Assessing the contributions of intraspecific and environmental sources of infection in urban wildlife: *Salmonella enterica* and white ibis as a case study: Supplemental Information

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S1. Field data

We here detail methods of ibis sampling, collection of environmental samples, and laboratory analyses to determine *Salmonella* positivity, as well as providing all site-level prevalence data. Ibis were captured with nylon slip-knot leg lassos [1] and modified manually operated flip traps [2]. Fecal samples were collected by transferring one gram of fresh feces to 10 mL of dulcitol selenite enrichment broth in the field and maintained at room temperature. Once per site and season, during the session where we caught most ibises, we collected three water samples (each 1000 mL) and three soil samples (4–7 g). Water samples were maintained at 4°C until they were vacuum filtered through 47-mm diameter 0.45 μm pore size mixed cellulose ester membranes within six hours of collection (Millipore, Burlington, MA). Membranes and soil samples were transferred to 10 mL 1% peptone (w/v) water and held at room temperature for pre-enrichment. Samples were shipped overnight to the University of Georgia within four days of collection for *Salmonella* isolation. 100 μL of growth from each enrichment tube was transferred to 10 mL of Rappaport-Vassiliadis (RV) broth (Oxoid, Thermo-Fisher) for selective enrichment for *Salmonella* and incubated at 42ºC for 24–48 h. A loop of growth was then streaked onto XLT4 agar (Remel) and incubated at 37ºC for 24 $\pm$ four hours. Presumptive *Salmonella* isolates (e.g., black colonies or yellow colonies with a black center) were streaked an additional two times to assure isolation. Isolated colonies were confirmed by characteristic growth on CHROMagar *Salmonella* Plus agar medium, and a subset of samples was confirmed with PCR [3].

We estimated white ibis flock size using visual scans, in which the number of ibis in one contiguous flock (<10 meters apart) was quantified once per hour from 15–25 meters away. Using these hourly data, we derived the average flock size per site and sampling time point.

1. Hernandez SM *et al.* 2016 Urbanized White Ibises (Eudocimus albus) as Carriers of Salmonella enterica of Significance to Public Health and Wildlife. *PLOS ONE* **11**, e0164402. (doi:10.1371/journal.pone.0164402)

2. Parker JM, Folk MJ, Baynes SB, Candelora KL. 2008 Use of clap traps in capturing nonmigratory whooping cranes in Florida. *Proceedings of the Tenth North American Crane Workshop* **10**, 141–146.

3. Luo Z, Gu G, Giurcanu MC, Adams P, Vellidis G, van Bruggen AH, Wright AC. 2014 Development of a novel cross-streaking method for isolation, confirmation, and enumeration of Salmonella from irrigation ponds. *Journal of microbiological methods* **101**, 86–92.

Figure S1. Observed patterns of *Salmonella* prevalence in ibis (orange) and environment (blue) per each urban capture site during the non-breeding season. Segments denote 95% confidence intervals. 2015–2016 data are shown as circles, and 2016–2017 data are shown as triangles.

