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The role of starlings in the spread of *Salmonella* within concentrated animal feeding operations

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Summary

1. Characterizing and mitigating the disease risks associated with wildlife use of concentrated animal feeding operations (CAFOs) can reduce the spread of micro-organisms throughout the environment while increasing agricultural productivity. To better understand the disease risks associated with bird use of CAFOs, we assessed the capacity of European starlings *Sturnus vulgaris* to spread *Salmonella enterica* to cattle, their feed and water.

2. We sampled starlings, cattle feed, cattle water and cattle faeces from 10 CAFOs in Texas, USA. Samples were screened for *Salmonella enterica* to investigate: (i) the prevalence of *S. enterica* in starlings using CAFOs, (ii) whether there was a relationship between cattle infections and starling numbers, and (iii) if *S. enterica* contamination of cattle feed and water was related to numbers of starlings observed on CAFOs.

3. We used generalized linear mixed logistic regression models to assess the importance of starlings, cattle stocking, facility management and environmental variables in the transmission of *S. enterica* to cattle, feed troughs and water troughs in CAFOs.

4. Starling gastrointestinal tract samples tested positive for *S. enterica* (2·5% prevalence; 95% CI = 0·3%, 8·6%) and starlings were retained as model covariates in the best supported logistic regression models for *S. enterica* contamination within cattle feed, water and faeces.

5. *Salmonella enterica* contamination of both cattle feed troughs and water troughs is significantly related to numbers of starlings. Contamination in cattle feed increased as more starlings entered feed troughs. Contamination in water troughs increased asymptotically as numbers of starlings on CAFOs increased. Starling variables in the cattle faecal shedding model were not significant.

6. *Synthesis and applications.* The numbers of European starlings better explained *S. enterica* contamination of cattle feed and water than other variables including cattle stocking, facility management and environmental variables. This suggests that starlings are a source of *S. enterica* contamination in CAFOs. Thus, starling management tools such as population control, habitat management, exclusionary devises and bird repellents may be used to reduce the amplification and spread of disease within livestock production systems.

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

Introduction

Concentrated animal feeding operations (CAFOs) have been implicated as sources for new, more infectious or resistant micro-organisms that can spread to humans and to the environment (Gilchrist *et al.* 2007). For example, food animals raised in CAFOs have been linked to antibiotic resistant *Salmonella* (White *et al.* 2001). Thus, managing disease in CAFOs is of paramount importance in our efforts to reduce the dissemination of micro-organisms throughout the environment. Virtually all CAFOs within the US experience gastrointestinal (GI) diseases within their herds (USDA 2000a) and

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domestic cattle *Bos taurus* are known reservoirs of many GI pathogens that are of concern to livestock producers, including the bacterium *Salmonella enterica* (Himathongkham *et al.* 1999; Wells *et al.* 2001). Identifying and mitigating the risk pathways that contribute to *S. enterica* in CAFOs is necessary to reduce production losses and contamination of human food products.

Salmonella enterica is a ubiquitous micro-organism, which is known to cause illness in cattle (Fedorka-Cray *et al.* 1998). In CAFOs, cattle typically acquire *S. enterica* from other infected livestock which spread the pathogen throughout the herd via contaminated cattle faeces (Wray & Davies 2000), cattle feed (Maciorowski *et al.* 2006) and water (Kirk *et al.* 2002a). Recent empirical evidence suggests that small mammals and birds may also be a significant source of *S. enterica* contamination in animal feed, which by itself is capable of accounting for the prevalence of clinical salmonellosis seen in cattle herds (Daniels, Hutchings & Greig 2003). This is a major concern to producers faced with peridomestic wildlife problems because *S. enterica* infections in cattle can translate into significant economic losses for producers and carcass contamination at the slaughter house (Wells *et al.* 2001; USDA 2007). Additionally, *S. enterica* in cattle is a source for human salmonellosis, which is responsible for an estimated 1·3 million human cases, 15,600 hospitalizations and 550 deaths each year (Mead *et al.* 1999).

