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The effect of European starlings and ambient air temperature on *Salmonella enterica* contamination within cattle feed bunks

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Abstract: European starlings (*Sturnus vulgaris*) are a known risk factor for the occurrence of microorganisms that are pathogenic to cattle and humans in concentrated animal feeding operations (CAFOs). Starling use of CAFOs is known to vary in response to weather; starling control operations on CAFOs often are timed to coincide with favorable environmental conditions to maximize take. The totality of this information suggests that disease risks in CAFOs associated with starlings may be influenced by environmental factors, such as temperature. In this study, we assessed the risk of *Salmonella enterica* contamination of cattle feed by modeling the interaction between starling numbers and ambient air temperatures using data previously reported from Texas CAFOs. We compared these interaction models to the previously published additive models for *S. enterica* contamination of cattle feed using an information-theoretic approach to model selection that ranked and weighted models in terms of their support by the data, using bias-adjusted Akaike's Information Criterion (AIC_c) and Akaike weights (Wi). Our results indicate that the interaction between European starlings and ambient air temperature better explained the occurrence of *S. enterica* in cattle feed than any of the previously reported models. Specifically, the risk of *S. enterica* contamination of cattle feed by starlings was greatest when winter temperatures were highest (10°C). Thus, we conclude that the risk of *S. enterica* contamination of cattle feed by starlings will be worst on the few winter days when daytime high temperatures are above freezing and large numbers of birds are present. Because these conditions will be most common in the late winter and early spring, we recommend that starling control operations on feedlots and dairies be conducted as early in the winter as possible to mitigate the risks of disease created by large foraging flocks of starlings.

Key words: cattle, European starlings, foodborne pathogens, human–wildlife conflicts, invasive species, peridomestic wildlife, *Salmonella enterica*, wildlife disease, zoonosis

EUROPEAN STARLINGS (*STURNUS VULGARIS*), originally native to Europe, Southwest Asia, and North Africa, were introduced into New York City in 1890 and, by 1942, they had spread across the North American continent (Cabe et al. 1993). With a population estimated at 200 million birds (Feare 1984), starlings are one of the most abundant avian species in North America (Linz et al. 2007). Currently, starlings can be found on every continent except Antarctica (Rollins et al. 2009). Due to their ability to successfully colonize new areas, starlings have become recognized as one of the top 100 "world's worst" invaders by the Invasive Species Specialists Group (Lowe et al. 2004).

Use of concentrated animal feed operations (CAFOs) by starlings varies seasonally, with most damage occurring during winter months when insects and other natural foods are typically unavailable (Besser et al. 1968, Palmer 1976, Dolbeer et al. 1978, Glahn and Otis 1981, Johnson and Glahn 1994). During the winter, use of CAFOs by starlings also varies, and it has been speculated that this may be a function of weather conditions (Feare 1984). Agricultural damage by starlings has been estimated at $800 million dollars per year within the United States (Pimentel et al. 2000, Pimentel et al. 2005). Starling damage occurs as a result of depredation to row crops (e.g., corn), fruit orchards, and winter use of CAFOs (e.g., dairies and feedlots). Within CAFOs, Besser et al. (1968) estimated annual cattle-feed losses of $84 ($526

in 2010 dollars) per 1,000 starlings. Lee (1987) surveyed producers and estimated that Kansas livestock facilities on average absorbed $12,340 dollars ($24,551 in 2010 dollars) in damage during 1986. Feed loss was cited as the greatest source of economic loss, and starlings were identified as the worst offending bird species. Lastly, Depenbusch et al. (2011) estimated that feed consumption by European starlings increases the daily production cost $0.92 per feedlot animal.

