

Laboratory Methods for Rearing Horn Flies (Diptera: Muscidae)

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Subject Editor: Karen Poh

Received 8 February 2020; Editorial decision 4 May 2020

Abstract

The horn fly, *Haematobia irritans* (L.), is an obligate hematophagous ectoparasite of cattle, and one of the most important pests of cattle causing unrealized gains or losses in meat and milk production. The present study describes the difficulties that arise when research programs have attempted to maintain this pest, both on-host and off-host, in a laboratory environment. Suggestions aimed at assisting future researchers in successfully colonizing horn flies in the laboratory are provided.

Key words: laboratory insect colonization, colony rearing, biting fly, cattle pest, filth fly

Horn flies, *Haematobia irritans* (L.), are one of the most damaging pests affecting cattle production. In pastured beef systems, horn flies can cause a number of production losses typically manifested through decreases in weight gain and feed efficiency as well as milk production indirectly measured through weaning weights of calves (Campbell 1976, Huston et al. 1979, Kinzer et al. 1984, Kunz et al. 1984, Quisenberry and Strohhahn 1984, Smythe et al. 2019). Similar losses are present in dairy systems where horn fly impacts on milk production can be measured directly along with an increased incidence of mastitis associated with the presence of this pest (Block and Lewis 1986, Owens et al. 1998). Production losses are usually attributed to irritation as a direct result of adult blood feeding, pathogen transmission, and defensive behavioral responses employed by the host animal (e.g., fly avoidance behaviors, reduced feed intake, etc.).

Horn flies are obligate hematophagous ectoparasites of cattle. Larvae develop exclusively in fresh cattle feces, and pupate within or slightly under manure pats. Following adult eclosion, horn flies locate a suitable host and immediately initiate blood feeding. Horn flies maintain this close relationship throughout the adult life stage typically leaving the host only momentarily to oviposit or to relocate when disturbed by defensive behaviors (tail switches, etc.). Adult horn flies feed between 20 and 38 times a day on cattle hosts (Harris et al. 1974, Lancaster and Meisch 1986). The intimacy of this parasitic interaction leads to complications when attempting to colonize horn flies in laboratory settings. Moreover, laboratory rearing of horn flies off-host becomes even more difficult. Successful establishment of laboratory colonies have been achieved only a few times by various research groups, utilizing either on-animal or on- and off-animal rearing techniques.

Major barriers to successful on-animal laboratory colonization include animal and facility availability while off-animal colonies struggle with feeding larvae and adults, without the direct use of an animal host. Initially, colonized horn flies were reared on caged animals with reintroductions of flies (McLintock and Depner 1954). Later, off-animal techniques utilizing amended diet and immature substrates were developed allowing for successful colonization (Harris 1962), with various groups establishing off-animal laboratory strains (Greer 1975, Bolton 1980, Okine and Butler 1995).

Regardless of the colonization method, numerous experiments benefit from the use of laboratory-reared horn fly strains. In addition to year-round access, potential influencing variables such as age and sex can easily be accounted for in colonized systems. Accounting for these variables become particularly important when conducting research concerned with general toxicological and physiological assessments of the flies. Colonized horn flies can additionally offer baseline susceptibility or laboratory comparisons when evaluating insecticide resistance in wild strains.

For purposes of the current protocol, the authors draw strongly from unpublished personal experiences from research groups out of the University of Florida and New Mexico State University. The following discussion is structured as a guide based on these experiences and we anticipate the unpublished failures and successes discussed herein to assist future researchers in successfully colonizing horn flies in the laboratory.

Field Collections

Many of the techniques used to colonize horn flies involve the use of live animals. Use of animals in research requires appropriate

regulatory approval. Typically, Institutional Animal Use and Care Committees (IACUC) oversee academic institutions and provide resources for researchers working with animals. It is the responsibility of the researcher to pursue and acquire any and all regulatory approval prior to initiating the research.