European starlings *Sturnus vulgaris* are native to Europe and North Africa and have successfully established populations on every continent except Antarctica (Rollins *et al.* 2009). Outside their native range starlings are considered to be one of the most destructive invasive bird species world-wide (Lowe *et al.* 2000). Starlings congregate in large roosting groups and exploit abundant and nutritious food sources on CAFOs (Feare, Douville de Franssu & Peris 1992; LeJeune *et al.* 2008). Damage to CAFOs is greatest during winter months because insects and other natural foods are typically unavailable (Linz *et al.* 2007). Moreover, starlings are known carriers of many human and cattle pathogens, including *S. enterica* (Feare 1984; Clark & McLean 2003). Thus, starlings have been implicated as sources of pathogens causing disease and economic losses to livestock producers (LeJeune *et al.* 2008; Gaukler *et al.* 2009).

Scientific literature linking starlings to the spread of *S. enterica* in CAFOs is limited and inconclusive (Gaukler *et al.* 2009), yet many publications have suggested that wild birds may contribute to the maintenance and spread of *S. enterica* (Krytenburg *et al.* 1998; Wells *et al.* 2001; Kirk, Holmberg & Jeffrey 2002b; Daniels, Hutchings & Greig 2003; Fossler *et al.* 2005; Pedersen *et al.* 2006). Currently no information exists on the mechanism by which starlings transmit pathogens or the magnitude of pathogen transmission. The overall objective of this study was to assess the role of starlings in the transmission of *S. enterica* to cattle, their feed and water in CAFOs. Specifically, we addressed the following research questions: (i) what is the prevalence of *S. enterica* in starlings using CAFOs? (ii) is there a relationship between *S. enterica* infections in cattle and starling numbers on CAFOs? and (iii) is *S. enterica* contamination of cattle feed and water related to the abundance of starlings within CAFOs?

Materials and methods

We selected 10 CAFOs located in Moore, Sherman and Hansford Counties, Texas, USA, based on the similarity of CAFO management practices and the presence or absence of starlings. We estimated starling numbers on CAFOs prior to sample collection 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. Based upon our own starling damage criteria, two of 10 CAFOs selected were experiencing severe problems with starlings ($>10\,000$ starlings day^{-1}), four were experiencing moderate problems with starlings (1000–$10\,000$ starlings day^{-1}), and four were experiencing minimal starling problems (<1000 starlings day^{-1}). We sampled CAFOs when starling numbers were greatest from 20 January to 19 February 2009.

Diagnostic samples were only collected from CAFOs when starlings were present, no samples were collected prior to starling arrival and none were collected after starlings returned to roosts. Also, the number of starlings observed in feed troughs, water troughs, and cattle pens were estimated when feed, water, and faecal samples were collected, respectively. This provided estimates of starling numbers at two spatial scales; numbers of starlings on CAFOs (facility level) and numbers of starlings in cattle pens, feed troughs or water troughs within CAFOs (pen level).

Feed samples were collected from cattle feed troughs and placed in sterile Whirl-Paks® (NASCO, Fort Atkinson, WI). Water samples were collected from cattle water troughs using sterile 125-mL plastic vials. We collected fresh faecal samples from individual cows. Samples were only collected when an animal was observed defecating to standardize environmental exposure between faecal samples and to eliminate cross-contamination from other faeces. All faecal samples were stored in sterile Whirl-Paks®. Starlings were captured opportunistically from CAFOs using modified Australian crow traps, which were baited with cattle feed, dog food and water. All captured birds were euthanized by cervical dislocation, a method conforming to agency policy as stated in USDA/APHIS/WS Directive 2.505 and approved by the National Wildlife Research Center's (NWRC) Internal Animal Care and Use Committee (IACUC). The GI tract (proventriculus to the cloaca) was removed from euthanized starlings and placed in sterile Whirl-Paks®. All samples were immediately stored at 4 °C and express shipped on the day of collection to the Colorado State University, Veterinary Diagnostic Laboratory (CSUVDL) in Fort Collins, Colorado for diagnostic testing.

Standard operating procedures were used for *Salmonella* culture. Briefly, 10-fold dilutions were made of each environmental sample type (10 g feed, 25 mL water) 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 Bacterius Ltd, Houston, TX) and incubated overnight at 35 °C (Dargatz *et al.* 2005). Faecal or intestinal samples were added at 10-fold dilutions to tetrathionate (Difco) broth and incubated overnight at 35 °C (Kim *et al.* 2001). For each sample type, 100 μL of the incubated tetrathionate suspension was transferred to 10 mL of Rappaport-Vassiliadis broth (Oxoid, Ogdensburg, NY, USA) 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 h at 35 °C. Up to three 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 (NVSL) in Ames, Iowa for serotyping.