Starling damage to CAFOs may not be isolated to feed loss. European starlings have been associated with many human and cattle pathogens, including *Escherichia coli*, *Mycobacterium avium paratuberculosis*, *Chlamydophila psittaci*, *Histoplasma capsulatum*, and *Salmonella enterica* (Johnson and Glahn 1994, Linz, et al. 2007, Gaukler et al. 2009). Starling use of CAFOs was associated with *S. enterica* contamination of cattle feed and water (Carlson et al. 2011*a*). The length of time cattle are exposed to rations in feedlots was associated with fecal shedding of *S. enterica* by cattle. This suggests that contaminated cattle rations contributed to infections in the herd (Fedorka-Cray et al. 1998). Lastly, control of starlings reduced the amount of *S. enterica* contamination found in cattle feed and water (Carlson et al. 2011*b*).

Salmonella enterica are rod-shaped, gram-negative bacteria that are ubiquitous in CAFOs (Maciorowski et al. 2006). It is one of the most economically significant pathogens in livestock production because of the high incidents of livestock morbidity and mortality (USDA 2007) and because it is a source for human salmonellosis, which is responsible for an estimated 1.3 million human cases and 550 human deaths each year (Mead et al. 1999). Cattle typically acquire *S. enterica* from other infected livestock that spread the pathogen throughout the herd via contaminated cattle feces (Wray and Davies 2000), cattle feed (Fedorka-Cray et al. 1998, Maciorowski et al. 2006), and water (Kirk et al. 2002). Lastly, fecal shedding of *S. enterica* by cattle is higher during the summer (Van Donkersgoed et al. 1999, Barkocy-Gallagher et al. 2003, Dargatz et al. 2003, Green et al. 2010) than during winter. This is likely due to the fact that warm summer months provide environmental conditions optimum for survival and amplification of this microorganism.

The overall objective of this study was to determine if *S. enterica* contamination of cattle feed is associated with European starlings and environmental conditions. Specifically, we addressed the research question: is there an interaction of effects between European starlings and ambient air temperature that influences the risk of *S. enterica* contamination of cattle feed in CAFOs during the winter?

Materials and methods

Data used for this analysis were previously published in Carlson et al. (2011*a*). We built on the previous statistical models by assessing the interaction between temperature and starling variables (Table 1). Specifically, we analyzed the interactions of the number of starlings within feed bunks (SB), the number of starlings on CAFOs (SS), and ambient air temperature (T).

Ten CAFOs were randomly selected from 15 facilities identified as acceptable for inclusion in this study. Acceptable CAFOs produced the same final commodity (feeder cattle), had comparable management practices (feeding, watering, cleaning, and housing practices), and were willing to participate in the study. All facilities were located in Moore, Sherman, and Hansford counties, Texas, USA. We sampled CAFOs when starling numbers were greatest: from January 20 to February 19, 2009.

We estimated starling numbers on CAFOs each day before collecting diagnostic samples by systematically driving through CAFOs and counting starlings observed in or flying above pens. We were careful to account for bird movement to eliminate duplication of numbers. Also, the number of starlings observed in feed bunks was estimated when feed samples were collected. This provided estimates of starling numbers at 2 spatial scales: numbers of starlings on CAFOs (facility level) and numbers of starlings in feed bunks within CAFOs (pen level).

Diagnostic samples were collected from CAFOs only when starlings were present. No samples were collected before starlings arrived on facilities, and no samples were collected after starlings returned to roost. All samples were collected between 0930 and 1530 hours, Monday through Thursday. Feed samples were collected approximately 15 minutes after feeding trucks filled bunks, thus, standardizing

Table 1. Cattle feed contamination models, number of estimable model parameters, 2 negative log-likelihoods (-2LogL), bias-corrected Akaike's Information Criterion (AIC_c), and Akaike weights (wi) for all logistic regression models assessed in the analysis of European starlings (*Sturnus vulgaris*) and ambient air temperature on *Salmonella enterica* contamination within cattle feed bunks. Data were collected within 10 concentrated animal feeding operations located in Moore, Sherman, and Hansford counties, Texas, 2009.