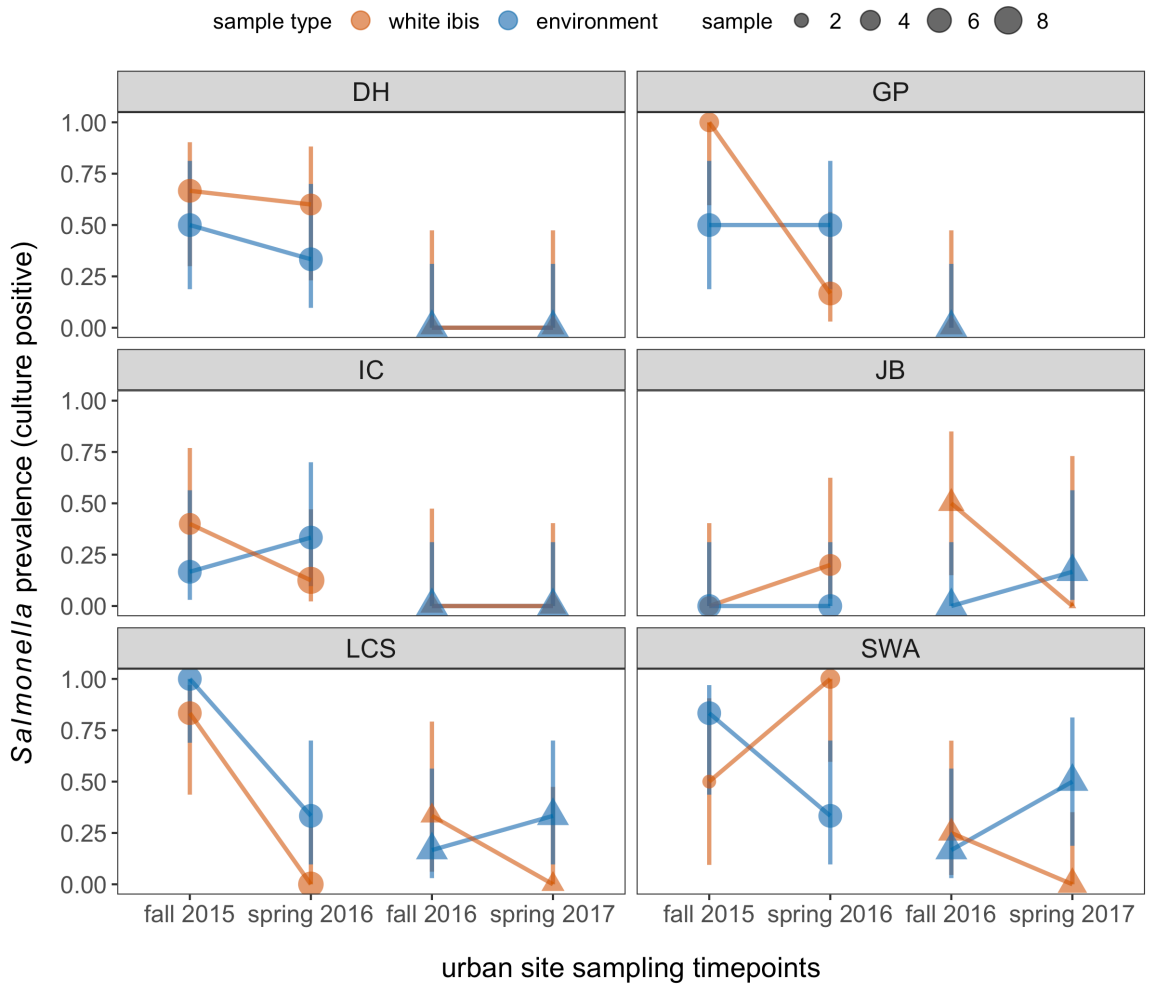
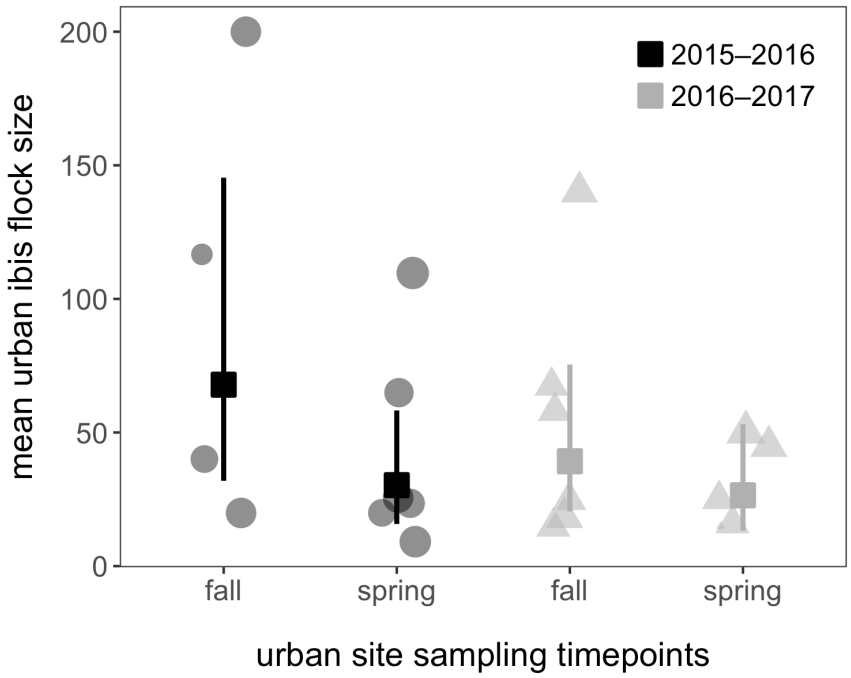


