



## The Impact of Nuisance Flies on Growing Dairy Heifers

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### ABSTRACT

The horn fly is an economically important external parasite of cows and horses. Cows with large numbers of flies on them have lower milk production and decreased feed conversion due to avoidance activity. These flies are not only a pest; they are also a vector for disease. Many commercial pesticides have lost efficacy in controlling horn flies. This increase in resistance and growing concern regarding food safety issues has prompted investigation into alternate methods of control. In this study, two groups of Holstein-Friesian heifers were observed for their level of horn fly infestation. The pedigrees and microbiomes of the cows with the five highest and five lowest levels of fly infestation were compared. Pedigree analysis showed that some of the heifers with low fly infestation had similar ancestors. Microbiome samples were obtained from three sites and the results are still being analyzed.

## INTRODUCTION

*Haematobia irritans*, also known as horn flies, live their lives on the bodies of cattle, biting and passing diseases to their host. These flies have been shown to lead to increased reproductive failure, as well as decreased meat and milk yields that result in significant economic losses to producers (Birkett, 2004). The adult female lays her eggs exclusively in the feces of cattle. Two days after they reach maturity, new females are able to reproduce. Adult horn flies must feed quickly and when they feed they are voracious, feeding on their host 24-38 times a day (Oyarzún, 2008).

The irritation and disease caused by horn flies have negatively impacted the economy on a worldwide basis. In the United States, the damage done by them has been estimated at one billion dollars per year. It has been estimated that a cow with 200 flies on her will have reduced milk production in the amount of 520mL/day or 4--12% (Oyarzún, 2008; Byford, 1992). In addition to

production losses the skin damage caused by bite marks, fiber separation, scars, black spots, and pits from inflammatory reactions, lower hide quality (Oyarzún, 2008).

In Argentina, a survey greater than 1 million hides indicated that the highest incidence of damage occurred in the summer months. The degree of damage done by the flies affects in descending order, bulls, cows, steers, heifers, and calves. There is a significant, but low correlation (< 0.6) between hide damage and fly infestation level. This suggests that other factors not directly related to levels of infestation are involved, such as individual variations in tissue reaction to bites (Guglielmone, 1999). The horn fly is also known as a vector for the filarial nematode, *Stephanofilaria stiles*, in the USA and Canada (Jubb, 1993). An indirect effect of *H. irritans* on milk production has been proposed, as the fly may serve a role as a vector of the bacterium *Staphylococcus aureus*, a causative agent for mastitis

(Owens, 1998; Gillespie, 1999). Furthermore, the horn fly has been reported to act as a reservoir for eggs of the human bot fly, *Dermatobia hominis* (Leite, 1998).

Most insects are known to navigate their environment using chemical information from various sources, such as plants, animals, and other non-living objects. However, very little information is known about how the horn fly and other cattle fly pests use the chemical nature of olfactory cues to find and select a host (Birkett, 2004). One known fact of cattle flies is that they are attracted to carbon dioxide, which is a component of a mammal's breath (Birkett, 2004).

Volatile semiochemicals are thought to determine how attractive cows are to horn flies. One theory states that some semiochemicals act like repellants or mask the volatile profile that attracts horn flies.<sup>1</sup> These different profiles are based on the physiological status of the host and genetics (Birkett, 2004). Some studies show that low-carrier phenotypes can be passed through heritability (Oyarzún, 2008).

Historically, horn flies have been controlled by insecticides, but resistance to the insecticides are now common and even when the insecticides are effective, the duration of effect has been reduced (Brown, 1992). With increased awareness of resistant populations of bacteria and parasites in combination with the impact of chemicals on food safety, concern is heightened in all areas of food production. The overall objective of this study is to identify differences in cattle that are highly attractive to flies compared with those that have a low attraction to these flies. We hypothesize that there are differences in the chemical make-up of cows that influence fly behavior. Our goal is to compare differences in cattle by examining genetic markers and pedigrees, analyzing microbiomes obtained from the rumen, respiratory system and skin, as well as emitted semiochemicals obtained from vapors above the skin.

