Project Title: Evaluating the Role of Horn Flies in the Transdermal Transmission of *Salmonella* to Cattle

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Background

Salmonella sequestered in bovine peripheral lymph nodes is proposed to occur via a transdermal route of entry, and may be a source of ground beef contamination. Several important characteristics of the horn fly, *Haematobia irritans*, lend itself to being a possible source for transdermal introduction of the bacteria to cattle. Horn fly adults are obligate blood-feeders that spend their entire adult stage somewhat permanently associated with their bovine host. Egg carrying females leave only intermittently to deposit eggs in freshly laid manure pats, and they can acquire *Salmonella* while probing the microbe-rich manure pat to assess its suitability for egg deposition. Cattle hides, often carrying *Salmonella*, are additional sources for exposure for horn flies to bacterial pathogens. Researchers have demonstrated that horn flies can acquire *Salmonella* upon contact with the bacteria, and a 15 minute period of grooming after contact with *Salmonella* can result in bacteria present on the fly mouthparts, as well as ingested and present within their digestive tract. It is conceivable that microbes present on the fly mouthparts and on the animals hide can then be inoculated into the host as the horn fly creates a feeding lesion, but this remains to be demonstrated. The purpose of the current study is to determine whether transdermal transmission of the bacteria by horn flies to cattle can indeed occur, resulting in cattle peripheral lymph node (PLN) contamination.

The objectives of this study were to answer the following questions: Are cattle PLN contaminated with *Salmonella* after infestation with *Salmonella*-colonized horn flies and are cattle PLN contaminated with *Salmonella* after horn fly infestation on animals with hides that have been artificially contaminated with a *Salmonella* isolate?

Methodology

The proposed objectives were addressed in a series of three experiments utilizing *Salmonella* Anatum (serogroup E1) and Salmonella Senftenberg (serogroup E4). Experiments 1 and 2 used Anatum and Senftenberg, respectively. These experiments were designed to 1) assess acquisition of each challenge strain by newly emerged horn flies from artificially contaminated hides and subsequent transmission to bovine peripheral lymph nodes (PLN) upon fly feeding, and 2) evaluate whether deposition of the challenge strain on hides via excretion in fly feces would result in transmission to PLN upon fly feeding. Six Holstein steers were utilized in each of these experiments, five of which had fly cages adhered to their shoulder area. Two steers were

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infested with either 400 (Animal 1) or 800 (Animal 2) horn flies that consumed blood-meal spiked with the challenge strain, while the remaining three steers had fly cages affixed over regions of hair that were artificially contaminated with autoclaved, conspecific manure that had been inoculated with the challenge strain. These three were infested with 0, 400 or 800 unfed, adult horn flies. Flies could feed on steers for 5 days, after which the flies were anesthetized with carbon dioxide, collected in separate tubes by cage and stored at -20 °C until processing. Hide swabs and cage swabs were obtained upon termination of the experiment, and each were cultured for the challenge strain. All steers were euthanized, and the left and right popliteal, scapular, femoral and mesenteric nodes were collected and cultured for the challenge strain.

Experiment 3 was designed to evaluate whether prolonged fly feeding on regions where *Salmonella*-fed flies deposited fecal excreta would promote transdermal inoculation of the challenge strain, *Salmonella* Senftenberg. The experiment was conducted over the course of 19 days and comprised three groups of Angus steers (N = 3 per group). Within each group, one steer was a control, inoculated with the challenge strain using the 10-microlancet apparatus while the other two steers each had fly cages adhered at either the shoulder or the rump. Group 1 steers with fly cages were infested with 800 horn flies fed a *Salmonella*-spiked blood-meal. On days 5 and 12 after infestation, flies in the cages were anesthetized with CO_2, aspirated from the cages and replenished with newly emerged, unfed horn flies. The infestation was terminated on day 19. Group 2 steers were infested in the same manner, and flies were replenished on day 5 after infestation; the infestation was terminated on day 11. Group 3 steers were infested similarly, and the infestation was terminated on day 5 without fly replenishment. All flies collected were stored at -20°C until processing. Hide swabs and cage swabs were obtained upon termination of the experiment, and each were cultured for the challenge strain. All steers were euthanized on the same day, and the left and right popliteal, scapular, femoral and mesenteric lymph nodes were collected and cultured for the challenge strain.