Figure S2. Distribution of mean urban ibis flock size per site and sampling timepoint (circles). Means and 95% confidence intervals for the start and end of each non-breeding season are shown with squares and were derived from the flock size GLMM (χ^2=5.6, p=0.13). Data from 2015–2016 are shown as circles, and data from 2016–2017 are shown as triangles.



S2. LHS routine sensitivity

Table S1. Simulations out of 1000 LHS where endpoint infection prevalence matched observed *Salmonella* prevalence of urban ibis and environmental samples during the non-breeding season. Results are shown using mean flock size and its 95% confidence interval to assign N, I_t, and I_a.

	P_{start}=41%		P_{start}=59%		P_{start}=75%	
N=32 (lower 95% CI)	$c_f \geq 0$	$c_f = 0$	$c_f \geq 0$	$c_f = 0$	$c_f \geq 0$	$c_f = 0$
$\psi \geq 0$	9	9	16	15	20	19
$\psi = 0$	7	8	13	13	17	17
N=68 (mean, main text)	$c_f \geq 0$	$c_f = 0$	$c_f \geq 0$	$c_f = 0$	$c_f \geq 0$	$c_f = 0$
$\psi \geq 0$	11	11	16	16	18	18
$\psi = 0$	6	6	9	9	12	12
N=145 (upper 95% CI)	$c_f \geq 0$	$c_f = 0$	$c_f \geq 0$	$c_f = 0$	$c_f \geq 0$	$c_f = 0$
$\psi \geq 0$	10	10	12	12	16	16
$\psi = 0$	5	5	5	5	9	9

Table S2. Simulations out of 1000 LHS where endpoint infection prevalence matched observed *Salmonella* prevalence of urban ibis and environmental samples during the non-breeding season, assuming infected ibis at the start of simulations are either all transiently shedding or evenly split between transiently shedding and colonized.