Model covariates	Model parameters	-2logL	AIC_c	Akaike weight
Number of starlings in feed bunks (SB)	3	103.08	109.21	0.117
Natural log of starlings on site (LNSS)	3	106.56	112.69	0.004
Number of cattle on site (CS)	3	106.71	112.84	0.003
Air temperature (T)	3	106.71	112.84	0.003
Intercept-only model	2	108.93	112.99	0.003
Antibiotic feed additives used (FA)	3	108.10	114.23	0.001
Number of starlings on sites (SS)	3	108.27	114.40	0.001
Number of cattle feeding from bunk (CFB)	3	108.91	115.04	0.000
Date of sample collection (TD)	3	108.91	115.04	0.000
SB+T+(SB*T)[a]	5	97.68	108.00	0.391
SS+T+(SS*T)	5	100.02	110.34	0.038
SB+CS[b]	4	100.65	108.87	0.166
SB+T	4	100.76	108.98	0.148
SB+T+CFB	5	99.58	109.90	0.059
SB+T+CS	5	100.06	110.38	0.036
SB+CFB	4	102.77	110.99	0.020
CFB+T	4	104.81	113.03	0.003
LNSS+CS	4	105.08	113.30	0.002
LNSS+T	4	105.62	113.86	0.001
CS+CFB	4	105.74	113.96	0.001
CS+T	4	106.04	114.26	0.001
SS+CS	4	106.35	114.57	0.001
CS+CFB+T	5	104.27	114.59	0.001
SS+T	4	106.49	114.71	0.000
SS+CFB	4	107.38	115.60	0.000
SS+CS+CFB	5	105.87	116.19	0.000
SS+CS+T	5	105.87	116.19	0.000

[a]The top-ranked model, based on Akaike weights, reported in this manuscript.
[b]The top-ranked model, based on Akaike weights, reported in Carlson et al. 2011*a.*

starling exposure time to cattle rations. All samples were aseptically collected from cattle feed bunks and placed in sterile Whirl-Paks®. All diagnostic samples were immediately stored at 4°C and express shipped on the day of collection to the Colorado State University Veterinary Diagnostic Laboratory in Fort Collins, Colorado, for testing.

Standard operating procedures were used for *S. enterica* culture. Briefly, 10-fold dilutions were made of each feed sample (10 g feed) in pre-enrichment broth (buffered peptone water,

Difco) and incubated overnight at 35°C. After pre-enrichment, 1 ml of the culture suspension was added to 10 ml of tetrathionate broth (Difco) and incubated overnight at 35°C (Dargatz et al. 2005). For each sample, 100 µl of the incubated tetrathionate suspension was transferred to 10 ml of Rappaport-Vassiliadis broth (Oxoid, Ogdensburg, N.Y.) and incubated overnight at 42° C. A swab of the culture suspension was plated for isolation on Brilliant green agar (Difco) and an XLT4 agar plate (BBL) and incubated for 24 hours at 35°C. Up to 3 suspect colonies based on colony morphology were picked and plated to blood agar plates. Following overnight incubation at 35°C, colonies were tested with polyvalent O-grouping antisera for agglutination. All positive samples were sent to the National Veterinary Services Laboratory in Ames, Iowa, for serotyping.

Data on the presence and absence of *S. enterica* in cattle feed were analyzed using generalized linear mixed effects logistic regression with PROC GLIMMIX in SAS version 9.2 (SAS Institute Inc., Cary, N.C., 2006). Fixed effects included the number of starlings at both spatial scales, temperature (C°) and the interaction between number of starlings and temperature. The response variable was binary (detection or no detection of *S. enterica*), and the CAFO of origin was included as a random effect. These models were compared to the previously published models for *S. enterica* contamination of cattle feed using an information-theoretic approach to model selection (Burnham and Anderson 2002) that ranked and weighted models, in terms of their support by the data, using bias-adjusted Akaike's Information Criterion (AIC_c) and Akaike weights (Wi). Following model selection, we estimated model fit using the Goodman-Kruskal gamma statistic, which is a measure of association between the predicted probabilities and observed responses. Pearson's correlation coefficients were used to test for associations between variables. Because starling numbers at different spatial scales were highly correlated (r = 0.71, $P < 0.0001$), they were assessed in competing models only. Pearson's correlation coefficients also were used to test for associations between starling numbers and temperature data.

