Project Title: Examination of the Potential Transmission of *Salmonella* from House Flies to the Peripheral Lymph Nodes of Cattle via the Mucous Membranes

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Background

Recent research suggests that the peripheral lymph nodes (PLN) in cattle may be a contributor to the contamination of ground beef with *Salmonella*. The source of *Salmonella* in the PLN has not been clearly defined. The gastrointestinal tract (GIT) in cattle is often asymptomatically colonized by *Salmonella*, and *Salmonella* has been reported in the mesenteric lymph nodes of seemingly healthy cattle, therefore conventional wisdom supports systemic distribution via an oral route from the GIT to the mesenteric lymph nodes, and subsequently to the PLN. Research utilizing an experimental model of infection, demonstrated *Salmonella* recovery from the PLN in cattle following oral *Salmonella* administration. However, significant challenge dose(s) were required, suggesting other routes of infection are involved.

One such route may involve the mucous membranes. Some fly species, such as the house fly (*Musca domestica*) are common pests on cattle production facilities, and often frequent the nose and eyes of cattle. These flies lay their eggs and develop in rotting and decomposing substrates, including manure, and may acquire bacteria internally and externally. Mechanical transmission of bacteria has been reported for house flies. Bacteria replication occurs within house fly mouthparts and these bacteria may be stored in the crop (facilitating recovery in the vomitus), or distributed throughout the GIT and subsequently shed in the feces. Taken together, the ability of house flies to transfer viable bacteria in their vomitus, feces, or via direct contact (external contamination) suggest the potential role of flies and the mucous membranes in dissemination of *Salmonella* to the PLN.

The objective of this study was to determine if *Salmonella* will reach the PLN of cattle following application of *Salmonella* to the mucous membranes. Initial proof-of-concept studies will apply *Salmonella* directly to the mucous membranes of the nose. If successful, follow-up studies will utilize externally-contaminated flies as the transmission vehicle to the membranes.

Methodology

Holstein steers (~BW = 100 kg) were housed in individual indoor pens (one steer per pen). For treatment administration, cattle were haltered and tied securely to restrict movement within the pen. A foam tipped swab was used to administer *Salmonella* Cerro (log phase; avg. concentration 12.1 x 10^8 CFU/ml) to the inside of each nostril of three steers designated for the treatment. Two control steers were treated similarly with trypitic soy broth only. Treatment administration was done in the afternoon on three (study 1) and

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four (study 2) successive days. Fecal samples were collected daily, and on day 5 all steers were euthanized, necropsied and peripheral and mesenteric lymph nodes were collected for bacterial culture.

A third experiment was completed. In study 3, Holstein steers (~BW = 100 kg) were housed in individual indoor pens (one steer per pen). For treatment administration, cattle were haltered and tied securely to restrict movement within the pen. A foam tipped swab was used to administer *Salmonella* Cerro (log phase; avg. concentration 8.5 x 10^8 CFU/ml) to the inside nostril of each of the three steers designated for the treatment group. The two control steers were treated similarly with trypitic soy broth only. In study 1 and 2, the steers were observed to lick their nostrils after halter removal, but typically did not exhibit this behavior when the halter remained on. Therefore, all steers remained haltered for 30 minutes following treatment. Treatements were administered in the afternoon on each of eight consecutive days. Fecal samples were collected on four occasions the week prior to *Salmonella* inoculation and daily during the 8 day treatment period. The day following the last treatment administration, all steers were euthanized and necropsied. The right and left sub-iliac, popliteal, superficial cervical, parotic, retropharangeal, mandibular lymph nodes and mesenteric lymph node were collected for bacterial culture.

Findings

The inoculated strain of *Salmonella* Cerro, as indicated by serogrouping of the isolates, was not recovered from any of the peripheral lymph nodes in study 1 or study 2. All isolates from one *Salmonella*-treated animal (Study 2) recovered following quantitative and qualitative culture of the mesenteric lymph node, were identified as serogroup K, indicative that it was the challenge strain Cerro. The majority of lymph node isolates belonged to serogroups C_1 (69.9%) and C_2 (26.5%) with one isolate identified as serogroup E_1. In study 3, the increase in number of inoculation events resulted in the successful recovery of the challenge strain, *Salmonella* Cerro (serogroup K) in the lymph nodes. Only a few PLN contained quantifiable concentrations of *Salmonella*, while many (44%) were positive following enrichment for the challenge strain. One of the *Salmonella*-treated steers had only one PLN culture positive for serogroup K. The mesenteric lymph node contained quantifiable concentrations of the challenge strain in all three treated steers. Lymph nodes from the head (parotid, retropharangeal and mandibular) were mostly positive for the challenge strain of *Salmonella*, in the exception of the parotid, which was positive only in one steer. Of the two steers that served as unchallenged controls in study 3, one steer had PLN test positive for *Salmonella* in the sub-iliac nodes. The right node tested positive for serogroups K and the left node tested positive for serogroup C_2.

Most steers in study 1 were shedding *Salmonella* in their feces daily. However, *Salmonella* was detected at very low levels and detected only following enrichment of the samples. Steers inoculated with *Salmonella* were all culture negative the first day, but started shedding primarily the challenge strain (serogroup K) on the second and third days, and continued to shed serogroup K throughout the experimental period. Fecal shedding results from study 2 were similar. The results of fecal samples in study 3 showed that all inoculated steers shed the challenge strain in their feces on multiple occasions throughout the experiment, with two of the three steers shedding serogroup K 24 hours post-inoculation. In study 3, the unchallenged controls also tested positive for *Salmonella* in the feces, with the identified serotypes being C_1 and C_2 with one steer shedding serotype K on days 6 and 7.

