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A Note on the Associations Between the Prevalence of Stable Flies (*Stomoxys calcitrans*) and the Behaviour of Dairy Cows under Semi-Arid Conditions

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Abstract: A study was conducted on the effect of an increasing natural infestation of stable flies (*Stomoxys calcitrans*) and its relation with the behaviour of dairy cows under semi-arid conditions. Twenty-one milking Holstein cows raised in pens in a semi-intensive system were used. The number of stable flies was determined every other day over 62 days by counting the number of flies present on the front legs of the cows every 30 min from 11: 00-14: 00 h. The number of cows lying and standing was also recorded at this time. The following behaviours of the cows were counted during nine 5 min episodes every 15 min during the same observation periods: head movements, ear flicks, skin twitches, stamp/kicks and tail movements. The maximum number of flies observed per cow per day was 55. The number of cows standing or showing fly dislodging behaviours was at maximum frequency when there were between 25 and 30 flies per cow, respectively. Up to this number of flies there was a linear relation between the number of flies and the number of cows lying and the number of tail movements. When the fly count rose from 0-15, standing cows increased from 0-62%, respectively. Tail movement was the most frequent behaviour observed in response to fly increment, with a maximum of 150 tail movements per hour of observation, followed in frequency by ear flicks, skin twitches, head shaking and stamp/kicks, with a correlation of $r_s = 0.91$ ($p<0.05$) among these five dislodging behaviours. It is concluded that: *S. calcitrans* induces restless in dairy cows and even though tail twitching may become maximized at certain fly population, it is the most frequent behaviour observed in response to fly population increment.

Key words: Pest avoidance behaviour, stable flies, dairy cattle, *Stomoxys*

INTRODUCTION

The stable fly (*Stomoxys calcitrans*) is an haemotaphagous pest of cattle (Morgan *et al*., 1983). As a result of weight loss and reduction in milk flow, financial losses in the United States due to this species have been estimated at $398.9 million per year (Drummond *et al*., 1981). Bruce and Decker (1958) listed several possible ways that stable flies might cause a loss of milk production in dairy cows. They were: annoyance from pain, blood losses, a possible anaphylactic reaction from fly-derived substances left in the animal, interference with normal eating habits and increased energy utilization by the animal in its effort to remove flies.

Animals may repel or dislodge biting flies by ear twitching, head-tossing, leg stamping, muscle flicking, muscle twitching and tail switching (Schofield and Torr, 2002) and it has been reported that when fly biting intensity is high, fly repelling or dislodging behaviour increases (Harris *et al*., 1987).

For cattle, most of the studies of fly defence strategies have been done using beef cattle kept on pasture (Dougherty *et al*., 1993a, b) or in feedlots (Wieman *et al*., 1992; Thomas *et al*., 1996). Most of those conducted on dairy cows used artificially released fly populations (Miller *et al*., 1973) and no studies have been conducted under semi-arid conditions. Thus, the purpose of this research was to conduct a study of the effect of an increasing natural

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infestation of stable flies (*Stomoxys calcitrans*) on the behaviour of dairy cows under semi-arid conditions.

MATERIALS AND METHODS

The experiment took place on a private farm in Aguascalientes Mexico, from July to August, the period where the highest infestation of *S. calcitrans* (L.) is observed in this region (Cruz-Vázquez *et al*., 2000). The farm was located at 20° 30′ N and 101° 50′ W and is situated at 2,052 m above sea level and classified as a semi-arid climate, with an average rainfall and temperature of 74 mm and 20°C, respectively for the months of July to August.

Twenty-one mature Holstein (*Bos taurus*) cows that were between 20-25 day postpartum were used. All animals were between 3 and 4 years old and in their second or third lactation. Cows were kept in a 50×50 m soil-surfaced pen.

All animals were machine milked twice a day (05:00 and 17:00 h) and a concentrate ration based on maize and alfalfa was offered *ad libitum* four times daily (06:00, 10:00, 16:00 and 21:00 h).