When colonizing horn flies, it is important to first establish and consider specific research objectives as this will dictate the colony characteristics desired and suitable techniques to employ (i.e., a susceptible population, a local population, a population resistant to a given or multiple insecticides). Colonies originating from a wild population require a horn fly-infested animal herd. Typically, adult stage capture is conducted with sweep nets passed across the back and bellies of animals held in chutes or working pens. Captured horn flies should then be transferred to a screened insect cage and if possible, immediately offered a blood meal. Multiple passes with sweep nets will be necessary to capture enough flies for colony establishment. Cages containing field-collected flies should be placed away from the working area and sheltered from direct sunlight. Estimating the number of captured flies is difficult to impossible using visual observation. However, we estimate that a collection of roughly 10,000 horn flies should be sufficient for colony establishment. Following field collection, horn flies should be immediately transferred to laboratory conditions. Upon arrival to the laboratory, a stanchioned or caged bovine should be considered for initial adult rearing. Though not always possible, the use of a live animal increases the chances of early colony success. The likelihood of a horn fly colony becoming immediately established using off-animal techniques is minimal.

On-Animal Colonization

Few facilities are equipped to establish and maintain on-animal horn fly colonies. An ideal scenario for on-animal horn fly colonization includes a temperature-controlled room with a stationary mechanism (e.g., headgate mounted stanchions) that limit host movement thereby increasing animal and researcher safety. Following field collection, horn flies can be released directly on to the confined animal. Feces produced by the animal within confinement should be collected a minimum of three times daily and stored in larval growth bins located within the room. Adult population size decreases during the first 7–10 d of colonization. However, immature development, if successful, produces viable adults that emerge from the larval growth bins and reinfest the host animal slowly progressing to self-sustainability.

On-animal techniques are labor intensive and require highly specialized facilities to properly and safely sustain horn fly colonies. Daily cleaning and feeding of the host animal is critical to adhere to the regulatory requirements of animal welfare. Additionally, when utilizing these techniques, researchers sacrifice the ability to monitor generational differences. Researchers in New Mexico successfully established an on-animal colony from horn flies collected from the New Mexico State University College Ranch in April of 2019 using the techniques described above. Subsets of the colony were isolated throughout the 12-wk colonization for general insecticidal screening (B.G.S., unpublished data).

Off-Animal Colonization

The following descriptions of off-animal colonization techniques are specific to established colonies. As mentioned previously, wild horn flies rarely, if ever, thrive in artificial conditions immediately

following collection. Modification of the procedures detailed below as well as those discussed in on-animal techniques can be utilized in cohort to ease the transition toward off-animal procedures.

Eggs

Once established, horn flies require little to no stimuli to induce oviposition. In fact, once sexual maturity is reached, female horn flies readily oviposit through screened mesh of the rearing cage and eggs drop below for collection. Eggs can easily be collected from underneath rearing cages by placing paper towels on water-soaked sponges allowing the edges of paper towels to wick water from below. This scenario creates a high humidity micro-environment that prevents desiccation of the collected eggs. Researchers should be careful to not over saturate the egg collection device as prolonged submergence may reduce viability. To avoid excess eclosion in the egg collection device, collections should not extend beyond 24 h and eggs should be transferred to rearing pans immediately. Collected eggs can be easily transferred to larval media by gently washing the egg collection device with a wash bottle.

Larvae

Various techniques for amending larval media have been published. All utilize fresh bovine feces collected from animals known to not have been treated with insecticides, parasiticides, or other chemicals that may inhibit fly development. Although collected fresh, feces is typically first frozen and then thawed before use. When in colony at the University of Florida horn flies were reared on a mixture of fresh feces and peanut hull pellets (Geden et al. 2006, Holderman 2012). This media was maintained at ambient laboratory conditions (photoperiod of 12:12 [L:D] h and ~50% RH). Researchers at New Mexico State University currently utilize a 1:4:1.5 combination of vermiculite:wheat bran:Animax (Purina) added at a rate of 5% of approximately 4,500 g of fresh feces to generate larval rearing media. Both research groups have found success with amending fresh feces substrates to supplement horn fly development.

