Epidemiology and Infection

cambridge.org/hyg

Risk factors for veterinary hospital environmental contamination with *Salmonella enterica*

B. A. Burgess[1,2] and P. S. Morley[2]

[1]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA, USA and [2]Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

Original Paper

Cite this article: Burgess BA, Morley PS (2018). Risk factors for veterinary hospital environmental contamination with *Salmonella enterica*. *Epidemiology and Infection* **146**, 1282–1292. https://doi.org/10.1017/S0950268818001164

Received: 4 October 2017
Revised: 22 February 2018
Accepted: 10 April 2018
First published online: 9 May 2018

Key words:
Infectious disease control; *Salmonella*; veterinary epidemiology

Author for correspondence:
Brandy Burgess, E-mail: brandy.burgess@uga.edu.

Abstract

Healthcare-associated infections in veterinary hospitals are commonly attributed to *Salmonella enterica*, particularly in large animal facilities, and are characteristically associated with widespread environmental contamination. The objective of this study was to investigate factors influencing the likelihood of identifying environmental contamination of a veterinary hospital with *S. enterica*, while exploring different analytic methods to model complex factors that may influence this ecology. Environmental surveillance samples were collected in a large veterinary hospital as part of a long-term infection control programme. Data were collected retrospectively from the electronic medical records database. Many easily measured variables were complex in nature (i.e., they represented variance that is unmeasured or unidentified as a specific factor) necessitating the use of alternative analytic methods (variable cluster and principal components analyses) to provide perspective regarding the complex data structure and latent factors that may be contributing to this ecology. Subsequently, multivariable logistic regression was performed using generalised estimating equations. Results suggest the probability of detecting *Salmonella* in the environment increased as demand on personnel increased (e.g., in a busy hospital). Veterinary personnel need to remain vigilant in implementing practices that we believe empirically will mitigate risk for widespread environmental contamination and sustained transmission among patients (i.e., rigorous hygiene for personnel and the environment).

Introduction

Epidemics of healthcare-associated infections (HCAIs) in equine and livestock veterinary hospitals are commonly attributed to *Salmonella enterica* and characteristically there is widespread environmental contamination identified during these events [1–4]. Further, isolates recovered from environmental and patient samples during the same timeframe will frequently be of the same phenotype (i.e., serotype and antimicrobial susceptibility), something that is appreciated during periods of both epidemic and endemic disease occurrences [2, 5]. Although more specific strain typing is less commonly utilised in this surveillance (e.g., PFGE, MLST or other types of genetic typing), these observations suggest that clonal dissemination of *Salmonella* strains between veterinary patients and their care environments is common. Further, these findings also suggest that there is likely a strong relationship between patients and their hospital environment with respect to the ecology of hospital-associated *Salmonella* infections. Despite incorporating rigorous personal and environmental hygiene practices into infection control programmes, widespread outbreaks still occur with high case fatality rates and at considerable financial cost [1–4, 6–8].

S. enterica is an important part of the microbial ecology of large veterinary hospitals, as has been particularly noted in publications regarding veterinary teaching hospitals [2–5, 7–10]. In these large facilities, personnel tend to work in multiple areas (rather than individual areas) and many times the people most likely to contact patients are veterinary students – generally considered to be novices with respect to patient management and infection control practices. Additionally, all hospitals congregate ill and injured animals from many different farms thereby increasing the risk for exposure to shedding patients and infectious disease transmission [11, 12].

Our imperfect understanding of *Salmonella* transmission in veterinary hospitals is typically based on snapshots of sample data that have been obtained during epidemics, or by use of targeted surveillance of animals (i.e., surveillance of high-risk groups) [9, 10, 12]. These data provide a very basic understanding suggesting that patients with severe GI disease (e.g., colic or diarrhoea) or other major systemic illness, and those that have experienced stressful situations are more likely to shed. However, this information does not fully elucidate transmission risks in a hospital as it does not account for the potential of personnel to deviate from standard

prevention protocols during demanding time periods (e.g., increased case-load or personnel shortages) thereby facilitating infectious agent transmission. By developing a deeper understanding of the forces affecting ecological shifts in microbial populations, it may be possible to identify an environmental or hospital 'profile' that marks heightened risks for widespread contamination and sustained transmission among patients. This would allow targeted prevention strategies to be implemented before an outbreak is even detected.

Key questions that may relate to this hospital-related ecology of *Salmonella* include: Are there factors that shift sporadic, endemic *Salmonella* shedding and periodic contamination, to promote nosocomial transmission and epidemic infections with associated widespread (i.e., hospital-wide) environmental contamination? Is the immediate contamination pressure most important (i.e., what happens the day prior to the occurrence of environmental contamination); or is cumulative contamination pressure more likely to drive occurrence of epidemics (i.e., what happens for 30 days prior to the occurrence of contamination)? Further, how do we best measure this contamination pressure in a veterinary hospital? Should we consider caseload (e.g., inpatients or outpatients) and types of patients (e.g., elective surgeries, systemically ill) – or should we consider factors related to the hospital (e.g., numbers of personnel, hospital type, season). When considering variables that we can easily measure, many are complex in nature (i.e., they are 'suitcase' variables that mark exposure to several different factors). In other words, these measured variables represent variance that is not specifically measured or is not identified in relation to a specific exposure factor, and are therefore referred to as latent variables. While we can easily count patients as a measure of caseload, it becomes inordinately more difficult to measure or tabulate the impact that increased caseload has on the actions of personnel. It is also difficult if not impossible to find markers of people's attitudes, such as whether they are likely to adhere to previously implemented protocols and procedures (i.e., poor compliance) and the impact a busy hospital may have on that decision.