The number of stable flies (*S. calictrans*) was determined every other day during the experimental period (62 days) by counting the number of flies present on the front legs on each of the cows. This methodology has been used in several experiments with *S. calcitrans* (Thomas *et al*., 1989; Lysyk, 1993; Dougherty *et al*., 1993a) and it has been estimated that, as the front legs are the preferred feeding sites for stable flies, the number of flies counted on both front legs represents about 67% of the flies found on the total body surface of the cow (Dougherty *et al*., 1993b). The cows were assessed in an order determined by random number generation. Animals were habituated to the presence of the observer, who counted the number of flies on each cow by walking calmly among the herd. The fly count took place every 30 min from 11:00-14:00 h. During this period, the temperature ranged between 21.1-26.4°C, with a mean of 24.7°C. This time of the day was selected to avoid feeding management disruptions and to favour the activity of the flies (i.e., feeding and mating), since flies tend to be more active between 20 and 38°C (Semakula *et al*., 1989; Gilles *et al*., 2005). In addition, according to Lysyk (1995), the number of flies found on the front legs and its relation to the total number of flies on the cow is not significantly affected by temperature (between 20-30°C), indicating that the number of flies on the front legs is a consistent proportion of the total number of flies on the animal. Using this schedule, the number of flies on each cow was counted a maximum of 217 times during the experimental period (31 days × 7 counts day^{-1}), depending on the number of times a cow was found lying down, as counts were conducted only on standing animals. The number of lying and standing cows were also recorded at this time. When a cow was found lying during the counting process, the recorder waited for her to stand. Cows that were lying during the whole counting period (11:00-14:00 h) were forced to stand at the end of it. The recorder counted the number of flies within 2-5 min after the cow stood.

All cows were identified with large numbers (15×15 cm) painted (oil-based orange paint) on their flanks so they could be observed at a distance. The behaviour of individual cows was recorded during nine 5 min episodes every 15 min during the same observation periods. With this schedule, the behaviours of the cows were assessed 279 times (31 days × 9 times day^{-1}). Behaviours recorded included: head movements, defined as the shaking or throwing of the head towards the legs or flanks, ear movements, including single twitches or continuous rotation of one or both ears, skin twitches, recorded as one event whether it happened in a localized area or was a continuous shiver over the whole flank for several seconds, stamp/kicks of the front or hind legs, which were recorded as one event when cows raised their legs or hit the ground and tail movement, defined as the travel of the tail from its resting position to one side. If the tail re-crossed its resting position, this was recorded as another movement (Dougherty *et al*., 1993a).

An analysis of variance was used to compare fly populations of the forelegs within observation periods and among experimental days (Gill, 1978). The Spearman rank-order correlation coefficient (Thorndike and Dinnel, 2001) was used to estimate the degree of association between each of the behaviours recorded. The sum of the behaviours recorded during the nine 5 min daily observation episodes was transformed to behaviours per h by a proportion relation. Regression methodology (Gill, 1978) was used to establish linear functions (quadratic) between the prevalence of *Stomoxys* and the number of cows lying or standing and between the number of flies and tail movements (the most frequent behavioural event observed).

RESULTS

The average numbers of stable flies recorded on the front legs of all cows for each observation period varied from 0-55 flies cow^{-1}, with a mean (± SE) of

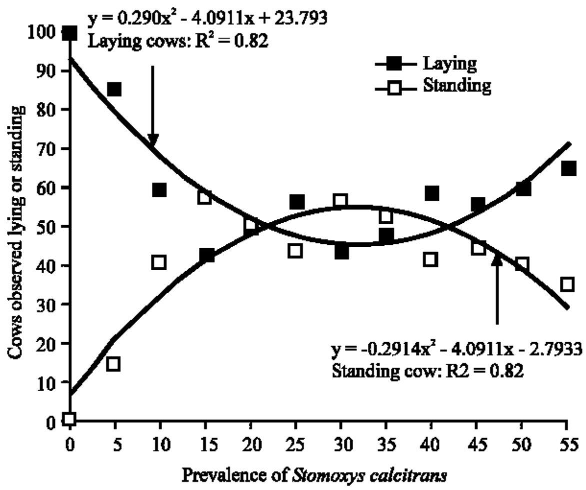

Fig. 1: Relationship between the number of stable flies present on the front legs of the cows and the percentage of cows standing or lying. Lines are regression equations corresponding to each behavioural state

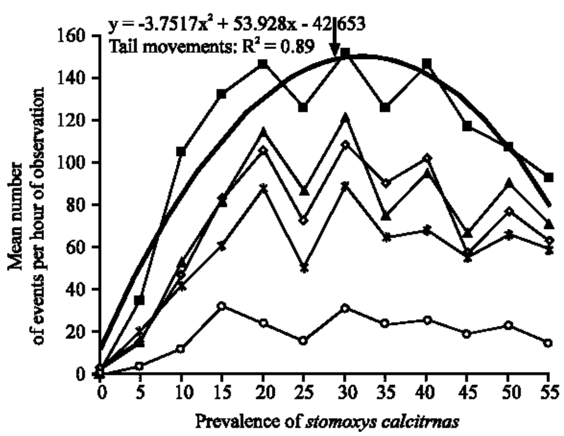

Fig. 2: Relationship between the number of stable flies present on the front legs of the cows and the average number of dislodging behaviours displayed. Regression equation solved for tail movements

11.39±0.95. No trend was observed during the observation periods. However, the analysis indicated significant ($p<0.05$) increases in alighted stable fly populations on the forelegs as the experiment progressed.