## LITERATURE REVIEW

Several studies have been done regarding horn fly attraction to cattle. A study done by investigators in Denmark (Jensen, 2004) tested the fly load on Holstein-Friesian heifers during two field seasons. Both fields were in permanent, low-lying, wind exposed pastures. Each year, two herds of cattle were studied. The herds were kept 500m apart. Three times a day flies were counted on 5

different areas of the heifers. The average number of flies was calculated every day. On day eight of fourteen, after the high carrier and low carrier cows were identified, four of the highest carriers in one herd were switched with four of the lowest carriers from the other herd. The new average was taken and after two days, the cows were switched back, and the average was again taken. In the second experiment, the same procedure was performed with two new herds. Three cows were switched between the herds and the averages were taken. The researchers discovered that when the cows were switched, the average fly count of the herd either rose, with the introduction of the fly attractive cows, or dropped when fly -resistant cows were co-mingled (Jensen, 2004). Interestingly, when the cows were returned to their original herds, the average fly count returned to the original number.

In another set of experiments done in Brazil, a series of three, two-year studies were conducted. The authors discovered that horn flies were not attracted to one breed of cow more than the other, but instead the level of infestation in a herd depends on the individuals in that herd (Bianchin, 2006).

Studies conducted by investigators in Kansas compared the attractiveness of horn flies between Angus bulls and steers (Christensen, 1979). Sections of skin from each animal were taken to determine a difference between sebaceous glands and horn fly counts that were taken daily. The results showed that horn flies were more attracted to the Angus bulls.

All of these investigations arrived at a similar conclusion that horn flies are attracted to individual animals. The objective of this study is to identify if there is a difference in microbiomes of the rumen, respiratory system or skin that attracts or repels horn flies. This information will hopefully lead to a more efficient way to control horn flies.

## METHODOLOGY

Thirty Holstein-Friesian cows and heifers housed at the Veterinary Medical Research Farm were selected for sampling based on horn fly loads that were calculated over two separate time periods from May 27 to July 9, 2014. Each cow was identified by a unique, numeric ear tag. Horn fly populations were monitored once per week during

the morning hours of 0900-1100 by obtaining five separate digital images of each animal taken at a 90 degree angle to the animal in addition to a head-on view for identification purposes. The images were then uploaded and the number of flies per side on each of the heifers was enumerated from each image. These observations and subsequent numerical calculations of flies per side of each animal were then used to determine and identify five animals with consistently high and five animals with consistently low numbers of flies. The number of flies attracted to each animal was then correlated with the animal's pedigree to determine if there was a genetic basis in fly attractiveness. As these data were developed those animals with either high or low attractiveness to flies were identified for further investigation into the mechanism of how this attraction occurs. Samples from various sites were collected from these two groups and will be compared to determine if there is a difference in the composition of their microbiomes. Three different samples were obtained: rumen fluid, deep pharyngeal epithelial swabs, and skin swabs. All of the samples were collected while the cattle were restrained in a squeeze chute for both the animal's and the sampler's safety.

Rumen fluid was obtained by means of rumenocentesis. These samples were obtained via percutaneous aspiration just caudal to the last rib at the level of the stifle. The site of collection was scrubbed three times with chlorhexidine diacetate then wiped clean three times with 70% isopropyl alcohol to cleanse the area. The rumenocentesis was then performed by introducing a 5 inch, 16 gauge needle attached to a 12cc syringe, through the skin, body wall and peritoneum, and into rumen. Once in the rumen, the syringe was held steady while the plunger was slowly pulled back until approximately 3cc of rumen fluid was collected. Once collected the sample was immediately deposited into a 1.8cc cryogenic tube and placed into liquid nitrogen.

The skin microbiome samples were obtained from an approximately 6cm x 6cm square at level of the transverse processes of the vertebrae, on a line directly dorsal to the olecranon on the left side of the cow. This is an area where the caudal aspect of the leg meets the shoulder blade and appears to be a favorite spot for horn flies to congregate based on our photographic evidence. The sample was collected by passing a sterile swab over

the described area approximately 50 times in a back and forth motion. Once the sample was collected the tip of the swab was broken off in a 1.8cc cryogenic tube and placed into the liquid nitrogen.

The respiratory microbiome was obtained from the deep pharyngeal epithelium. To obtain the sample one individual restrained the animal with nose tongs and stretched the head and neck forward to allow for passage of a guarded-tip culture swab. A second individual then passed a guarded-tip culture instrument through the ventral meatus of the nasal cavity to the deep pharyngeal region where the swab was extended past the guard and the area swabbed for 3-5 seconds. The swab was then retracted back into the guard and removed from the nostril. The tip of the swab was then broken off into a 1.8cc cryogenic tube and placed into liquid nitrogen.

Following collection, all samples were then transported back to the laboratory, removed from the liquid nitrogen, and placed into a -80°C freezer until they can be sequenced.