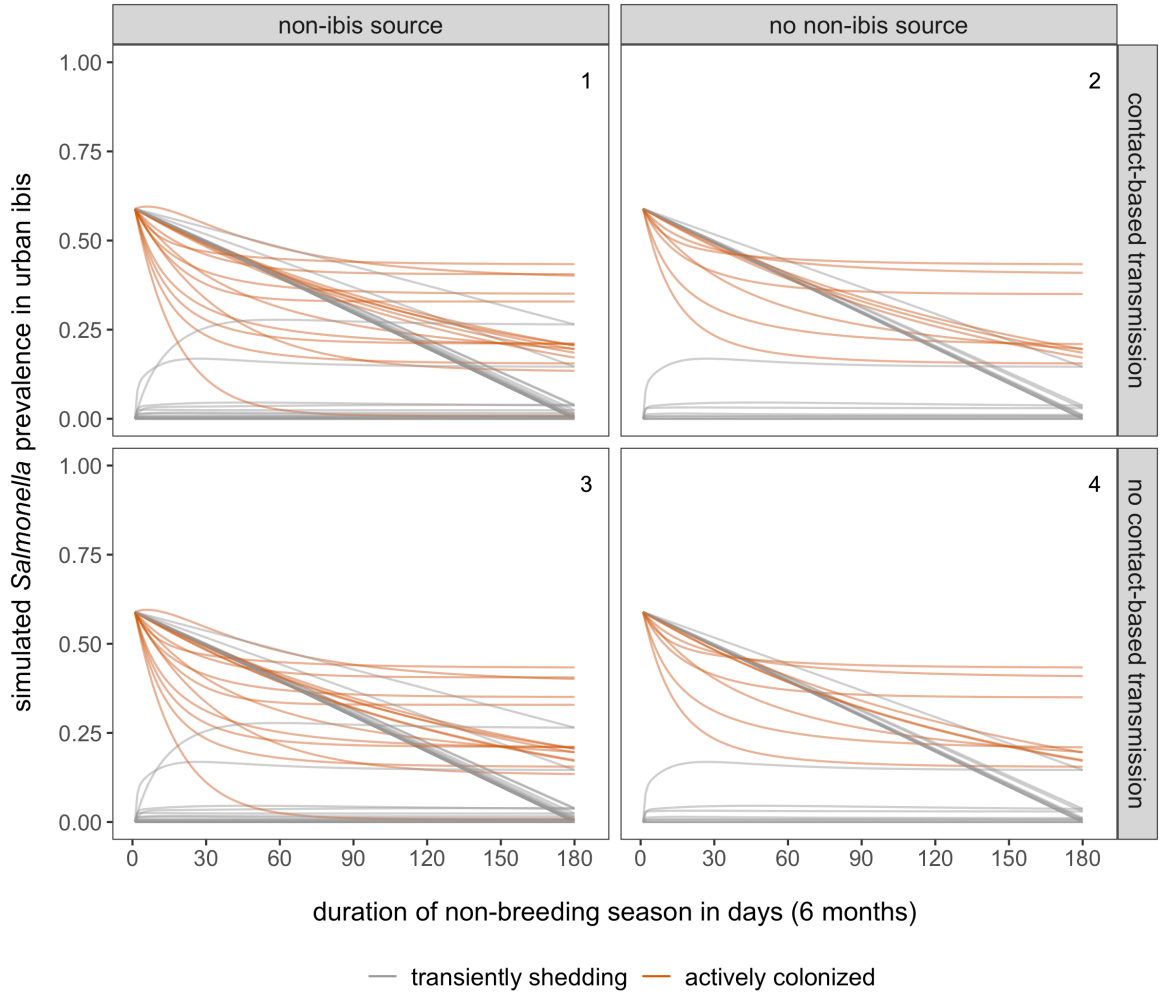
	I_t=20, I_p=20		I_t=40, I_p=0	
N=68 (mean flock size)	$c_f \geq 0$	$c_f = 0$	$c_f \geq 0$	$c_f = 0$
$\psi \geq 0$	11	11	11	11
$\psi = 0$	6	6	6	6

Table S3. Simulations out of 1000 LHS where endpoint infection prevalence matched observed *Salmonella* prevalence of urban ibis only.

N=68 (mean flock size, main text)	**Contact-based transmission ($c_f \geq 0$)**	**No contact-based transmission ($c_f = 0$)**
Non-ibis transmission ($\psi \geq 0$)	104	102
No non-ibis transmission ($\psi = 0$)	87	85

S3. Transiently shedding versus actively colonized ibis

Figure S3. Simulated *Salmonella* prevalence in ibis during the non-breeding season for parameter combinations retained from our LHS procedure. Lines show the simulated infection prevalence time series for transiently shedding (I_t, grey) and actively colonized ibis (I_a, orange) using plausible parameter combinations per model scenarios (indicated by 1–4). Rows show simulations where contact-based transmission was included ($c_f \geq 0$; top) or excluded ($c_f = 0$; bottom). Columns show simulations where non-ibis sources of *Salmonella* were included (left in each panel; $\psi \geq 0$) or excluded (right in each panel; $\psi = 0$). Transiently shedding hosts rarely contribute to the total ibis prevalence compared with actively colonized hosts.



S5. Plausible parameter sensitivity

Figure S4. Violin plots of plausible parameter values from restricting LHS results by observed *Salmonella* prevalence in ibis and the environment. Estimates are shown across model scenarios (indicated by 1–4), including if contact-based transmission was included or excluded ($c_f \geq 0$ and $c_f = 0$) and if non-ibis sources of *Salmonella* were included or excluded ($\psi \geq 0$ and $\psi = 0$). Filled circles indicate the median parameter values for each treatment. Note that for most of the parameters, the median and range of plausible estimates were similar across the four model scenarios (Table S4), with the exception of the shedding rate for colonized ibis (ϕ; χ^2=15.91).

