

The role of muscid flies in bovine pathogen dissemination

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Project overview

Hypothesis: Pathogen dispersal on dairy farms

Aim 1: Characterize fly-associated pathogens

Field sampling

Sequencing

Aim 2: Assess pathogen colonization, persistence, and dissemination in fly hosts

Inoculate larvae

Determine abundance

We hypothesize that muscid flies have the capacity to acquire and disseminate pathogenic bacteria within and between dairy barn environments.

Bovine mastitis

Bovine mastitis is an infection characterized by the inflammation of the mammary gland tissues.¹

Bacterial invasion of mammary gland

Inflammation of mammary gland

Loss of milk quality and production

Research has historically focused on control of contagious pathogens; however, environmental pathogens are of growing concern.

Important environmental pathogens include:

- Staphylococcus* spp.
- Coliform bacteria
- Streptococcus* spp.

Muscid flies

Stable flies

House flies

Piercing mouthparts

Sponging mouthparts

Adult

Eggs

Pupae

Larvae

Microbial acquisition via adult behaviors

Microbial acquisition via larval feeding

House and stable flies closely associate with manure throughout their lifecycle.²

Research implications

Increased understanding of pathogen dissemination by flies

Increased understanding of factors shaping fly microbiota

Development of a new model system

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Aim 1: Characterize fly-associated pathogens

Background:

- Dairy barns are home to large populations of biting and nonbiting flies, with fly abundance positively correlating with mastitis incidence.^{2,3,4}
- Cow manure is likely to harbor an array of bovine pathogens.^{5,6}
- Flies have previously been shown to spread and modulate antimicrobial resistance gene (ARG) profiles on swine farms.^{7,8}

Knowledge gaps:

- Current knowledge is highly biased towards culture-dependent methods.
- Past research has focused near exclusively on house flies.

Progress:

- Fly and environmental samples were collected from the Dairy Cattle Center (Madison, WI) and Emmons Blaine Dairy Research Center (Arlington, WI) on a weekly basis between July–September 2021.

Sample collection

Homogenize

Isolate clinically relevant taxa

Taxonomic identification

Gut dissection

Bacterial diversity via 16S rRNA gene amplicon sequencing

Distribution and abundance of ARGs and virulence factors via metagenomic sequencing

Photos by Michael P. King/UW-Madison CALS

Aim 2: Assess pathogen colonization, persistence, and dissemination in fly hosts

Background:

- Adult flies can acquire microbes via feeding or transstadial transmission from larvae.^{9,10}
- Bacteria colonizing the digestive tract can then be spread via regurgitation or defecation.^{9,11}

Knowledge gaps:

- A suitable model in which to study the mechanisms underlying fly-microbe interactions is currently lacking.
- There is little research on colonization dynamics of dairy barn pathogens.

Progress:

- A house fly colony has been established to perform laboratory experiments.
- We have optimized methods for extracting and amplifying DNA from field-collected and lab-reared flies.
- Methods have also been developed for the generation of axenic (microbe-free) and gnotobiotic flies (colonized by known microbes).

Aim 2.1: Characterize microbiota acquisition and persistence across fly life history

Sample diet

Sample larvae

Conventional pupae

Surface-sterilized pupae

Non-sterile conditions

Sterile conditions

Sample adults and eggs

Sample adults

Sample newly emerged adults to assess transstadial transmission

16S rRNA gene amplicon sequencing

Relative abundance

Aim 2.2: Assess the ability of pathogens to colonize, persist, and be disseminated by fly hosts with different microbial backgrounds

Flies exposed to bacteria-inoculated manure

Flies transferred to sterile containers

Determine bacterial abundance

Visualization via fluorescent microscopy

Fly host backgrounds:

- Gnotobiotic
- Conventional
- Antibiotic-treated
- Fluorescent
- Non-fluorescent