In all cases, diet fed to the animals affects the quality of feces for horn fly rearing. Fresh feces from animals on fresh forage (not hay) is preferred, but not always available. Animals with loose watery feces or those fed feedlot rations (high protein diets) should be avoided. Fresh feces are recommended to be frozen to reduce the potential for introduction of predators and parasites of horn fly eggs or larvae.

Pupae

The pupal stage of horn fly development requires little care. Larval development and pupation occur within the rearing bins requiring anywhere from 6 to 7 d to complete at approximately 26°C. During this developmental period, rearing bins should be placed in a secure elevated location, preferably covered with tulle to prevent interlopers from gaining access to the media (Formicidae, Phoridae, other Muscidae, etc.). Fully developed pupae can be collected from the larval media by water flotation. Typically, larval bins are submerged in a water bath where larval media can be gently agitated to dislodge pupae. Viable pupae float to the top of the bath where they can be collected and dried to remove excess water. Compressed air drying systems are utilized by researchers out of New Mexico; however, collected pupae can be spread across absorbent paper and air-dried. If pupae are not dried adequately, they tend to aggregate into hardened masses that decrease adult eclosion rates. Following drying, pupae are transferred to adult cages.

Adults

Newly eclosed adults require immediate access to a blood meal typically offered as citrated bovine blood. Sodium citrate is added to blood immediately upon collection at a rate of around 12 g/l to prevent coagulation. Alternative anticoagulants have been reported previously and rates of these anticoagulants that do not affect horn fly rearing have been previously published (Guerrero et al. 1993). Adult horn fly blood meals should be offered at least twice a day in the laboratory. Blood meals are typically offered by soaking cotton pads in citrated blood prior to placement directly on top of the screened adult cages. Blood meals can be warmed prior to feeding to help the transition from on-animal colonization. Citrated blood can be held refrigerated for up to 2 wk without issue. After this 2-wk period, blood sources appear to sour putting off a foul odor which appears to decrease horn fly feeding activities. Researchers in New Mexico currently freeze blood sources for up to 6 mo rationing out and thawing quantities of blood to fulfill production needs at 2-wk intervals with no observable effects on fly production. Volume of blood consumed peaks when females are ovipositing their first eggs (C.J.H., personal observation). In the laboratory, photoperiod is usually maintained at 12:12 (L:D) h cycle. Diapause in horn flies is thought to be triggered both by temperature and photoperiod exposure of a female, which causes subsequent diapause of her eggs as they reach pupation (Showler et al. 2014). Laboratory temperatures are typically maintained at 75–80°F and 12:12 (L:D) to prevent diapause.

Colonization Failures

The authors of this manuscript have made numerous unsuccessful colonization attempts of field collections in excess of 10,000 horn flies (C.J.H. and B.G.S., unpublished data). In Florida, one attempt resulted in approximately 500 eggs being collected from wild adult horn flies with only with only around 50 of those maturing to the adult stage. It was suspected that the blood source was not palatable enough for the flies to create eggs, though blood was observed to have been ingested. As such, the attempt to colonize a wild adult horn fly colony directly into an off-animal scenario was immediately unsuccessful. Similar attempts in New Mexico produced similar results. Modification of standard techniques including heated blood meals and ovipositing substrates resulted in little success in these instances.

Additional attempts to modify existing protocols for established off-animal horn fly colonies have led to unfavorable results. Mimicking a more natural production routine for colony flies may help retain certain behavioral traits that may be desirable to the researchers. Many colonized horn flies utilize amended larval substrates for development. However, attempts to maintain colonies on nonamended feces have resulted in extremely desiccated substrates from which pupae could not be extracted. Furthermore, survival on feces-only substrate samples is consistently less than 60% (unpublished data).