Prevalence of *S. enterica* within starling GI tracts was estimated and comparisons were made to the samples of cattle faeces, feed and water that tested positive. Data on the presence and absence of *S. enterica* in cattle feed, water and faeces were analyzed using generalized linear mixed effects logistic regression with PROC GLIMMIX in SAS version 9.2 (SAS Institute 2006). We performed separate analyses of multivariate logistic regression models for data on cattle feed, cattle water and cattle faecal samples. For all three models the response variable was binary (detection/no detection of *S. enterica* in samples) and CAFO was included as a random effect.

The explanatory variables assessed in these models were selected because they have been identified as or suspected of contributing to *S. enterica* in CAFOs (Fedorka-Cray *et al.* 1998; USDA 2000b; LeJeune *et al.* 2001; Huston *et al.* 2002; Fossler *et al.* 2005). These variables included numbers of starlings at both spatial scales (in CAFOs and in pens within CAFOs), cattle stocking (number of cattle in CAFO, number of cattle in pens, number of cattle using water troughs, number of cattle using feed troughs), environmental factors (temperature, date of sample collection) and CAFO management factors (water troughs clean: yes/no, type of water trough: open or free floating ball actuator, use of antibiotic feed supplements: yes/no).

Water troughs recorded as clean were free of visibly detectable algae, cattle faeces and bird faeces. After 1 day post-cleaning all water troughs contained visually detectable amounts of cattle faeces and bird faeces. Thus, water troughs free of faecal material were assumed to have been cleaned within the past 24 h. Two types of water trough were used within our selected CAFOs: open and free floating ball actuator water troughs. Open troughs have no covering, a basin is automatically filled with water and cattle drink directly from the basin. Ball actuator troughs have a floating ball that covers the opening of the water trough and cattle have to depress the float to drink water. Cleaning and type of water trough variables were only assessed in the water trough model. Antibiotics were added to cattle feed in some of our selected CAFOs. Thus, the antibiotics in feed variable was assessed in the cattle feed and faecal contamination models.

Multiple *a priori* hypotheses concerning the effects of explanatory variables on detection of *S. enterica* in samples were developed and an information-theoretic model selection approach (Burnham & Anderson 2002) was used to rank and weight models in terms of their support by the data using bias-adjusted Akaike Information Criterion (AICc) and Akaike weights (W_i). 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. Odds ratios and their 95% confidence intervals were estimated for each explanatory variable included in the best models for *S. enterica* contamination of cattle feed, cattle water and cattle faeces. Odds ratios were a measure of effect size, which represented the odds of *S. enterica* being detected in a sample when the explanatory variable increased, given that all other explanatory variables are held constant. Because the numbers of starlings on CAFOs were correlated to the numbers of starlings observed in feed troughs ($r = 0.711$, $P < 0.0001$) and the numbers of starlings observed in water troughs ($r = 0.623$, $P < 0.0001$), we did not include numbers of starlings at the different spatial scales in the same models.

Results

There was considerable daily variation in starling numbers within CAFOs (CVs ranged from 0.07 to 1.05) and starling numbers between CAFOs (CV = 1.908; Fig. 1). Despite variability in CAFO use by starlings, 70% of sites continued to
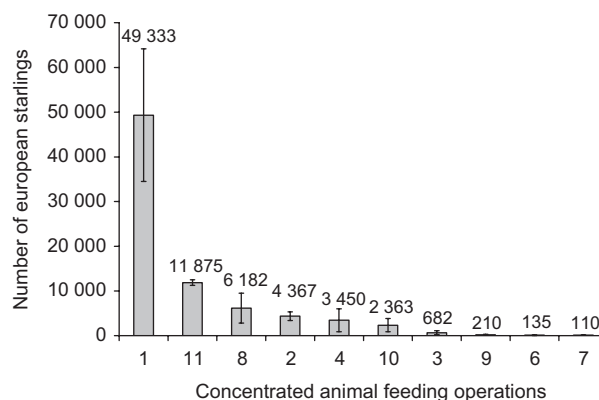
Fig. 1. Estimated daily European starling numbers ($\bar{x} \pm$ SE) observed on 10 concentrated animal feeding operations in Moore, Sherman and Hansford Counties Texas, 2009. Data labels above bars denote the mean European starling population size and vertical lines represent the standard errors for the means.

experience the same degree of problem throughout data collection. Based on our starling damage criteria, two sites (CAFO site 4 and 10) experienced minimal to moderate starling problems and CAFO site 8 experienced moderate to severe problems. Starlings were also detected within animal pens ($\bar{N} = 109$, SE = 15), feed troughs ($\bar{N} = 67$, SE = 8) and water troughs ($\bar{N} = 3$, SE = 0.5). A total of 81 starlings were trapped from 3 CAFOs (sites 1, 2 and 8) and sampled for *S. enterica*. *Salmonella enterica* was recovered from 2.5% (2/81; 95% CI = 0.3%, 8.6%) of the starling GI tracts.