Applying traditional regression modelling techniques to large complex datasets with variables that are not fully discriminatory (i.e., represent variance which is not identified with a specific factor) can result in unstable statistical models due to multicollinearity which occurs when individual variables or linear combinations of variables are highly correlated (i.e., not statistically independent) [13]. This can result in unstable parameter estimates (estimates that change drastically during the modelling process), large standard errors (incorrect variance estimates) and difficulties with model convergence. Therefore, consideration should be given to using alternative techniques to aid in model development.

To reduce the impact that multicollinearity may have on statistical modelling, variable reduction techniques such as variable cluster analysis and principal component analysis can be utilised. Both of these approaches can be used to help understand which variables have important effects on the outcome of interest [14, 15]. Variable cluster analysis is a method that groups variables in a manner such that those in a cluster are more similar to each other than to variables in another cluster. In other words, they are occupying similar space in the variance structure. As such, a variable can be selected that best represents the variance that is embodied by that cluster thereby reducing the number of variables used in multivariable modelling while still accounting for the majority of the variance. Principal components analysis is a technique that creates new variables, which are uncorrelated (i.e., orthogonal). Each principal component represents a proportion of variance from many different variables based on the variance space each variable occupies. The proportion of variance from each variable on a component suggests relationships among variables, which may be indicative of latent variables. The new variables created (i.e., the principal components) represent the same amount of variance but do so with fewer variables thus reducing the number of variables used in the multivariable model building process while still describing the majority of the variance. The objective of this study was to investigate factors influencing the likelihood of identifying environmental contamination of a veterinary hospital with *S. enterica*, while exploring different analytic methods to model complex factors that may influence this ecology.

Methods

Study overview

A total of 5273 environmental samples were collected as part of routine, targeted surveillance for *S. enterica* at the Colorado State University Veterinary Teaching Hospital (CSU-VTH). Enriched cultures were performed on environmental samples collected at predetermined sites throughout the small animal, equine and livestock hospitals for the detection of *S. enterica*. Data regarding additional variables of interest were obtained from the electronic medical records database. Multiple analytical approaches were used to investigate factors that may affect environmental contamination, including variable cluster analysis, principal components analysis and generalised linear modelling.

Veterinary teaching hospital operations

The CSU-VTH is an integrated multidisciplinary practice with three interconnected care areas – small animal hospital (including exotics), equine hospital and livestock hospital – operating in connected buildings. While some personnel work in only one of these areas, there is a large degree of integration as some personnel (in particular, 3rd and 4th year veterinary students) move from one hospital area to the others, as do some supplies and equipment. Additionally, there are central services utilised by all three care areas (e.g., central supply, laundry, pharmacy) and some specialties (e.g., cardiology, ophthalmology, dermatology) that work throughout the hospital. While presence of infectious disease or environmental contamination in one care area may be most likely to affect patients managed in that same area, the large degree of integration across the hospital means that these events can also impact disease transmission for other species in other care areas.

Environmental samples

Environmental samples were collected as part of routine, targeted surveillance conducted at the CSU-VTH from March 2003 through January 2013. A total of 5273 environmental samples were collected on 167 different dates at predetermined sites throughout the small animal ($n = 19$), equine ($n = 24$) and livestock ($n = 15$) hospitals (each area of the hospital was sampled approximately monthly on the first Monday through Wednesday of the month) using a commercially available electrostatic dust wipe (Swiffer®, Proctor & Gamble, Cincinnati, Ohio, USA) [5]. There was a median of 30 samples collected at each unique sampling time (range 11–68). Sampling preferentially focused on common use

and high-traffic areas such as hallways, treatment rooms, aisle ways, rounds rooms and staff offices. Sampling locations included hand-contact surfaces, floors and composite samples of hand-contact surfaces and floors (i.e., one wipe was used to sample the floor was cultured together with a different wipe that was used to sample hand-contact surfaces).

All environmental samples were cultured using standardised enriched techniques for detection of *S. enterica*, as previously described [5, 16, 17]. Briefly, samples were pre-enriched in 90 ml buffered peptone water (Becton Dickinson & Company, Cockeysville, Maryland, USA) for 24 h at 43 °C, then 1 ml was passed into 9 ml tetrathionate enrichment broth (Becton Dickinson & Company, Cockeysville, Maryland, USA) for 18 h at 43 °C, then 0.10 ml was passed into 10 ml Rappaport–Vassiliadis R10 broth (RV; Remel, Lenexa, Kansas, USA) for 18 h at 43 °C, then streaked for isolation on XLT4 agar media (Hardy Diagnostics, Santa Maria, California, USA) and incubated for 18 h at 43 °C. Suspect colonies were subcultured on trypticase soy agar containing 5% sheep blood (TSA; BD Diagnostic Systems, Sparks, Maryland, USA) and incubated 18 h at 43 °C and tested for agglutination using commercial polyvalent and O group-specific antisera. Serotype determination was performed by the USDA National Veterinary Services Laboratory (Ames, IA).

Fecal samples

Faecal samples were collected at standardised time points from every large animal inpatient (i.e., all equine and livestock species) throughout the study period as part of routine, targeted surveillance conducted at the CSU-VTH. These samples were collected upon admission of hospitalised patients (i.e., not out-patients), then three times weekly (Mon, Wed, Fri) for the duration of their hospitalisation from February 2003 to June 2003 and twice weekly (Tues, Fri) from July 2003 to June 2013. Additionally, faecal samples were collected from patients that exhibited signs of illness that could be attributed to *Salmonella* infection and cultured to detect *Salmonella*.

All faecal samples were cultured using standardised enrichment techniques to detect the presence of *S. enterica*. Briefly, 1 g faecal samples were enriched in 9 ml tetrathionate broth for 18 h at 43 °C, then streaked for isolation on XLT4 agar media and incubated for 18 h at 43 °C. Suspect colonies were subcultured on trypticase soy agar containing 5% sheep blood and incubated 18 h at 43 °C and tested for agglutination using commercial polyvalent and O group-specific antisera. Serotype determination was performed by the USDA National Veterinary Services Laboratory (Ames, IA).

