (or male aggressive behavior) and the presence of infectious virions in the environment have a greater impact on MdSGHV transmission than oral infection through consumption of virus-containing food sources (Keesling et al., unpublished). These significant theoretical findings will need to be tested in order to identify options to use MdSGHV for house fly control.

4. Potential of MdSGHV as a biocontrol agent

Development of resistance to insecticides by *M. domestica* is an increasingly pressing issue in the control of this ubiquitous insect pest (Kaufman et al., 2010; Keiding, 1999), and alternative methods such as use of biological control agents to suppress fly populations will need to be incorporated into future management strategies. Besides the adult-specific MdSGHV, a number of natural enemies have been or currently are being investigated. These include generalist egg and larval predators such as the beetle *Carcinops pumilio* (Erichson) and the black dump fly, *Hydrotaea aenescens* (Wiedeman), (Carlson et al., 2001; Kaufman et al., 2000), pupal parasitoids within the pteromalid genera *Muscidifurax, Nasonia*, and *Spalangia* (Geden and Hogsette, 2006; Kaufman et al., 2001; Skovgard and Nachman, 2004), the entomopathogenic fungus *Beauveria bassiana* (Geden et al., 1995; Kaufman et al., 2005), and the adult-specific mycopathogen *Entomophthora muscae* (Cohn) (Mullens et al., 1987).

MdSGHV, owing to its sterilizing effect and apparent mating disruption, may have potential as a biocontrol agent by reducing the intrinsic rate of increase in house fly populations. This virus maintains itself within fly populations (Geden et al., 2008; Lietze et al., 2012) and has a global distribution (Prompiboon et al., 2010). However, unknown factors regulate the maintenance of infected hosts within field populations at very low levels, which by preliminary results from a mathematical model (Keesling et al., unpublished) are predicted to facilitate a stable equilibrium. If transmission efficiency of MdSGHV is enhanced, this virus may have potential to suppress house fly populations below nuisance level, but repeated field applications may be required. At present, we suggest the following two options for utilizing *Muscavirus* as a biocontrol agent: Firstly, to increase oral infectivity, MdSGHV may be formulated with PM-disrupting materials and incorporated into food baits. A number of compounds that affect PM integrity have been shown to increase oral infectivity of insect pathogenic viruses (e.g., Undeen and Fukuda, 1994; Webb et al., 1996; Zhu et al., 2007). Secondly, abrasive materials such as desiccant dusts in combination with aerosol applications of MdSGHV may enhance infection of flies through a damaged cuticle. For example, synergistic interaction between diatomaceous earth and entomopathogens has been shown to increase the infection efficacy of the mycopathogen *B. bassiana* (Lord, 2001).

5. Conclusions

Identifying additional routes of MdSGHV transmission within house fly populations may allow us to develop better techniques to utilize this virus as a biological control agent. Preliminary results from our generated theoretical MdSGHV transmission model indicate that male aggressive behaviors and environmental contamination with infectious virus contribute more to a stable equilibrium than oral infection from virus-containing food sources. This article was written to stimulate a discussion about the importance of cuticular damage and/or other as yet unknown factors in enhancing efficacy of horizontally transmitted insect viruses.

Disclosures

The authors Verena-Ulrike Lietze, James E. Keesling, Jo Ann Lee, Celeste R. Vallejo, Christopher J. Geden, and Drion G. Boucias report no conflicts of interest to be declared.

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