CONTAMINATION OF CATTLE FEED

We collected 191 cattle feed samples from 10 CAFOs (14–22 pens/CAFO) and *S. enterica* was detected in 8.4% (16/191; 95% CI = 4.9%, 13.3%) of feed samples. The best logistic regression model explaining *S. enterica* contamination in cattle feed (Table 1) was:

$$\Pr(\hat{S}) = \frac{1}{1 + \exp[-(-3.927 + 0.006(\mathrm{SB}) + 0.00003(\mathrm{CS})]},$$

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 troughs and CS was the number of cattle on CAFOs. The association of predicted probabilities and observed responses was 47.5%. Within this model the probability of *S. enterica* contamination increased as the number of starlings in feed troughs increased and as the number of cattle on CAFOs increased (Fig. 2). Based on 95% confidence intervals, the estimated slope of the SB variable was relatively precise and differed from zero (95% CI = 0.001, 0.011), suggesting we could reliably detect increased *S. enterica* contamination within feed troughs exposed to starlings. The slope of the CS variable was not significantly different than zero (95% CI = −0.000011, 0.000062), suggesting the magnitude of the effect attributed to increasing numbers of cattle on CAFOs could not be reliably determined.

Table 1. Model structure, number of estimable parameters (K), bias-corrected Akaike Information Criterion (AIC$_c$) and Akaike weight (W_i) for the three top-ranked logistic regression models explaining the probability of *Salmonella enterica* contamination in cattle feed troughs, cattle water troughs and cattle faeces, based on data collected within 10 concentrated animal feeding operations located in Moore, Sherman and Hansford Counties, Texas, 2009

Model structure	K^a	AIC$_c$	W_i
Cattle feed troughs			
$\beta0 + \beta1(SB) + \beta2(CS)$	4	108·87	0·290
$\beta0 + \beta1(SB) + \beta2(T)$	4	108·98	0·260
$\beta0 + \beta1(SB)$	3	109·21	0·206
Cattle water troughs			
$\beta0 + \beta1(LNSS) + \beta2(C)$	4	124·72	0·658
$\beta0 + \beta1(LNSS)$	3	126·07	0·172
$\beta0 + \beta1(LNSS) + \beta2(TD)$	4	126·78	0·084
Cattle faeces			
$\beta0 + \beta1(LNSS)$	3	29·68	0·339
$\beta0 + \beta1(LNSS) + \beta2(T)$	4	29·75	0·315
$\beta0 + \beta1(T)$	3	31·26	0·070

SB, number of European starlings observed within cattle feed troughs; CS, number of cattle within CAFOs; T, ambient air temperature (°C); LNSS, natural log transformation of number of European starlings observed on CAFOs; C, water trough recently cleaned (Y, N); TD, type of water trough (open trough, ball actuator).
[a]Number of estimable parameters based on the number of logistic regression coefficients plus an estimated covariance from the random effect of CAFOs.
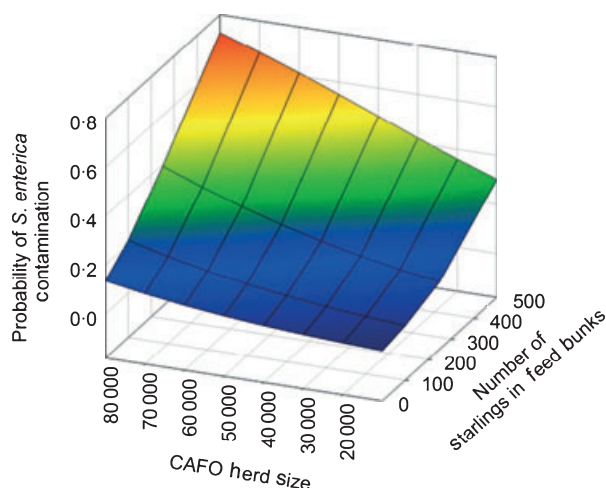
Fig. 2. Predicted probability of *Salmonella enterica* contamination within cattle feed troughs as a function of number of cattle on the concentrated animal feeding operation (CAFO herd size) and the number of starlings observed in feed troughs. Data were collected on 10 CAFOs in Moore, Sherman and Hansford Counties Texas, 2009.