Faecal culture results for *Salmonella* were interpreted in parallel to classify a patient's culture-status for every hospitalisation (i.e., if $\geqslant$1 culture was positive then the animal was classified as *Salmonella*-positive). The vast majority of in-patients were hospitalised only a single time during the 10-year study period. However, in the occasional instances that animals were admitted and cultured on more than one occasion, each hospital visit was considered an independent event, as it was deemed unlikely that the impact of a specific patient on environmental contamination was likely to cluster by patient, beyond its *Salmonella* culture status. This assumption is based on our experience of monitoring shedding in several hundred horses over the past two decades of our surveillance programme, and, in that time, we have not found that horses which shed *Salmonella* during one visit have an increased likelihood of shedding during subsequent visits, especially when these visits are not temporally proximate. Culture-status for patient groups was evaluated as summary measures (e.g., number of culture-positive patients for a given period) which could be indicative of the contamination pressure on the hospital.

Potential risk factors

Independent variables were derived from invoice data contained within the electronic medical records database as well as data contained within the Infection Control Programme database, both maintained at the CSU-VTH. Data regarding environmental temperatures were obtained from the National Oceanic and Atmospheric Administration (NOAA). See Table 1 for independent variable definitions.

Factors related to hospital characteristics

Variables that related to hospital characteristics included hospital area (small animal hospital, equine hospital, livestock hospital), room use (single animal use (i.e., areas cleaned and disinfected between uses), multiple animal use (i.e., areas that might be used in the care of more than one patient without being cleaned and disinfected between uses such as halls, aisle ways and treatment rooms), personnel use (i.e., areas inaccessible to patients such as offices and records rooms)), sample type (hand-contact surface sample, floor sample, composite of hand and floor), year (2003 through 2013), season (July–October, November–February, March–June), faecal sample frequency (three times per week, two times per week), footwear hygiene (footbath (i.e., areas using dedicated footwear and disinfectant footbaths such as the livestock hospital and equine isolation facility), footmat (i.e., areas using disinfectant footmats with or without dedicated footwear such as inpatient areas of the equine hospital), none (i.e., areas not requiring dedicated footwear or specific footwear hygiene practices such as the small animal hospital)) and occurrence of HCAIs with *Salmonella* during the study period. HCAIs associated with *Salmonella* were defined as events where two or more *Salmonella* isolates with the same phenotype (i.e., serotype and antimicrobial susceptibility) were obtained from patients (not from the same herd/flock) hospitalised within 10 days of each other (i.e., discharge date for one patient was $\leqslant$10 days of admission date for another patient). These were then counted for the week and the month prior to an environmental sampling date. It is important to note that subclinical infections and shedding of *Salmonella* in the absence of disease is much more common than clinical infections among equine and livestock species [2, 18]. As such, the detection of animal shedding among surveillance samples does not necessarily equate to a HCAI.

Factors related to patient population characteristics

Variables representing patient population characteristics that described the immediate contamination pressure (i.e., the day prior to environmental sample date) and the cumulative contamination pressure (i.e., during the month prior to environmental sample date) on the veterinary hospital environment were evaluated.

Variables describing patient population characteristics included the following:

- Immediate contamination pressure – the number of faecal culture-positive inpatients;
- Cumulative contamination pressure – number of culture-positive inpatients and total number of hospitalisation days; and

Table 1. Variable descriptions

Variable		Definition
Hospital factors	Hospital area	Small Animal Hospital, Equine Hospital, Livestock Hospital
	Room use	Personnel use areas, single animal use areas, multi-animal use areas
	Sample type	Type of environmental sample (hand-contact surfaces, floor surfaces, composite samples of hand and floor surfaces)
	Footwear hygiene	footmat, footbath, none
	Faecal culture frequency	Three times per week (March to June 2003) and two times per week (June 2003–2013)
	Season	July–October, November–February, March–June
	Year	2003–2013
	Average temperature	Average outdoor air temperature the month prior to environmental sampling date (minimum, maximum and monthly change)
Population factors by species[a],[b] and species group[c] for the day prior to and the month prior to environmental sampling date		
Case load	Inpatients	Total number of inpatients
	Outpatients	Total number of outpatients
	Total case load	Total case load (inpatients and outpatients)
	Positive patients	Total number faecal culture-positive patients
	Healthcare-associated infections	Total number of identified healthcare-associated infections in patient population
Severity of disease	Level of care days	Total days at each level of care[d] (1–4)
	Hospital days	Total number of hospitalisation days

[a]Species = amphibian, avian, bovine, canine, caprine, exotic large animal, equine, feline, small mammals, New World camelids, other[b], reptile.
[b]Other = caprine, ovine, porcine.
[c]Species Groups = small animal, exotics, equine, food animal.
Small Animal Species Group = canine, feline.
Exotics Species Group = amphibian, avian, small mammals, reptile.
Equine Species Group = equine.
Food Animal Species Group = bovine, caprine, exotic large animal, New World camelid, ovine, porcine.
[d]Level of care: categorised as level 1 (e.g., no intravenous catheter, awaiting elective surgery or discharge, routine care); level 2 (e.g., intravenous catheter, daily treatment and/or assessment approximately every 6 h); level 3 (e.g., intravenous catheter, systemic illness, recovering from surgery, daily treatments and/or assessments approximately every 4 h); and level 4 (e.g., critical care requiring intravenous therapy and daily treatments and/or assessments approximately every 2 h or more frequently).

- Both immediate and cumulative contamination pressure – inpatient case load, outpatient case load, total case load and level of care.

Criticality of a patients conditions were assessed via measures of care intensity or 'level of care', which was categorised as level 1 (e.g., no intravenous catheter, awaiting elective surgery or discharge, routine care); level 2 (e.g., intravenous catheter, daily treatment and/or assessment approximately every 6 h); level 3 (e.g., intravenous catheter, systemic illness, recovering from surgery, daily treatments and/or assessments approximately every 4 h); and level 4 (e.g., critical care requiring intravenous therapy and daily treatments and/or assessments approximately every 2 h or more frequently), as indicated by the medical record.