Results

Based on Pearson Correlation Coefficients, the number of starlings at CAFOs decreased as temperature increased (r = -0.32, $P < 0.0001$), but the number of starlings observed in feed bunks was not associated with temperature data (r = -0.026, $P = 0.72$). Of the 191 cattle feed samples collected from 10 CAFOs (14 to 22 pens per CAFO), we detected *S. enterica* in 8.4% (Carlson et al. 2011*a*). The probability of detecting *S. enterica* in cattle feed was associated with the number of starlings in feed bunks and ambient air temperature ($F_{1,178} = 4.00$, $P = 0.05$). The best logistic regression model explaining *S. enterica* in cattle feed (Table 2) was:

$$\Pr(\hat{S}) = \frac{1}{1+\exp[-\{-3.157+0.006(SB)-0.149(T)+0.0007(SB*T)\}]}$$

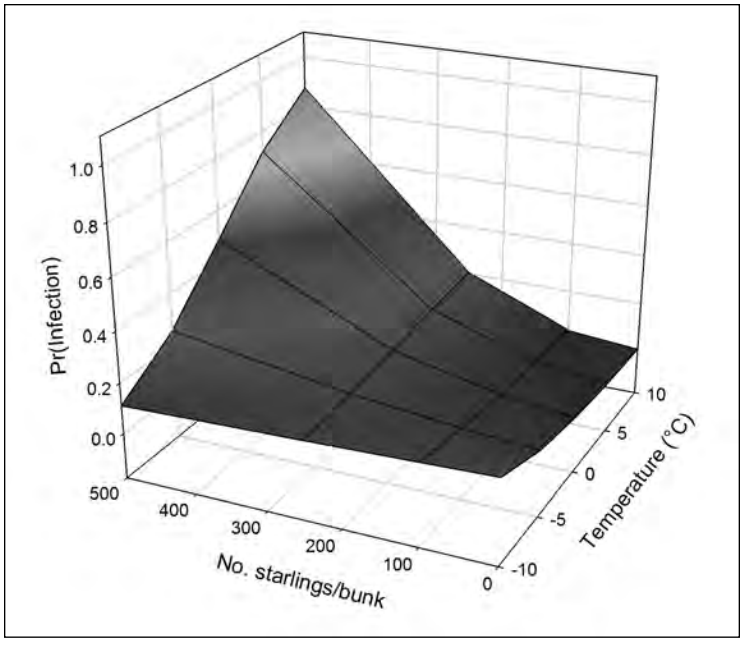

Figure 1. Predicted probability of *Salmonella enterica* contamination within cattle feed as a function of number of European starlings observed in feed bunks and ambient air temperature (C°). Data was collected on 10 CAFOs in Moore, Sherman, and Hansford counties, Texas, 2009.

Table 2. Model structure, number of estimable parameters (K), bias-corrected Akaike's Information Criterion (AIC_c), and Akaike weight (wi) for the 5 top-ranked logistic regression models assessing numbers of starlings and temperature data on the probability of *Salmonella enterica* contamination in cattle feed bunks. Data were collected within 10 concentrated animal feeding operations located in Moore, Sherman, and Hansford counties, Texas, 2009.

Model structure[a]	K[b]	AIC_c	w_i
β0 + β1(SB) + β2(T) + β3(SB*T)	5	108.004	0.391
β0 + β1(SB) + β2(CS)	4	108.865	0.166
β0 + β1(SB) + β2(T)	4	108.975	0.148
β0 + β1(SB)	3	109.208	0.117
β0 + β1(SB) + β2(T) + β3(CFB)	5	109.904	0.059

[a] Variable acronyms in model structures are: SB = number of European starlings observed within cattle feed bunks; T = ambient air temperature (C°); CS = number of cattle within CAFOs; and CFB = number of cattle accessing feed bunk.
[b] Number of estimable parameters based on the number of logistic regression coefficients plus an estimated covariance from the random effect of CAFOs.

where $\Pr(\hat{S})$ was the probability of a feed sample being contaminated with *S. enterica*, SB was the number of starlings observed in feed bunks, T was the ambient air temperature (C°), and SB*T was the interaction between number of starlings in feed bunks and ambient air temperature. The association of predicted probabilities and observed responses was 45%. Within this model, the probability of detecting *S. enterica* in feed was greatest when large numbers of starlings (500 birds) were present in feed bunks on the warmest winter days (≥10° C; Figure 1).