The probability of *S. enterica* contamination increased as starlings entered feed troughs (odds ratio = 1·006; 95% CI = 1·001, 1·011) and effectively doubled for every 123 starlings that entered feed troughs (odds ratio = 2·01; 95% CI = 1·068, 3·766). The estimated odds ratio for the number of cattle on CAFOs was not significant (Table 2).

CONTAMINATION OF WATER TROUGHS

We collected 169 water trough samples from 10 CAFOs (11–21 troughs/CAFO) and *S. enterica* was detected in 13·6% (23/169; 95% CI = 8·8%, 19·7%) of water troughs. The best logistic regression model explaining *S. enterica* contamination in cattle water troughs (Table 1) was:

$$\Pr(\hat{S}) = \frac{1}{1 + \exp[-(-5\cdot740 + 0\cdot509(\text{LNSS}) + 1\cdot304(C)]},$$

where $\Pr(\hat{S})$ was the probability of a water trough being contaminated with *S. enterica*, LNSS was the natural log of the number of starlings observed on CAFOs and C was the categorical variable identifying water troughs that had not been recently cleaned. The association of predicted probabilities and observed responses was 55·9%. Within this model *S. enterica* contamination increased when the natural log of the number of starlings on CAFOs increased and when water trough had not been recently cleaned (Fig. 3). Based on 95% confidence intervals, the slope of the LNSS variable was relatively precise and differed from zero (95% CI = 0·157, 0·844), suggesting we could reliably detect increased *S. enterica* contamination within water troughs exposed to starlings. The slope of the C variable was not significantly different from zero (95% CI = −0·914, 3·524), suggesting the magnitude of the effect attributed to water trough cleaning could not be reliably determined.

The probability of *S. enterica* contamination increased as the natural log of the number of starlings on CAFOs increased (odds ratio = 1·663; 95% CI = 1·189, 2·325), suggesting the odds of *S. enterica* contamination of water troughs increase when CAFOs are exposed to starlings. The estimated odds ratio for water trough cleaning was not significant (Table 2).

CATTLE SALMONELLOSIS

We collected 61 cattle faecal samples within nine CAFOs (2–13 samples/CAFO) and *S. enterica* was detected in 6·5% (4/61; 95% CI = 1·8%, 16·0%) of these samples. The best logistic regression model explaining *S. enterica* faecal shedding by cattle (Table 1) was:

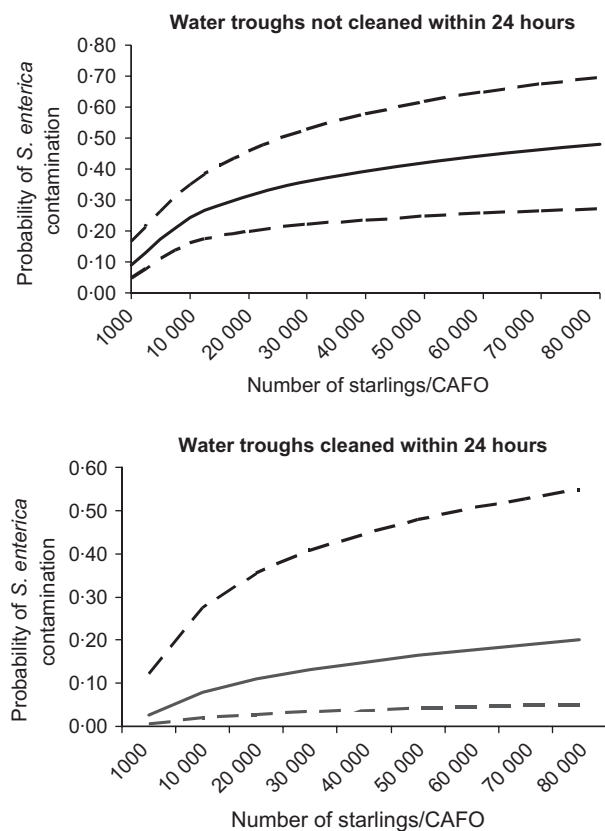$$\Pr(\hat{S}) = \frac{1}{1 + \exp[-(-9\cdot2850 + 0\cdot757(\text{LNSS})]},$$

where $\Pr(\hat{S})$ was the probability of a cattle faecal sample being contaminated with *S. enterica* and LNSS was the natural log of the number of starlings observed on CAFOs. The association of predicted probabilities and observed responses suggests this model explained 76·2% of the variability in the data set. Within this model the probability of *S. enterica* contamination increased as the natural log of the number of starlings on CAFOs increased (Fig. 4). Neither the slope nor the odds ratio for LNSS was significantly different from zero (Table 2).