In addition to amended larval substrates, researchers have utilized additives to blood sources such as beef purge (intra- and extracellular fluid that drains from cut beef muscle cells in meat processing), antibiotics, and antifungals (Okine 1996). The addition of beef purge was used to closer mimic natural horn fly blood meals as flies imbibe both blood and cellular fluid that escapes muscle as the mouthparts cut through host tissues. At the University of Florida, when beef purge was added to blood treated with antibiotics and antifungals, microbial growth was observed in the blood and horn fly development was negatively affected; thus, the blood amendment was discontinued because an aseptic source of beef purge was not available. Furthermore, blood components (whole, plasma, erythrocytes) were evaluated separately and confirmed erythrocytes or some component thereof are required for oogenesis to occur (Okine and Butler 1995).

Colonization Successes

The authors were not aware of a published critical evaluation of laboratory colonies compared with field horn flies. Colonization events in insects typically bottleneck genetically, and are often not expected to be identical to field insects, but represent a snapshot in time of population genetics and behaviors that can exist under laboratory conditions (Ochieng'-Odero 1994). As such, a series of data collection events were conducted to establish baseline colony performance of the New Mexico State University permethrin resistant (PR) and susceptible (SS) strains of horn flies under the standard rearing methods.

Off-Animal Procedures

Weekly larval bins (33 cm × 45 cm × 12 cm) were set containing approximately 1.5 ml of eggs, 4,400 g of larval media, and pupae were harvested following a 6-d incubation period. Larval bins were floated, and resultant pupae were measured in milliliter (total production), which was averaged by bin. A subset of pupae (1 ml) from each weekly collection was counted and used as a metric of pupal and adult fly size. Furthermore, 10 individual pupae from weekly harvests were separated and allowed to eclose as adults that were then sexed. This procedure provided a value of percent eclosion and sex ratios of the two colonies. Summary statistics were generated in Microsoft Excel, Version 1911.

Results

The SS and PR horn fly strains were evaluated for 33 and 34 wk, respectively, in 2019. In both cases, the colonies were reared at an average of 9.9 bins each week. Production metrics show that slightly more SS flies were produced in each bin, though they were slightly smaller in size, and eclosed successfully at a higher rate (Table 1). The sex ratio between the two strains was nearly identical.

Table 1. Production metrics of two horn fly colonies

Strain (<i>n</i>)	Average (SEM)			
	Pupae per bin	Pupae per ml	Percent female	Percent adult emergence
SS (33)	55.5 (1.78)	131.2 (2.47)	50.6 (0.33)	93.3 (1.46)
PR (34)	54.3 (2.29)	127.2 (2.53)	50.9 (0.29)	88.2 (0.33)

PR, permethrin resistant; SS, susceptible strain.

Discussion

Colony rearing of any obligate ectoparasite is difficult in a laboratory setting. This is particularly true when attempting to establish horn fly colonies, which has historically been difficult to accomplish. The techniques described above are intentionally presented as a broad overview of a variety of approaches that could be used to better existing protocols. Horn fly colony maintenance without the direct involvement of a living bovine animal (off-animal colonization) may offer benefits in terms of reduced labor, costs, and regulatory oversight associated with colonization but fail to incorporate host animal involvement in existing protocols. Host animals are still required for fresh feces collection utilized in larval production and blood for adult feeding. Furthermore, it is unknown what colonization impacts, if any, have occurred with existing laboratory strains of horn flies. Due to many of these challenges, most horn fly colonies are often not maintained indefinitely, and many colonies referenced in previous literature have since been terminated.

Rearing horn flies without the use of animals (directly or indirectly) is unlikely to be successful with flies directly collected from the field. Horn fly colony production metrics presented herein are provided with our hope that researchers can utilize this information for comparisons to future horn fly colonies utilizing similar or modified techniques, leading to more precise colonization protocols, and healthy horn fly colonies that more accurately represent wild population characteristics.

Acknowledgments

The authors wish to extend an appreciation for inclusion of this manuscript into this special issue. This research was not specifically supported by any funding agencies.

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