Patient population characteristic variables were classified by species (ordered from highest to lowest case load), including canine, feline, equine, bovine, small mammals, avian, New World camelid, other livestock (caprine, ovine, porcine), reptiles, exotic large animals and amphibians (see Table 2 for average case load by species). Additionally, patient population characteristics were classified by aggregating in species groups, which included small animal (canine, feline), equine, livestock (bovine, caprine, New World camelid, ovine, porcine, exotic large animal) and exotics (avian, amphibian, reptile, small mammal).

Environmental temperature data

Environmental temperature data were obtained for the study period from the National Climate Data Center from the National Oceanic and Atmospheric Administration (NOAA). Based on data from two different weather stations (Station GHCND: USC00053005 (Fort Collins, CO, USA) and Station GHCND: USC00053006 (Fort Collins, 4 E, CO, USA)), the average minimum, average maximum and average daily change in environmental temperatures were calculated for the month prior to each environmental sampling date.

Data analysis

Data were entered in a spreadsheet, entries were validated (e.g., assessed for accuracy, missing data retrieved), and summarised using descriptive statistics. Continuous variables were assessed for the assumption of linearity in relation to the logit of the *S. enterica* culture status of samples; variables not meeting this assumption were categorised based on distributional quartiles or

Table 2. Median monthly case load (in-patients and out-patients) by species during the study period (2003–2013)

Species	Median number of patients	Range
Amphibian	0	(0–3)
Avian	19	(7–37)
Bovine	32	(19–59)
Canine	1146	(755–1493)
Equine	152	(73–253)
Exotic large animal	0	(0–4)
Feline	206	(127–288)
New World camelid	17	(3–35)
Other (caprine, ovine, porcine)	9	(1–27)
Reptile	8	(0–19)
Small mammal	30	(7–55)

breakpoints with biological relevance. Repeated measures logistic regression was performed using generalised estimating equations to evaluate factors that might be associated with the occurrence of a positive environmental culture; potential clustering of environmental sample results by date was controlled as a repeated measure. The dependent variable for this analysis was a positive environmental culture (yes/no).

In total, there were 272 independent variables that were developed for consideration in logistic regression model development, which necessitated the use of a variable reduction strategy. Initially, all variables were grouped into variable subsets including one subset representing hospital factors and two subsets representing patient population factors consisting of a caseload and a severity of disease subset (Table 1). Univariable screening was performed on all variables with a critical $\alpha \leqslant 0.25$ to be included in subset multivariable screening. Within each variable subset, multivariable screening using backwards selection was performed using a critical $\alpha \leqslant 0.20$ for retention in the subset multivariable model. Consideration was given to *P*-values and quasi-information criteria (QIC) in variable subset selection for inclusion in the final multivariable model-building process. All variables that passed univariable screening were also subjected to variable cluster analysis (PROC VARCLUS) to elucidate the underlying data structure. Variables with the lowest 1-R^2 ratio were selected as the best cluster representative when traditional modelling exhibited model instability (e.g., erratic or non-sensical standard error or parameter estimates) [14]. In addition, all variables that passed univariable screening were also subjected to principal component analysis (PROC PRINCOMP) to assess multicollinearity. Variables loading on principal components with an Eigenvalue >1.0 were also considered when traditional modelling exhibited model instability. Thus, final multivariable model development was based on *a priori* knowledge, biological sense, univariable associations, variable cluster analysis 1-R^2 ratio, variable loading on principal components, QIC and *P*-values.

The final multivariable model was identified using backwards selection with a critical $\alpha \leqslant 0.05$ for retention in the final model. Confounding was identified by ⩾20% change in parameter estimates when previously excluded variables were individually offered back to the multivariable model. When identified, confounding variables were forced into the multivariable models regardless of *P*-values. First-order interaction terms for main effects variables included in final models were also evaluated. Final models were assessed for model stability (e.g., standard errors, model convergence) and a lack-of-fit (e.g., R^2, QIC). Odds ratios (OR) and profile likelihood 95% confidence intervals (95% CI) were calculated using the least-squares mean estimates. All analyses were performed using SAS v9.3 (SAS, Inc., Cary, North Carolina, USA).

Results

During the study period, a total of 5273 environmental samples were collected throughout the VTH (including the small animal, equine and livestock hospitals) at 167 unique sampling dates (generally 32 samples per each unique sampling time (range 11–68; median 30)). The preponderance of which were collected from the equine hospital (41.8%; $n = 2204/5273$) and the remaining being divided between the small animal (30.7%; $n = 1619/5273$) and livestock (27.5%; $n = 1450/5273$) hospitals (Table 3). These samples included 3067 floor surfaces samples, 1321 hand-contact surface samples and 885 samples that were composites of floor and hand-contact surfaces. Of the samples collected, 8.3% ($n = 434/5273$; 95% CI 7.5–9.0) were culture-positive for *S. enterica*. *Salmonella* was detected most frequently in samples collected from the livestock hospital (13.0%; $n = 188/1450$; 95% CI 11.2–14.7) with *S. enterica* contamination being less prevalent in the small animal and equine hospitals, 9.8% ($n = 158/1619$; 95% CI 8.3–11.2) and 4.0% ($n = 88/2204$; 95% CI 3.2–4.8) of samples, respectively.

Many variables characterising the hospital (Table 3), patient population (Table 4) and severity of disease (Table 5) passed univariable screening and were subjected to both variable cluster analysis (Table 6) and principal component analysis (Table 7). Based on data exploration, descriptive statistics and univariable analyses, variables representing the immediate hospital pressure (i.e., day prior to environmental sample date) were eliminated from inclusion in further model development due to the sparse nature of the data. In addition, data was too sparse for modelling of individual species contained within the small animal and exotics species groups. As such, none of the individual small animal and exotics species passed into the multivariable model building process; however, data regarding individual species were considered for equine, bovine and New World camelid patients.

