PROJECT SUMMARY
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Prevalence of Filarial Nematodes in Cattle Production Systems: A Role in Transdermal *Salmonella* Transmission?

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Background

Salmonella sequestered in bovine peripheral lymph nodes may be a major source of ground beef contamination (Arthur et al. 2008, Gragg et al. 2013), and this infection is proposed to occur via transdermal introduction of the bacteria that is then shuttled to the nodes by lymphatic drainage (Edrington et al. 2013). Studies funded by the Beef Checkoff highlighted a role for horn flies as important reservoirs for, and mechanical vectors of, *Salmonella* (Olafson et al. 2014, Olafson et al. 2016). Interestingly, horn flies are an intermediate host of *Stephanofilaria stilesi*, a skin-dwelling, filarial nematode that produces lesions on cattle resulting in dermatitis (bovine stephanofilariasis). Nematodes can acquire bacterial pathogens from their environments, including *Salmonella*, and these pathogens can persist and be transmitted to subsequent nematode generations (Kenney et al. 2005, Lacharme-Lora et al. 2009, Diaz and Restif 2014); acquisition of *Salmonella* by skin-dwelling filarial nematodes via the hide or the intermediate arthropod host may provide an additional source of transdermally introduced pathogen. Several other fly species are also known to vector arbonematodes that impact livestock, including *Musca autumnalis* (face fly), *Musca domestica* (house fly), and *Stomoxys calcitrans* (stable fly). These non-biting and biting dipteran species are frequently encountered on conventional and pasture settings, they exhibit seasonal variation in population density, and they have varied geographic distributions within the US. Collectively, these may account for the seasonal and regional differences observed in PLN prevalence. The frequency and diversity of skin-dwelling filarial nematodes on cattle that inhabit conventional and pasture settings is not clearly documented in the US, nor is the prevalence of filariae harbored by flies parasitizing these cattle and/or inhabiting these settings. Filling these gaps is critical to assessing an impact on *Salmonella* transmission due to interactions between the bovine host and these macroparasites.

Objectives

The objective of the study was to screen for the presence of skin-dwelling filarial nematode species from cattle hides and from fly pests/ectoparasites inhabiting cattle production settings.

Methods

Horn, stable, and house fly populations were collected on conventional and pasture settings from seven states (n=1,177) by either aerial sweep net or sticky traps (Fig. 1). Genomic DNA was isolated from individual flies by maceration in a DNA Isolation buffer. These DNAs were used as template to detect nematode DNA using pan filarial primer pairs in PCR assays targeting nuclear rDNA. Reaction conditions consisted of optimized buffer (Advantage® HF2 PCR Buffer, Takara, Mountain View CA) for use with the Advantage® High Fidelity 2 Polymerase (Takara) system. Positive control genomic DNA templates from *Dirofilaria immitis*, *Brugia malayi*, and *B. pahangi* (BEI Resources, Manassas VA) and 'no template' controls were included in all experiments. Amplified products were purified and sequenced in both directions, and resulting sequence data was compared to publicly available nematode sequences deposited in the Genbank database.

Findings

Less than 1% (n = 6) of the horn flies screened were positive for nematode DNA using the nuclear rDNA assay (Table 1), and no products were amplified from house flies or stable flies. These indicate a low overall prevalence of nematode infection in filth fly populations sampled in March – July. Biologically, stable flies and house flies do not associate with cattle for as long a period of time as horn flies, and this may account for the difference in detection of filarial nematode DNA between the species. Two of the six sequenced nuclear rDNA products displayed similarity to

publicly available filarial nematode sequences, while four were similar to non-filarial species. Despite this, all six isolates remain 'unidentified' as they do not have high enough levels of sequence coverage to assign concrete identifications. This is, in part, due to a majority of the publicly available filarial nematode sequences being from the *Onchocercidae*, as they are associated with human diseases. Indeed, there are only a handful of sequences deposited for *Filariidae* nematodes, with no sequences deposited for *S. stilesi*, the nematode known to be vectored by horn flies.

Industry Impact

These data support molecular monitoring of filth fly populations at livestock settings as an indicator for the prevalence of nematodes within cattle herds; however, limited nematode sequences in publicly available databases can preclude identification. These data suggest that future sampling should be extended for the duration of the warmer season (June – October) when there is a reported increase in prevalence of *Salmonella* in peripheral lymph nodes, and it should focus on symbovine species, i.e. horn flies.

Figures/Tables

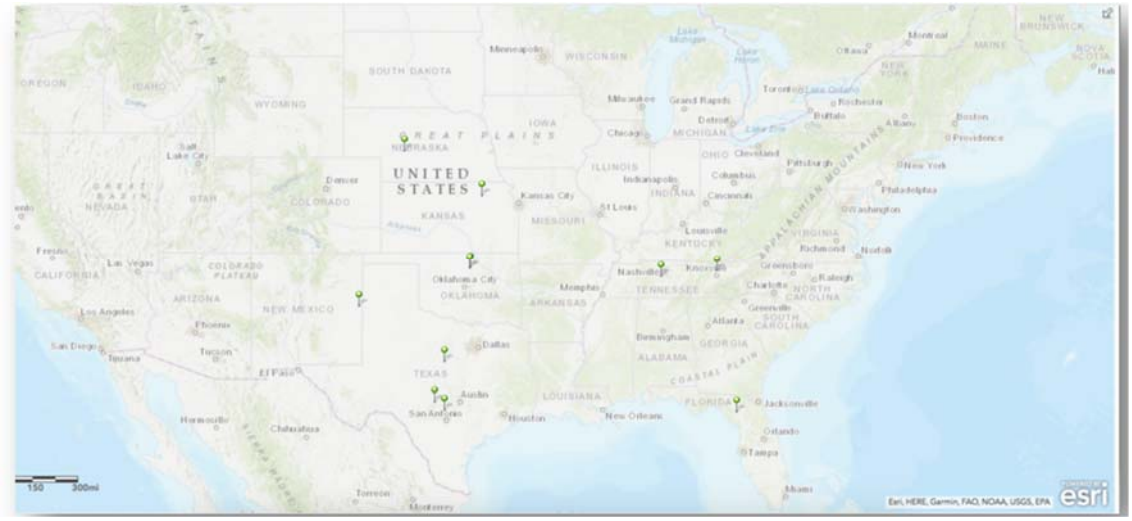

Figure 1. Flies were collected from livestock production settings in seven states within the USA and screened for the presence of filarial nematode DNA. Green pins represent collection sites located within each state.

Figure 2. Representative Angus herd, heavily infested with horn flies (Comanche County, Texas). Flies were collected by sweep-net from numerous animals within the herd.

Table 1. Molecular monitoring for the presence of filarial nematode DNA in filth fly species from seven states within the U.S.

Fly Species	State (County)	Total Flies Screened	PCR Positive
Haematobia irritans (horn fly)	Texas (Bexar)	312	1
	Texas (Kerr)	200	0
	Texas (Comanche)	167	2
	Oklahoma (Payne)	50	3
Stomoxys calcitrans (stable fly)	Florida (Gilchrist)	30	0
	Kansas (Riley)	30	0
	Oklahoma (south Noble)	30	0
	Nebraska (Lincoln)	70	0
	Tennessee (Knox)	88	0
	Tennessee (Marshall)	139	0
Musca domestica (house fly)	New Mexico (Curry)	61	0

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