## FINDINGS

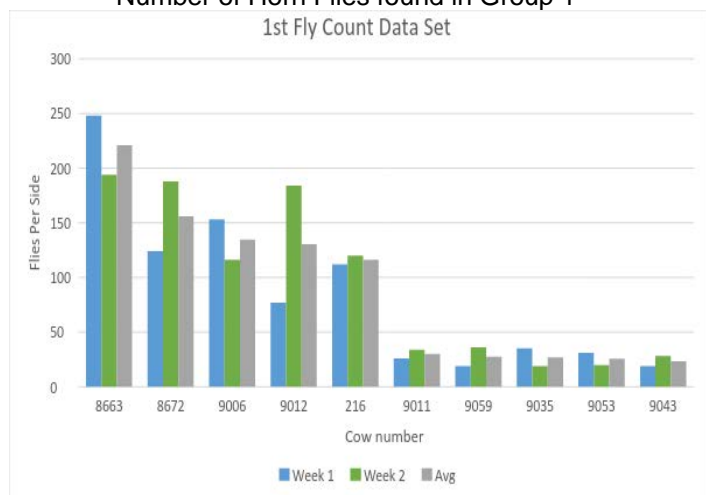
Weekly fly counts per side were obtained on individual animals during the first observation period. The animals shown in Figure 1 consist of the five highest and the five lowest fly count averages during the first observational period.

Weekly fly counts per side were obtained on individual animals during the second observation period. The animals shown in Figure 2 consist of the five highest and the five lowest fly count averages during the second observational period.

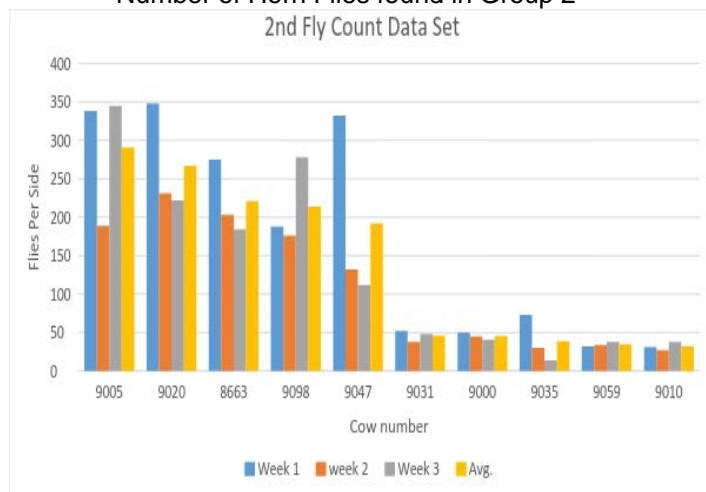
Analysis of pedigrees was performed and animals that had low fly numbers were found to have similar ancestors in the bull Shamrock and/or the bull Regancrest Elton Durham. The animals with high fly burdens did not have a common ancestor as far back as the great great grand sire or dam.

Overall, our findings are in agreement with past literature. The animals in both group 1 and group 2 stayed within their group, either a high or low, for the duration of the study. This shows that it is the individual cow that is attracting the horn flies since the cattle were all kept in the same area during the same time period.

**Figure 1**  
Number of Horn Flies found in Group 1



**Figure 2**  
Number of Horn Flies found in Group 2



The graphs both show there are major differences between the number of flies a high animal carries versus the burden that a low animal carries. In the first group there were 5x more horn flies on the highly affected animals than there were on those with low fly burdens. In group 2, the difference is much higher at 8x more flies on the highly affected individuals.

After studying the pedigrees of the cows, it appears that there is a genetic trend. Those with low fly burdens had similar ancestors in the bull Shamrock and/or the bull Regancrest Elton Durham. The highly affected animals did not have a common ancestor as far back as the great grand sire or dam. This could be a coincidence and definite answers may be possible at a later date as the results from the microbiome samples or DNA data become available.

## CONCLUSIONS

This study confirmed that horn flies are attracted to individual cows and not a herd as a whole. Also, cows with either a low infestation or a high infestation may have a common ancestor and, by that, a genetic connection. Unfortunately, the results of the microbiome samples and the DNA samples could not be accessed at this time. After analysis of the samples, testing ways to alter fly burdens and the effect it has on cattle will be the next step of this study. That can mean something as simple as changing the feed or something as complicated as trying to change the microbiome in the respiratory system.

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