The final multivariable model included bovine positive patient days, New World camelid inpatient caseload, equine outpatient caseload, equine care level 1 caseload and New World camelid care level 2 caseload as main effects and an interaction between sample type and hospital area (Table 8). Season was forced into the model, irrespective of *P*-value, as a potential confounding variable. Final models gave no indications for a lack-of-fit.

Controlling for effects of other variables in the model, the odds of detecting *Salmonella* in the environment was almost two times greater if the preceding month had at least three bovine positive patient days as compared with two or fewer (OR 2.10; 95% CI 1.05–4.22); almost two times greater if the preceding month had at least seven New World camelid inpatients as compared with six or fewer (OR 1.72; 95% CI 0.99–2.98); and almost two times greater if the preceding month had at least six New World camelid patients at a care level 2 as compared with five or fewer (OR 1.87; 95% CI 1.04–3.38). The odds of detecting *Salmonella* in the environment was 1.7 times greater if the

Table 3. Univariable logistic regression results for hospital characteristics associated with hospital environmental contamination with *Salmonella enterica* (listed variables passed initial screening)

Variable	Category	Number positive	Total samples	% positive	95% CI	OR	95% CI	*P*-value
Sample type	Both	78	885	8.8	6.9–10.7	2.23	1.51–3.31	<0.0001
	Floor	296	3067	9.7	8.6–10.7	2.21	1.63–2.99	
	Hand	60	1321	4.5	3.4–5.7	ref		
Hospital	Livestock	188	1450	13.0	11.2–14.7	3.25	1.92–5.49	0.001
	Small animal	158	1619	9.8	8.3–11.2	2.61	1.67–4.07	
	Equine	88	2204	4.0	3.2–4.8	ref		
Use	Multi	246	2514	9.8	8.6–10.9	2.02	1.40–2.91	0.001
	Personnel	158	2038	7.8	6.6–8.9	1.52	1.00–2.30	
	Single	30	721	4.2	2.7–5.7	ref		
Season	July–October	180	1592	11.3	9.7–12.9	2.17	1.17–4.01	0.07
	March–June	153	1739	8.8	7.5–10.1	1.43	0.79–2.57	
	November–February	101	1942	5.2	4.2–6.2	ref		
Healthcare-associated infections	⩾3	33	232	14.2	9.7–18.8	2.15	0.82–5.65	0.13
	1–2	154	1520	10.1	8.6–11.7	1.74	1.01–3.01	
	0	247	3521	7.0	6.2–7.9	ref		

CI, confidence interval; OR, odds ratio; ref, reference.

Table 4. Univariable logistic regression results for patient population characteristics associated with hospital environmental contamination with *Salmonella enterica* (listed variables passed initial screening)

Variable subset	Variable	Category	Total samples	OR	95% CI	*P*-value
Outpatients per month	Bovine	>19	2684	1.61	1.00–2.58	0.06
		⩽19	2589	ref		
	Equine	>86	2579	1.84	1.14–2.96	0.02
		⩽86	2694	ref		
	NWC	>9	2761	1.95	1.20–3.17	0.01
		⩽9	2512	ref		
Inpatients per month	Equine	>50	3935	1.60	0.86–2.96	0.11
		⩽50	1338	ref		
	NWC	>7	2609	1.67	1.03–2.71	0.05
		⩽7	2664	ref		
Positive patients days per month	Bovine	>2	3800	2.01	1.14–3.56	0.01
		⩽2	1473	ref		
	Equine	>1	2613	1.90	1.10–3.28	0.01
		⩽1	2660	ref		
Positive patients per month	Bovine	>1	3739	1.70	1.01–2.88	0.04
		⩽1	1534	ref		
	Equine	>1	2715	1.80	1.04–3.10	0.03
		⩽1	2558	ref		
	NWC	⩾1	787	1.81	0.79–4.12	0.26
		0	4486	ref		

CI, confidence interval; NWC, New World camelid; OR, odds ratio; ref, reference.

Table 5. Univariable logistic regression results for patient severity of disease variables associated with hospital environmental contamination with *Salmonella enterica* (listed variables passed initial screening)

Variable subset	Variable	Category	*N*	OR	95% CI	*P*-value
Level of care[a] (total days at level of care for the month prior to sampling date)	Equine level 1	>73	2729	1.51	0.93–2.45	0.10
		⩽73	2544	ref		
	Equine level 2	>20	4000	2.03	1.19–3.43	0.01
		⩽20	1273	ref		
	Equine level 3	>16	2445	1.52	0.94–2.46	0.10
		⩽16	2828	ref		
	NWC level 2	>5	2548	1.62	0.99–2.64	0.07
		⩽5	2725	ref		
	NWC level 3	⩾1	2459	1.43	0.88–2.32	0.17
		0	2814	ref		
	Other level 1	⩾1	1163	1.84	1.00–3.39	0.11
		0	4110	ref		
	Exotics level 1	>1	3419	0.71	0.44–1.15	0.17
		⩽1	1854	ref		
	Small animal level 2	>87	3953	1.43	0.85–2.39	0.16
		⩽87	1320	ref		
	Small animal level 3	>21	2411	2.05	1.28–3.28	0.01
		⩽21	2862	ref		
	Small animal level 4	>2	2281	0.69	0.43–1.11	0.13
		⩽2	2992	ref		
Hospitalisation days per month	Equine	>219	3910	2.21	1.15–4.26	0.01
		⩽219	1363	ref		
	NWC	>14	3860	1.99	1.14–3.48	0.01
		⩽14	1413	ref		
	Small animal	>655	2583	1.47	0.91–2.37	0.12
		⩽655	2690	ref		

CI, confidence interval; NWC, New World camelid; OR, odds ratio; ref, reference.
[a]Level of care: categorised as level 1 (e.g., no intravenous catheter, awaiting elective surgery or discharge, routine care); level 2 (e.g., intravenous catheter, daily treatment and/or assessment approximately every 6 h); level 3 (e.g., intravenous catheter, systemic illness, recovering from surgery, daily treatments and/or assessments approximately every 4 h); and level 4 (e.g., critical care requiring intravenous therapy and daily treatments and/or assessments approximately every 2 h or more frequently).

preceding month had at least 86 equine outpatients as compared with 85 or fewer (OR 1.86; 95% CI 1.01–3.43); and was approximately two times greater if the preceding month had least 74 equine patients at a care level 1 as compared with 73 or fewer (OR 2.24; 95% CI 1.25–4.00).