Discussion

We investigated the role of European starlings and temperature in the spread *S. enterica* within CAFOs. Models with interactions between starlings and temperature were not assessed in Carlson et al. (2011*a*) because, at the time, there was no biological justification for including these models in their analysis. Subsequently, information has emerged, suggesting that starling use of CAFOs varies as environmental conditions change and that starling numbers are greatest on the coldest winter days (Feare 1984, Carlson et al. 2011*b*). Based on this information, we decided to revisit our original data set and model the interaction between the number of starlings and ambient air temperature. Our results indicate that European starlings and temperature are associated to increases the risk of *S. enterica* contamination within cattle feed bunks. The probability of detecting *S. enterica* in cattle feed was greatest on the warmest winter days (10°C) in feed bunks containing the greatest number of starlings (≥500 birds). Based on Akaike weights (wi), the top-ranked model reported in this manuscript is a better predictor for *S. enterica* contamination of cattle feed than competing additive models published in Carlson et al. (2011*a*).

The relationship among starlings, temperature, and *S. enterica* contamination of CAFOs is complicated. *Salmonella enterica* contamination of cattle feed was greatest on the warmest winter days in feed bunks containing the greatest number of starlings. Additionally, our results indicate that starlings were most common on CAFOs on the coldest winter days. This suggests that even though starlings are more abundant on the coldest days, they are a greater risk for spreading *S. enterica* to cattle feed on the warmest winter days. Thus, the combination of warm temperatures and large numbers of starlings in feed bunks produces a disproportionally large risk of *S. enterica* contamination of cattle feed in CAFOs.

We believe that this information provides important management implications related to farm-side biosecurity and starling control within CAFOs. Typically, USDA-APHIS-Wildlife Services operational biologists will time

starling control operations to coincide with cold days following winter storms. This approach increases target efficacy, bait consumption, and starling take (R. L. Gilliland, Texas Wildlife Services, personal communication). We believe that high-risk conditions for *S. enterica* transmission to cattle feed by starlings will be most common in late winter and early spring when daytime high temperatures are above freezing but prior to starling dispersal for breeding. Thus, we recommend that future starling control operations be scheduled to occur following the first winter storms of the season. This approach will reduce starling numbers at a time before they have had less of a chance to create risks of disease in CAFOs. Also, scheduling starling control for early winter will have additional benefits to producers because CAFOs are known to lose significant amounts of feed to starlings while experiencing physical damage to structures and fecal contamination of machinery (Besser et al. 1968, Dolbeer et al. 1978, Lee 1987). Thus, controlling starlings as early in the winter as possible will maximize the value of the control operations for livestock producers.

We hypothesize that the amount of *S. enterica* contamination in CAFOs during winter and early spring will influence its prevalence in herds during the summer. Previous publications have shown that herd prevalence for *S. enterica* in CAFOs varies seasonally with peak fecal shedding by cattle occurring in the summer months and the lowest occurring during the winter (Wells et al. 2001). This variability is likely due to environmental changes that are conducive to survival and amplification of *S. enterica* within media that contributes to cattle infections (i.e., cattle feed, water, and feces). Thus, improved biosecurity during the winter may reduce the risk of *S. enterica* cattle infections during the summer.

In conclusion, it is unlikely that the ecological interactions between European starlings, *S. enterica*, and cattle are the only disease risks that can be attributed to peridomestic wildlife use of CAFOs. Starlings may contribute to the maintenance and spread of other pathogens in CAFOs and other wildlife species may contribute to the maintenance and spread of *S. enterica*. Thus, identification of high-risk wildlife, the pathogens they introduce, and their ecological interactions with domesticated animals is needed to characterize the disease risks, production costs, and environmental impacts associated with wildlife use of CAFOs.

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