Findings

Anatum was recovered from scapular nodes of five of the six animals in Experiment 1, one of which was the negative control (Animal 3, 2 cages, 0 flies). This indicated that the study animals may have had pre-existing node contamination with Anatum, which is possible given the high prevalence of Anatum reported in U.S. feedlots and dairies. This complicated the interpretation of the positive node results. No Senftenberg was isolated from any of the nodes in Experiment 2, including the positive control. This challenge strain has been used successfully in prior animal inoculation studies, and it is unclear what may have resulted in no recovery from the positive control nodes. A high frequency of surface contaminated horn flies and a consistent recovery of the challenge strains from cage and hide swabs was seen in Experiments 1 and 2, and this led researchers to consider whether an extended duration of fly feeding would increase probability of transdermal inoculation of the bacteria (Experiment 3).

Senftenberg was selected for Experiment 3 because it is a serotype rarely isolated from field-collected nodes, enabling it to be distinguished from more prevalent strains. Interestingly, longer durations of fly infestation appeared to be associated with an increased prevalence of positive nodes (Table 1). Animals infested in the shoulder area consistently had more positive nodes than those infested in the rump area, although we cannot rule out that this may be a result of minor challenges with fly cages adhered in this area. These data suggest that a prolonged duration of fly feeding on a hide contaminated by bacteria via fly fecal excreta provides enough of a transdermal inoculum to result in transmission to bovine PLN.

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Given the horn fly feeds almost hourly, the frequency of transdermal inoculation of bacteria on the host's hide while taking a meal is increased relative to biting insects that may feed less regularly, i.e. stable flies. The prolonged feeding approach used in Experiment 3 was an attempt to more accurately reflect the extent of horn fly exposure/feeding during the peak of fly season, and this addition to the experimental design was critical. Horn flies are typically located in the shoulder, rump and under belly of cattle, all of which are regions drained by PLN, and the placement of fly cages in the experiments was intended to mimic the fly's biology.

Implications

Horn flies are a pest of pastured cattle. Dairy operations that cycle their cattle on pastures may create an opportunity for increased exposure to horn flies during peak fly season, and other breeds of pastured beef cattle run the same risk prior to market sale. The current study demonstrated that biting insects have a role in the transdermal transmission of *Salmonella* to bovine PLN and supports the need for implementation of fly control programs in livestock production systems. Future research will include identifying the threshold quantity of deposited bacteria needed to promote the transdermal transmission in the presence of horn flies and the level of fly infestation that results in transmission, as this will reflect on the economic impact of this livestock pest to cattle producers.

Table 1. *Salmonella* recovery from peripheral lymph nodes of fly-infested steers within Experiment 3. A reported value indicates a *Salmonella* positive node and represents the concentration recovered (log_{10} CFU/g lymph node).

	Group 1 (19 d exposure)			Group 2 (11 d exposure)			Group 3 (5 d exposure)		
Lymph	Rump	Shoulder	Microlancet	Rump	Shoulder	Microlancet	Rump	Shoulder	Microlancet
Popliteal									
Right	-	-	-	-	-	-	-	-	-
Left	-	0.1	-	-	-	-	-	-	-
Scapular									
Right	-	0.8	0.8	-	0.4	0.1	-	-	-
Left	-	1.8	0.9	0.1	0.1	0.1	-	0.1	-
Femoral									
Right	0.5	0.8a	0.8	0.6	1.3	0.6b	-	0.6a	-
Left	-	-	0.9	0.4	0.5	-	-	-	0.6
Mesentric	-	-	-	-	-	-	-	-	-

a: Three presumptive isolates were serogrouped, and all were serogroup B.
b: Three presumptive isolates were serogrouped. 1/3 was B and 2/3 were E

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