The minimum number of cows lying and the maximum number of cows standing occurred when the number of flies counted on the front legs corresponded to around 30 flies per cow. With this prevalence, almost 60% of the herd was standing while all cows were found lying when fly count was near zero. The correlations between the number of flies and the number of cows standing or lying were 0.72 ($p<0.05$) and 0.80 ($p<0.05$), respectively. The regression equation (quadratic) for cows standing and lying had an $R^2 = 0.82$ (Fig. 1).

Fly dislodging behaviours showed an initial peak when the number of flies counted on the front legs of each cow was about 20. Up to this prevalence, there was a quadratic relation (bell shape) between the number of cows standing, fly dislodging behaviours frequencies and the fly population (Fig. 2).

All fly dislodging behaviours followed a similar pattern in response to the increase in fly population, with an $r_s = 0.91$ ($p<0.05$) among them. Tail movements was the most frequent event observed, with a maximum of 150 tail movements per hour shown, followed by, ear flicks, skin twitches, head shaking and stamps/kicks. The regression equation (quadratic) for tail movements had an $R^2 = 0.89$ (Fig. 2).

DISCUSSION

The average load of flies counted on the front legs of the cows during the present study is larger than that reported by Mullens *et al.* (2006). They found that fly numbers varied, peaking at 3.0-3.5 flies per leg of dairy cows in California.

Dougherty *et al.* (1993b, 1994) and Skoda *et al.* (1991) studying beef cattle and Todd (1964) studying grazing dairy cows, found a maximum of 40-50 flies per leg (80-100 on both legs), whereas in the current study the maximum number of flies counted on both front legs per animal was 55, suggesting that perhaps environmental conditions in the present experiment, like low rainfall and/or confinement-type operations, could have limited *Stomoxys* prevalence (Mullens and Peterson, 2005).

The lack of a trend in the number of flies counted in the present study within the observation periods could be due to the consistently high environmental temperature during the 3 h periods in which the numbers of flies were determined. However, the increases in stable fly population on the forelegs as the experiment progressed could indicate a daily fluctuation in *S. calcitrans* population, even though the entire experiment took place during the high fly incidence season. Furthermore, this variation might affect, at least in part, the number of flies recorded daily on each cow.

In the present study the maximum number of cows standing occurred when the number of flies counted on the front legs corresponded to around 30 flies per cow. This finding is consistent with the fact that more dislodging behaviours were observed at this fly population and strengthened the hypothesis that these behaviours were displayed to reduce the effects of blood suckling feeding habits of *S. calcitrans*. In addition, Cockram (1991) found that stressed herds spend more time walking and standing than groups of animals in more stable conditions.

Dougherty *et al.* (1993b) found that cattle grazing in enclosures in the absence of stable flies did not stamp their feet, throw their head towards their legs, twitch their ears or skin, or move their tails, while Schofield and Torr (2002) found that the frequency of all these defensive actions and the density of *Stomoxys* were positively correlated. Furthermore, Torr and Mangwiro (2000) found that more biting flies elicit more leg movements. In present experiment, increases from 1-89 and 0-146 movements h^{-1} of observation for head and tail movements, respectively, were observed when the number of flies rose from 0-20. Similarly, Dougherty *et al.* (1993 a) found that, releases of up to 250 stable flies every 15 min resulted in linear increases in tail movements and hind leg movements in beef cattle of up to 57.5 and 1.7 events min^{-1}, respectively.

The fact that tail movement was the most frequent event observed in response to fly increment is in accord with Mullens *et al.* (2006) and could be because more violent responses, such as lifting, stamping and kicking of legs, head throws and fleeing, are probably caused by biting trauma, whereas movements of the tail, ears or skin may be caused not only by biting but by minor irritation due to alighted flies. Another reason could be related to energy costs. It can be speculated that tail movements and skin twitches demand less energy from the cow than more violent movements. Similarly, Mullens *et al.* (2006) also established that behavioural responses to flies could be divided into less frequent and more energy-intensive acts (head throws and stamp kicks) or more frequent and less energy-intensive acts (tail twitches). However, in response to a high population of flies, behavioural and physiological mechanisms need to be activated, with a consequent energy cost (Byford *et al.*, 1992). According to Schwinghammer *et al.* (1986) these physiological mechanisms may include increase in heart and respiration rates, urine production, urinary output of nitrogen and blood cortisol concentration.

Weiman *et al.* (1992) suggested that, in beef cattle, the direct effects of biting flies and the energy loss involved in dislodging flies may account for a 28.5% of reduced weight gain in 320-363 kg beef cattle over a 21 day period.