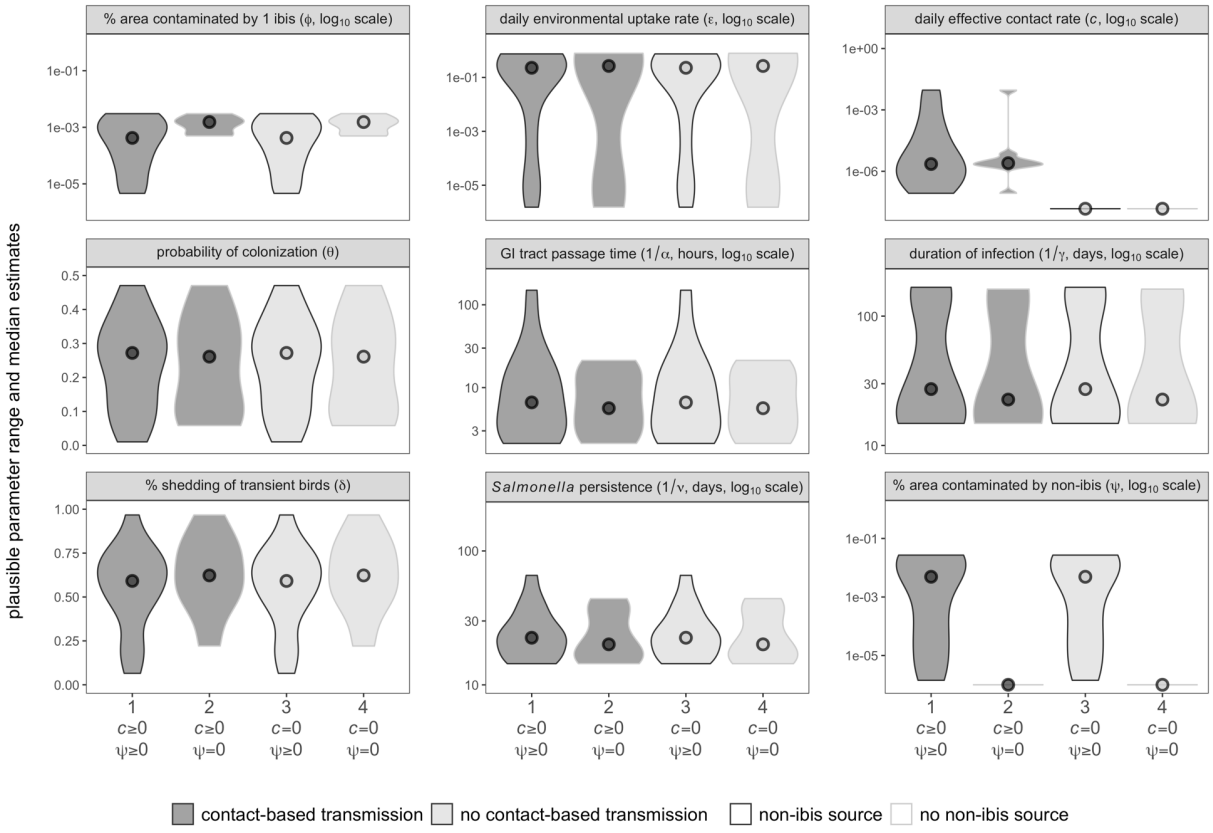


Table S4. Test statistics (χ^2) from GLMs or beta regression models of plausible parameter values predicted by transmission treatments and alternative source treatments.

Treatment	ε	ϕ	θ	α	γ	δ	ν	c_f	ψ
Contact-based transmission	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.00
Non-ibis source	0.09	15.91	0.01	0.01	0.02	1.36	0.27	0.03	NA
Source * transmission	0.00	0.00	0.00	0.00	0.00	0.44	0.00	NA	NA