Table 2. Parameter estimates and odds ratios, with their 95% confidence intervals, for variables from best logistic regression models explaining *Salmonella enterica* contamination of cattle feed, cattle water and cattle faecal samples collected in 2009 from 10 concentrated animal feeding operations in Moore, Sherman and Hansford Counties, Texas

Model covariates	Parameter estimate (95% CI)	Odds ratio (95% CI)
Cattle feed model		
Starlings in feed trough	0·006 (0·001, 0·011)	1·006 (1·001, 1·011)
Number of cattle on CAFO	0·00003 (−0·00001, 0·00006)	1·000 (0·989, 1·064)
Cattle water trough model		
LNSS	0·509 (0·173, 0·844)	1·663 (1·189, 2·325)
Water trough not cleaned[a]	1·304 (−0·914, 3·524)	3·687 (0·401, 33·906)
Cattle faecal model		
LNSS	0·757 (−0·099, 1·612)	2·131 (0·906, 5·014)

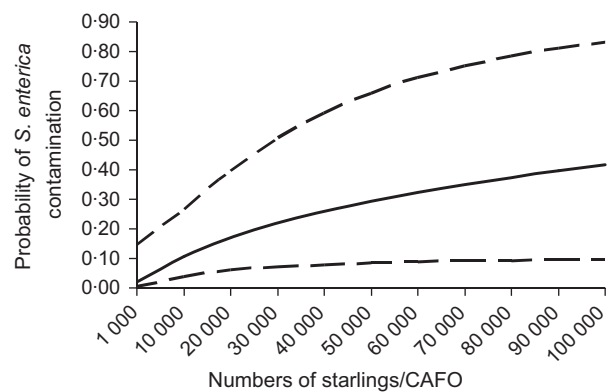LNSS, natural log transformation of the number of European starlings on CAFOs.
[a]Water troughs were visually confirmed to be recently cleaned based upon the presence or absence of starlings faeces and cattle faeces.

Fig. 4. Predicted probability of *Salmonella enterica* contamination in cattle faeces as a function of number of starlings observed on concentrated animal feeding operations (CAFO). Dashed lines represent the 95% confidence limits. Data were collected on 10 CAFOs in Moore, Sherman and Hansford Counties Texas, 2009.

Fig. 3. Predicted probability of *Salmonella enterica* contamination within water troughs as a function of number of starlings observed on concentrated animal feeding operations (CAFO) and cleanliness of water troughs. Dashed lines represent the 95% confidence limits. Data were collected on 10 CAFOs in Moore, Sherman and Hansford Counties Texas, 2009.

SEROGROUPS AND SEROTYPES

We identified four serogroups (B, C1, C2 and E) from 45 isolates (Table 3) and 17 serotypes from 42 isolates (Table 4). The most common serogroup was C1 (53·3% of isolates), it was detected in cattle feed, water and faecal samples. Serogroup E (24·5%) was also common and was isolated from starlings, cat-

tle feed, water troughs and cattle faecal samples (Table 3). Montevideo was the most common serotype (20·0% of isolates), it was isolated from cattle feed and water samples. Mbandaka (17·8% of isolates) was also common and was isolated from cattle feed, water and faecal samples (Table 3). We found the Saint Paul serotype only in starlings and not in any of the other sample type (Table 4).

Discussion

We investigated the potential for European starlings to spread *S. enterica* within CAFOs. Numbers of starlings were included as variables in the best logistic regression models from analyses of *S. enterica* contamination within cattle feed troughs, cattle water troughs and cattle faeces. Based on the odds ratio analysis, starlings contribute to *S. enterica* contamination of cattle feed and water. This relationship was not as clear in the cattle faecal shedding analysis, even though number of starlings on CAFOs was the best explanatory variable among all of the variables assessed.