In general, environmental samples collected in the livestock hospital, and those collected from floors, had a greater likelihood of being culture-positive. Within the livestock hospital, the odds of detecting *Salmonella* was almost two times greater for floor samples (OR 1.81; 95% CI 1.11–2.95) and for composite samples (OR 2.79, 95% CI 1.48–5.27) as compared with hand-contact surface samples. Within the equine hospital, the odds of detecting *Salmonella* was also greater for floor samples (OR 1.50; 95% CI 1.07–2.10), but was less likely for composite samples (OR 0.81; 95% CI 0.27–2.43), as compared with hand-contact surface samples. Within the small animal hospital, the odds of detecting *Salmonella* in the environment was considerably greater for floor samples (OR 6.12; 95% CI 2.42–15.48) and composite samples (OR 2.90, 95%CI 0.85–9.96) as compared with hand-contact surface samples – likely related to central services incorporated into the small animal hospital that also service both the equine and livestock hospitals.

Discussion

This study demonstrates the complex ecology of *Salmonella* in a veterinary hospital emphasising the role that latent (unmeasured) factors may play in driving low-level endemic environmental contamination to become hospital-wide and ultimately promote epidemic disease development. In general, the probability of detecting *Salmonella* in the hospital environment was associated with type of veterinary hospital and patient population characteristics. Some of the factors describing contamination risk are readily definable, such as hospital type (e.g., the livestock hospital) or species (i.e., large animal species) and many that can be easily measured and quantified, such as the number of days a hospitalised patient was

Table 6. Variable cluster analysis for variables associated with veterinary hospital environmental contamination with *Salmonella enterica*

Cluster	Variable	R^2 with Own Cluster	R^2 with Next Closest Cluster	$1–R^2$ Ratio
Cluster 1: equine patient population	Equine outpatients	0.5061	0.1715	0.5961
	Equine inpatients	0.5535	0.0988	0.4954
	Equine care level[a] 1	0.3586	0.086	0.7017
	Equine care level 2	0.4617	0.0731	0.5808
	Equine care level 3	0.3941	0.0846	0.6619
	Equine hospitalisation days	0.5974	0.2651	0.5479
	Small animal hospitalisation days	0.3083	0.1479	0.8117
Cluster 2: livestock patient population	Healthcare-associated infections	0.4761	0.1323	0.6038
	Bovine positive days[b]	0.6666	0.0498	0.3509
	Bovine positive patients[c]	0.7281	0.0815	0.296
	NWC positive patients	0.2276	0.0477	0.8111
Cluster 3: equine patient shedding	Small animal care level 2	0.0361	0.0094	0.973
	Equine positive days	0.9699	0.0749	0.0325
	Equine positive patients	0.9742	0.0929	0.0284
Cluster 4: New World camelid patient population	Sample type[d]	0.1692	0.0543	0.8785
	NWC outpatients	0.519	0.2384	0.6316
	NWC inpatients	0.7163	0.1841	0.3477
	NWC hospitalisation days	0.6381	0.1582	0.4298
Cluster 5: severity of disease	NWC care level 2	0.5961	0.1714	0.4874
	NWC care level 3	0.4889	0.0504	0.5382
	Other care level 1	0.4982	0.0491	0.5277
	Small animal care level 3	0.4782	0.2008	0.6529
Cluster 6: season	Season	0.5769	0.0561	0.4483
	Bovine outpatients	0.5558	0.062	0.4736
	Small animal care level 4	0.1792	0.0315	0.8475
Cluster 7	Use[e]	1	0.1405	0
Cluster 8	Hospital[f]	1	0.1405	0
Cluster 9	Exotics care level 1	1	0.0139	0

NWC, New World camelid.
[a]Level of care: categorised as level 1 (e.g., no intravenous catheter, awaiting elective surgery or discharge, routine care); level 2 (e.g., intravenous catheter, daily treatment and/or assessment approximately every 6 h); level 3 (e.g., intravenous catheter, systemic illness, recovering from surgery, daily treatments and/or assessments approximately every 4 h); and level 4 (e.g., critical care requiring intravenous therapy and daily treatments and/or assessments approximately every 2 h or more frequently).
[b]Positive days = number of hospital days attributed to *Salmonella*-positive patients.
[c]Positive patients = case load attributed to *Salmonella*-positive patients.
[d]Sample type = hand-contact surfaces, floor surfaces, composite samples (both hand-contact and floor surfaces).
[e]Use = used by multiple patients (multi), used by a single patient (single), used by personnel only (personnel).
[f]Hospital = small animal, equine or livestock; NWC = New World camelid.

shedding *Salmonella* (i.e., positive patient days; specifically, bovine) and caseload (i.e., New World camelid inpatients and equine outpatients). The results of this study also suggest that the probability of detecting *Salmonella* in the environment increases as the demand on personnel increases (i.e., a busy hospital). We considered factors related to increased demand to be latent (unmeasured) variables which contribute to the complex hospital ecology but that cannot be easily measured – the so-called 'human effect.' For example, how personnel respond to increased demand with respect to cleaning frequency and number of patient contacts, which are integral in infectious agent transmission, and compliance with established protocols. While we have previously been limited in our investigations to data derived from epidemic disease and that generated from targeted surveillance, the study reported here provides some insight into the complicated nature of the environment in which we practice veterinary medicine on a daily basis.