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It is unclear what route(s) were used that resulted in *Salmonella* ultimately reaching the PLN. It would appear that the mucous membranes functioned as expected and the challenge strain of *Salmonella* was captured in the mucous, swallowed and deposited into the gastrointestinal tract, and subsequently shed in the feces. It has been suggested that *Salmonella* in the PLN of cattle may originate from the gastrointestinal tract and transport to other PLN is facilitated by the mesenteric lymph nodes.

Seeking other viable explanations, the lymph nodes of the head were examined and the challenge strain of *Salmonella* was recovered by the majority (78%) of them. All three nodes examined receive afferent vessels from nasal cavity and muzzle, therefore, it was not surprising that the challenge strain was recovered in these nodes. It is hypothesized that the added inoculations in study 3 may have exceeded the ability of these nodes in the head to effectively handle all of the *Salmonella*, thus resulting in its culture from the PLN. Detection of the challenge strain of *Salmonella* in the control animals further confounds interpretation of the results. Cross-contamination, exposure through aerosolized *Salmonella*, or any number of other routes of infection may be responsible for these results.

Implications

Results of the third experiment, in which the number of inoculation events was doubled and resulted in uptake of the challenge strain of *Salmonella* by the PLN following exposure the mucous membranes suggests that a threshold was met and exceeded that allowed for the acquisition of *Salmonella* by the PLN. However, a threshold is hard to explain when looking at the ease in which the control animal became contaminated with the challenge strain in this experiment. It appears from this research and other projects conducted, that the PLN rapidly and easily acquire *Salmonella* when exposure is "natural" versus laboratory strains inoculated directly into the animal.

Results of this research demonstrated uptake of *Salmonella* by the PLN of cattle following application of *Salmonella* to the mucous membranes of the nose. Therefore, while it is feasible that filth flies may serve as a vector for *Salmonella* transmission to the PLN via contaminating mucous membranes, the dose required in this research would suggest that a substantial number of fly-related inoculation events would be required. Unless, as supported by this and other research, "natural" *Salmonella* strains are easily acquired and far more invasive than laboratory strains. The route in which *Salmonella* is transported to the PLN remains somewhat of a mystery.

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Table 1. *Salmonella* concentration [CFU (log_{10})/g lymph node], prevalence (% positive) and serogroup distribution following quantitative (direct) and qualitative (enriched) culture of peripheral and mesenteric lymph nodes (Study 2). Three isolates, when available, from each sample were serogrouped. Steers 98 and 167 served as controls; steers 99, 164, and 169 were inoculated with *Salmonella* Cerro.

	Culture Method			
	Direct		Enriched	
Steer	avg. conc.	SG	% positive	SG
98	1.03	3 C_2	57	6 C_1, 6 C_2
167	1.83	3 C_2	42.9	8 C_1, 1 E_1
99	1.1	3 C_1	14.3	3 C_1
164	2.1	3 K*	14.3	3 K*
169	1.2	3 C_1	28.6	6 C_1

*All isolates confirmed as serogroup K were cultured from the mesenteric lymph node.

Table 2. *Salmonella* recovery (concentration, prevalence [positive {POS}or negative {N}], and isolate serogroup [3 isolates from each positive sample examined]) following quantitative (direct) or qualitative (enriched) culture of lymph nodes (right and left) in control steers and steers inoculated with *Salmonella* Cerro (serogroup K) on the mucous membranes of the nose for 8 successive days.

	Controls			Treated					
	94	157		93		159		163	
Lymph node[a]	Enriched	Direct	Enriched	Direct	Enriched	Direct	Enriched	Direct	Enriched
Popliteal - R	N	N	N	N	N	N	POS; 3/3 K	N	N
Popliteal - L	N	N	N	N	POS; 3/3 K	N	POS; 3/3 K	N	N
Sup. Cerv. - R	N	N	N	0.5; 1/1 K	POS; 3/3 C_1	N	POS; 3/3 K	N	N
Sup. Cerv. - L	N	N	N	N	N	N	N	N	N
Sub-iliac - R	N	N	POS; 3/3 K	1.2; 1 K, 1 C_2	POS; 2 C_2, 1 K	N	N	N	N
Sub-iliac - L	N	0.7; 1/1 C_2	POS; 3/3 C_2	N	POS; 3/3 K	0.9; 2/2 K	POS; 3/3 K	N	POS; 3/3 K
Mesenteric	N	N	N	2.7; 6/6 K	POS; 3/3 K	2.5; 6/6 K	POS; 3/3 K	2.4; 6/6 K	POS; 3/3 K
Parotid - R	N	N	N	N	N	N	POS: 3/3 K	N	N
Parotid - L	N	N	N	N	N	N	POS; 3/3 K	N	N
Retrophar. - R	N	N	N	2.0; 6/6 K	POS; 2 K, 1 C_1	0.4; 1/1 K	POS; 3/3 K	N	POS; 3/3 K
Retrophar. - L	N	N	N	2.2; 3/3 K	POS; 3/3 K	N	POS; 3/3 K	N	POS; 3/3 K
Mandib. - R	N	N	N	1.9; 6/6 K	POS; 3/3 K	N	POS; 3/3 K	1.1; 2/2 K	POS; 3/3 K
Mandib. - L	N	N	N	1.0; 1/1 K	POS; 3/3 K	1.1; 2/2 K	POS; 3/3 K	0.7; 1/1 K	POS; 3/3 K

[a]Retrophar. = retropharangeal; Mandib. = mandibular

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