Table 3. *Salmonella* serogroups isolated from *Salmonella enterica* positive European starling gastrointestinal tract, cattle faecal, cattle feed and cattle water trough samples. Positive samples were collected in 2009 on 10 concentrated animal feeding operations sampled within Moore, Sherman and Hansford Counties, Texas

Percent of *Salmonella* positive samples by serogroup

Serogroup	Starling samples (n = 2)	Cattle faeces (n = 4)	Feed troughs (n = 16)	Water troughs (n = 23)	Total (n = 45)
B	50·0			21·7	13·3
C1		50·0	68·7	47·8	53·3
C2			12·5	8·8	8·9
E	50·0	50·0	18·8	21·7	24·5

Table 4. *Salmonella enterica* samples serotyped in 2010 from positive European starling gastrointestinal tract, cattle faecal, cattle feed and cattle water trough samples. All samples were collected on 10 concentrated animal feeding operations within Moore, Sherman and Hansford Counties, Texas, 2009

Percent of *Salmonella* positive samples by serotype

Serotype	Starling samples (n = 2)	Cattle faeces (n = 4)	Water troughs (n = 23)	Feed troughs (n = 16)	Total (n = 45)
Agona			4·4		2·2
Altona				6·3	2·2
Anatum		25·0	21·6		13·3
Cerro			4·4		2·2
Mbdanka		50·0	17·3	12·4	17·8
Meleagridis		25·0		6·3	4·4
Meunchen				6·3	2·2
Meunster				6·3	2·2
Montevideo			30·3	12·4	20·0
Reading			4·4		2·2
Rough:gms:-				12·4	4·4
Rough:i:z6			4·4		2·2
Rough:z:r6				6·3	2·2
Saint Paul	50·0				2·2
Seftenberg				6·3	2·2
Tennessee			4·4	18·7	8·9
Typhimurium var 5-			4·4		2·2
Nonviable[a]	50·0		4·4	6·3	6·7

[a]Samples that were positively identified as *Salmonella* and serogrouped in 2009 but could not be reisolated and serotyped in 2010.

Our inability to identify any significant explanatory variables for the faecal shedding analysis underscores the complexity of the *S. enterica* infection process in cattle. According to Wells *et al.* (2001) the interactions among *S. enterica*, affected cattle, and their environment are complex. For example, herd size (Huston *et al.* 2002), age of cattle (Tsolis *et al.* 1999), manure handling and disposal methods (Kabagambe *et al.* 2000; Fossler *et al.* 2005), feed rations and storage (Fossler *et al.* 2005; Green *et al.* 2010), access to environmental waters (Fossler *et al.* 2005), season (Wells *et al.* 2001), purchasing cattle from dealers (Evans & Davies 1996), method of cattle penning (Fossler *et al.* 2005), and exposure to wild birds and rodents (Evans & Davies 1996; Warnick *et al.* 2001) have all been implicated as herd-level risk factors for *S. enterica* infections. To understand the relative importance of starlings for *S. enterica* infections in cattle, we need information that characterizes how starlings contribute to the spread of *S. enterica* in CAFOs.

Although starlings were associated with *S. enterica* in cattle feed and water the serotype data did not suggest starling faeces contributed to the contamination process. Only one serotype was successfully isolated from starling faeces, S. Saint Paul. This serotype is pathogenic to cattle but it was not isolated from cattle feed, water troughs or faecal samples. Based upon our data and behavioral observations of starlings we hypothesize that starlings mechanically transmit contaminated cattle faecal material from cattle pens to other locations within CAFOs, especially feed troughs and water troughs. Starlings captured within CAFOs had visible amounts of cattle faeces on their feet and feathers. This faecal material was probably being disseminated in feed troughs and water troughs when the birds fed and drank. Also, starlings were regularly observed bathing in the open, shallow water within the troughs. As a consequence of this starling behaviour, cattle faecal material is being moved from the animal pens to cattle feed and water, and this will be likely to increase *S. enterica* loads in both media. The ability of starlings to mechanically transmit disease is not well documented. Previous studies have considered starling faeces as a possible source for *S. enterica* in CAFOs (Gaukler *et al.* 2009) but they did not consider mechanical transmission. Thus, mechanical transport of pathogens by birds in CAFOs is a potential source for disease that deserves a closer examination.