Modelling complex relationships naturally leads to complex data structures – something that must be accounted for in statistical model development. By using variable cluster analysis, we can gain an appreciation for the data's structural complexity. In the present study, variables regarding the same species tended to cluster together (Table 6). For example, equine outpatient

Table 7. Principal component analysis for variables associated with veterinary hospital environmental contamination with *Salmonella enterica*

Variable	Variable type	Prin1	Prin2	Prin3	Prin4
Bovine outpatients	Case load	0.109	−0.047	−0.023	0.202
Equine inpatients	Case load	0.291	−0.087	−0.007	0.162
Equine outpatients	Case load	0.338	0.027	0.003	0.060
NWC inpatients	Case load	0.223	0.075	0.038	0.016
NWC outpatients	Case load	0.243	0.013	0.110	0.008
Equine care level[a] 3	Disease severity	0.233	−0.113	0.118	0.219
Equine care level 2	Disease severity	0.260	−0.095	−0.030	0.215
Small animal care level 2	Disease severity	0.021	0.073	0.004	0.192
Exotics care level 1	Disease severity	0.003	−0.002	−0.228	0.174
Other care level 1	Disease severity	−0.032	0.224	−0.110	0.144
Equine care level 1	Disease severity	0.210	−0.153	0.118	0.115
Small animal care level 3	Disease severity	0.030	0.387	−0.053	0.091
Equine hospitalisation days	Disease severity	0.314	−0.031	0.056	0.049
NWC hospitalisation days	Disease severity	0.188	0.126	−0.031	−0.011
NWC care level 2	Disease severity	0.021	0.319	−0.056	−0.043
NWC care level 3	Disease severity	−0.024	0.273	−0.074	−0.065
Small animal hospitalisation days	Disease severity	0.225	−0.170	−0.031	−0.095
Small animal care level 4	Disease severity	−0.059	0.034	0.022	−0.132
Sample type[b]	Hospital	−0.100	0.084	0.282	0.679
Season	Hospital	−0.142	0.092	0.164	0.243
HCAIs	Hospital	0.095	0.311	0.055	0.007
Use[c]	Hospital	0.010	−0.008	0.597	−0.124
Hospital[d]	Hospital	0.006	−0.013	0.594	−0.221
NWC positive patients[e]	Positive patients	0.010	0.231	0.117	0.097
Equine positive patients	Positive patients	0.160	0.196	−0.098	0.033
Equine positive patient days[f]	Positive patients	0.176	0.193	−0.106	−0.008
Bovine positive patients	Positive patients	−0.063	0.387	0.101	−0.033
Bovine positive patient days	Positive patients	0.025	0.331	0.110	−0.149

HCAIs, healthcare-associated infections; NWC, New World camelid; Prin, principle component.
[a]Level of care: categorised as level 1 (e.g., no intravenous catheter, awaiting elective surgery or discharge, routine care); level 2 (e.g., intravenous catheter, daily treatment and/or assessment approximately every 6 h); level 3 (e.g., intravenous catheter, systemic illness, recovering from surgery, daily treatments and/or assessments approximately every 4 h); and level 4 (e.g., critical care requiring intravenous therapy and daily treatments and/or assessments approximately every 2 h or more frequently).
[b]Sample type = hand-contact surfaces, floor surfaces, composite samples (both hand-contact and floor surfaces).
[c]Use = used by multiple patients (multi), used by a single patient (single), used by personnel only (personnel).
[d]Hospital = small animal, equine, or livestock; NWC = New World camelid.
[e]Positive patients = case load attributed to *Salmonella*-positive patients.
[f]Positive days = number of hospital days attributed to *Salmonella*-positive patients.

caseload and inpatient caseload resided in the same cluster as did New World camelid outpatient caseload and inpatient caseload; as a result, multivariable models containing the previous two or the latter two demonstrated characteristics of model instability. Thus, a single variable from each was selected as the best representative to move forward in the model-building process. While the use of variable cluster analysis does reduce the number of variables considered in the analysis there can still be unidentified variables at play in the underlying data structure.

The use of principal components analysis was very beneficial in understanding the correlation in variance structure related to similar variables, and showed that factors in this study that were easily measured tended to be complex in nature (i.e., 'suitcase' variables that represented exposure to several different factors). While we were limited to these imperfect measures, use of alternative analytic methods (such as principal components analysis) allowed us to gain an understanding of which variables actually represented unmeasured latent variables that may be contributing to the overall ecology. In the present study, there were four principal components associated with the outcome – each explaining the variance as described by a unique constellation of variables (Table 7). For example, the first-principal component represented equine and New World camelid caseload and disease severity; the second represented presence of culture-positive patients and recognised occurrence of HCAIs; the third represented hospital factors including the type of use for an area and

Table 8. Final multivariable logistic regression model of factors associated with veterinary hospital environmental contamination with *Salmonella enterica*