Torr and Mangwiro (2000) concluded that the frequency of skin movements had the greatest effect on dislodging *Stomoxys*, while tail movements had the least. However, Ladewig and Matthews (1992) reported that docking of tails of New Zealand dairy calves doubled the number of alighted flies from 4-8 per side.

Tail movements occur mainly when stable flies are on the legs (Dougherty *et al.*, 1993b) while skin movement on lower front or hind legs is rare (Oliveras *et al.*, 1989). Furthermore, the front legs are apparently the preferred feeding sites for stable flies, as about 60% of the *Stomoxys* flies on the total body surface of the cow are found there (Dougherty *et al.*, 1993a), perhaps because tail movement is less likely to interrupt their feeding and because of the physiological characteristics of this area including thinner hair coat and skin and capillaries closer to the surface than on most of the rest of the body surface (Bishopp, 1913).

Todd (1964) found that tail movements were the only index of irritability when there were fewer than 15 alighting stable flies per cow. In the present experiment, dislodging behaviours increased as the fly population increased, displaying a first peak when about 20 flies per cow were counted on the front legs. Todd (1964) also found no increase in rate of tail movements above this level of infestation, which accords with our results. These findings suggest that the maximum frequencies of dislodging behaviours may be reached at this point. In addition, Mullens *et al.* (2006) found that these behaviours tended to peak at about 2 head throws, 3 leg stamps, 8 tail flicks, or 16 skin twitches per 2 min interval. Similarly, Douherty *et al.* (1994) found that the fly reactions of cows involving heads, ears, forelegs, hind legs and tails increased linearly with the number of flies released in screened enclosures. However, skin twitches increased with the number of stable flies released, but as time progressed within grazing bouts the response changed, suggesting that this behaviour may reach maximal levels with the increasing number of flies.

The decline in such reactions to the presence of flies or to the injury inflicted by biting flies declines at certain fly population levels might be due to muscular fatigue or heat stress related to fly-induced muscle activity (Dougherty *et al.*, 1994). Muscular fatigue depends on the duration and intensity of the work (Worth, 1985) and chronic fatigue can develop due to stress or anxiety (Morehouse and Miller, 1976).

Another possible explanation for the occurrence of this asymptote is habituation. This is one of the simplest forms of learning and leads to an animal not reacting to cues that initially triggered a behaviour pattern (Alcock, 1989). It can refer to the loss of instinctive responses and/or cessation of learned behaviours (Craig, 1981). The study of Mullens *et al.* (2006) suggests habituation to pain associated with fly biting over the 12 weeks of their study. Furthermore, Dougherty *et al.* (1993b) observed decreased rates of behaviours over 1 h periods that might have involved habituation (Mullens *et al.*, 2006). In present study, no signs of habituation were observed during the observation periods, nor as the experiment progressed, but perhaps this process could happen in those individuals that, as the morning progressed, accumulated more than 20 flies on their front legs (asymptotic point).

Using a mouse model, *Stomoxys* biting has been investigated from both a learned behaviour (habituation and induced (biochemical) analgesia perspective (Colwell and Kavaliers, 1992; Kavaliers *et al.*, 2003). Similar factors could be operating in cattle (Colwell *et al.*, 1997). Responses involving habituation or analgesic factors might further underscore the major impact that stable flies can have on the behaviour and physiology of the target animal (Kavaliers *et al.*, 1998).

Studies in the field have shown that different individual cattle, at the same time and place, vary considerably in the number of stable flies that are found on them (Mullens and Meyer, 1987). Torr and Mangwiro (2000) found a correlation of -0.89 between the mean feeding success of a haematophagous fly on a particular cow and that cow's mean rate of leg movements, suggesting that those cows that display fewer dislodging behaviours could be those with larger loads of flies. Alternatively, as the level of fly-repellent behaviour becomes maximized, this allows more flies to complete their meals. Unfortunately, the present data collection methods did not allow us to discriminate between these two possibilities.

More evidence is needed to support these hypothesis and it is probable that other factors might also be involved. For example, it is unknown if the skin in different body areas varies in its sensitivity to alighted flies and/or bite trauma, or if the skin receptors become desensitized by biting injury. In present experiment only the number of flies was examined. It would be of interest also to determine how the duration of fly exposure and the intensity and pattern of exposure impact the behaviours displayed.

CONCLUSION

It is concluded that *S. calcitrans* induce restless in dairy cows and even though tail twitching may become maximal at certain fly population, it is the most frequent behaviour observed in response to fly population increment.

CONCLUSION

The percentage of animals standing and the number of tail twitches are signs that can be monitored more easily than counting the flies on the cows to determine when treatment might be required. This management technique can not only improve animal welfare but also may benefit milk producers economically by restricting insecticide applications to times when fly-prevalence actually induces discomfort in the cows.

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