The presence of *S. enterica* in cattle water troughs and feed troughs was associated with starlings at two different spatial scales; CAFOs and pens within CAFOs. The spatial scale of observation is important when viewed in the context of our behavioral observations of starlings. After daily filling of the feed troughs, cattle and birds quickly ate all the feed; no feed was carried over in the troughs to the next day. Thus, the number of starlings in feed troughs was more strongly associated with the occurrence of *S. enterica* in cattle feed than the number of starlings on CAFOs. In comparison, contamination of water troughs were subject to carry-over effects because the troughs were not cleaned daily and contamination could accumulate over multiple days. This is a likely explanation for why the number of starlings on CAFOs was more strongly associated with the occurrence of *S. enterica* than the number of starlings observed in water troughs prior to sample collection.

Managing starling populations on CAFOs may be an effective means of reducing cattle infections that occur because of feed and water contamination. For example, the best water trough model suggests that reducing starling numbers on CAFOs in conjunction with daily water trough cleaning may reduce *S. enterica* contamination within water troughs by 50% or more. This provides producers with an inexpensive and effective means of managing *S. enterica* contamination

within CAFOs. Substantial reductions in *S. enterica* contamination of feed and water would be expected to produce unseen benefits through reductions in subclinical infections and possibly in clinical infections and mortalities.

Starling damage to CAFOs has been documented in the United States (Linz *et al.* 2007), England and northwest France (Feare, Douville de Franssu & Peris 1992), and Australia (Bentz *et al.* 2007). Within the United States and Australia starling management focuses on lethal control of starlings because they are an invasive species that causes environmental and economic damage (Linz *et al.* 2007; Tracey *et al.* 2007). Lethal starling control is carried out with the use of chemical toxicants (West 1968; Cummings *et al.* 2002; Bentz *et al.* 2007) and shooting (Tracey *et al.* 2007). Use of DRC-1339, a chemical toxicant registered for use in the United States, has been effective for reducing starling damage (Besser, Royall & DeGrazio 1967; West 1968; Cummings *et al.* 2002). However, trial use in Australia was found to be ineffective because of poor bait acceptance (Bentz *et al.* 2007).

Within regions where starlings are a species of conservation concern, managing damage in CAFOs will be far more complex. For example, in England starlings have been placed on the IUCN Red List as a species of highest conservation concern (Gregory, Noble & Custance 2004). Thus, reducing starling use of CAFOs in England will require the use of non-lethal management techniques. Based upon published reports, nonlethal chemical repellents (Glahn, Mason & Woods 1989), facility management and habitat alteration (Twedt & Glahn 1982; Kirk 2009), exclusionary devices (Lee 2005; Bentz *et al.* 2007), frightening devices (Conover & Perito 1981; Marsh, Erickson & Salmon 1992), acoustical devices (Palmer 1976), live traps (Palmer 1976) and feeding cattle rations as extruded pellets (Depenbusch *et al.* 2009) have all been used to reduce starling damage in CAFOs.

We believe non-lethal deterrents will be most effective when applied at the specific locations starlings cause damage. Unfortunately most exclusionary devices are impractical for repelling starlings from feed troughs and water troughs because they interfere with cattle feeding and facility operations. Instead, we recommend feeding cattle large extruded pellets while using predator models, acoustical devices and legal chemical repellents. Starling habituation to frightening and acoustical devices is a known problem (Johnson, Cole & Stroup 1985; Marsh, Erickson & Salmon 1992). To improve efficacy of these tools, they should be used in tandem and switched on a regular basis (Palmer 1976; Berge *et al.* 2007).

In facilities experiencing severe starling problems a secondary zone of management, outside the animal pens, should also be considered. Habitat modification in and around CAFOs, use of exclusionary devices for protecting stored feed supplies, buildings and other roosting sites, and use of baited drop in traps may be effective for reducing starling numbers on CAFOs. If used effectively, non-lethal techniques may reduce the number of starlings on CAFOs, contact with livestock feed and water, and any associated *S. enterica* contamination.

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, pathogens they introduce and their ecological interactions with domesticated animals is needed to characterize the disease risks, production costs and environmental impacts associated with peridomestic wildlife use of CAFOs.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Summary of the competing models assessed in the analysis of *Salmonella enterica* contamination of cattle feed.

Appendix S2. Summary of the competing models assessed in the analysis of *Salmonella enterica* contamination of cattle water troughs.

Appendix S3. Summary of the competing models assessed in the analysis of *Salmonella enterica* contamination of cattle feces.

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