Variable	Category	OR	95% CI	*P*-value
Bovine positive patient days (mos prior to sampling date)	⩾3 days	1.84	0.99–3.42	0.04
	⩽2 days	ref		
NWC inpatients (mos prior to sampling date)	⩾7 patients	1.99	1.18–3.36	0.01
	⩽6 patients	ref		
Equine outpatients (mos prior to sampling date)	⩾86 patients	1.79	1.01–3.19	0.057
	⩽85 patients	ref		
Equine care level 1 (mos prior to sampling date)	⩾74 patients	2.24	1.25–4.00	0.01
	⩽⩽73 patients	ref		
New World camelid care level 2 (mos prior to sampling date)	⩾6 patients	1.87	1.04–3.38	0.04
	⩽5 patients	ref		
Sample type	Composite	Interaction		0.03
	Floor			
	Hand-contact	ref		
Hospital	Livestock	Interaction		0.11
	Small animal			
	Equine	ref		
Season (Confounder)	July–October	1.36	0.66–2.82	0.65
	March–June	1.08	0.52–2.23	
	November–February	ref		
Sample type × hospital				0.002
Hand-contact sample	Livestock	2.43	1.05–5.63	
	Small animal	0.69	0.23–2.05	
	Equine	ref		
Composite sample	Livestock	8.38	2.63–26.73	
	Small animal	2.48	0.78–7.92	
	Equine	ref		
Floor sample	Livestock	2.92	1.59–5.36	
	Small animal	2.81	1.74–4.56	
	Equine	ref		
Equine	Floor sample	1.50	1.07–2.10	
	Composite sample	0.81	0.27–2.43	
	Hand-contact sample	ref		
Livestock	Floor sample	1.81	1.11–2.95	
	Composite sample	2.79	1.48–5.27	
	Hand-contact sample	ref		
Small animal	Floor sample	6.12	2.42–15.48	
	Composite sample	2.90	0.85–9.96	
	Hand-contact sample	ref		

CI, confidence interval; NWC, New World camelid; OR, odds ratio; ref, reference.

type of hospital; and the final component also represented hospital factors including the type of environmental sample collected and season that were associated with the probability of detecting environmental contamination.

The results of this study reflect the complexity of ecological factors that can perhaps change endemic levels of contamination to become hospital-wide and ultimately promote development of epidemics of HCAIs. This study suggests that the probability of

detecting *Salmonella* in the environment of the CSU-VTH is associated with livestock caseload, patient disease severity, the presence of patients shedding *Salmonella*; and its detection is affected by the types and locations of environmental samples tested. It is important to note that the livestock caseload at the CSU-VTH represents a patient population with a relatively high prevalence of *Salmonella* shedding, emphasising the important role the presence of positive patients may play in hospital-wide contamination. While not all hospitals will have a livestock caseload, each hospital should appropriately manage any patient group that has a high prevalence of *Salmonella* shedding to decrease the likelihood of extensive environmental contamination and resultant HCAIs.

The results of this study also demonstrated the complexity of this relationship by highlighting the difficulties in using imperfect measures upon which to base interpretations. In actuality, there are unmeasurable latent factors that likely represent the human effect – that increased demand on personnel during times of high caseload and when caring for compromised patients' likely affects compliance with infection control practices and creates more opportunity to transmit infectious agents between patients and among facilities. It is at these times that veterinarians and facility managers need to remain vigilant that infection control practices are being rigorously conducted, especially those related to improving hygiene of the hospital environment and personnel, and also those that promote segregation of high-risk patients as a means of mitigating widespread environmental contamination of the veterinary hospital environment.

Acknowledgement. This work was completed at Colorado State University with approval by the Institutional Animal Care and Use Committee.

Disclaimers. None.

Financial support. This study was supported by the James L. Voss Veterinary Teaching Hospital, Colorado State University, and the Storm Cat Career Development Award, Grayson-Jockey Club Research Foundation, Lexington, KY.

Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of animals.

References

1. **Benedict KM, Morley PS and Van Metre DC** (2008) Characteristics of biosecurity and infection control programs at veterinary teaching hospitals. *Journal of the American Veterinary Medical Association* **233**, 767–773.
2. **Steneroden KK *et al.*** (2010) Detection and control of a nosocomial outbreak caused by *Salmonella newport* at a large animal hospital. *Journal of Veterinary Internal Medicine* **24**, 606–616.
3. **Dallap Schaer BL, Aceto H and Rankin SC** (2010) Outbreak of salmonellosis caused by *Salmonella enterica* serovar Newport MDR-AmpC in a large animal veterinary teaching hospital. *Journal of Veterinary Internal Medicine* **24**, 1138–1146.
4. **Tillotson K *et al.*** (1997) Outbreak of Salmonella infantis infection in a large animal veterinary teaching hospital. *Journal of the American Veterinary Medical Association* **211**, 1554–1557.
5. **Burgess BA, Morley PS and Hyatt DR** (2004) Environmental surveillance for *Salmonella enterica* in a veterinary teaching hospital. *Journal of the American Veterinary Medical Association* **225**, 1344–1348.
6. **Dargatz DA and Traub-Dargatz JL** (2004) Multidrug-resistant *Salmonella* and nosocomial infections. *Veterinary Clinics of North America Equine Practice* **20**, 587–600.
7. **Carter JD *et al.*** (1986) Salmonellosis in hospitalized horses: seasonality and case fatality rates. *Journal of the American Veterinary Medical Association* **188**, 163–167.
8. **Mainar-Jaime RC *et al.*** (1998) Influence of fecal shedding of *Salmonella* organisms on mortality in hospitalized horses. *Journal of the American Veterinary Medical Association* **213**, 1162–1166.
9. **Ekiri AB *et al.*** (2009) Epidemiologic analysis of nosocomial *Salmonella* infections in hospitalized horses. *Journal of the American Veterinary Medical Association* **234**, 108–119.
10. **Kim LM *et al.*** (2001) Factors associated with *Salmonella* shedding among equine colic patients at a veterinary teaching hospital. *Journal of the American Veterinary Medical Association* **218**, 740–748.
11. **Christley RM and French NP** (2003) Small-world topology of UK racing: the potential for rapid spread of infectious agents. *Equine Veterinary Journal* **35**, 586–589.
12. **Burgess BA and Morley PS** (2013) Factors associated with large animal inpatient shedding of *Salmonella enterica* in a veterinary teaching hospital. In *Proceedings of the 94th Annual Conference of Research Workers in Animal Diseases*. Chicago, p. 136.
13. **Dohoo I, Martin W and Stryhn H** (2009) Detecting highly correlated (collinear) variables. In *Veterinary Epidemiologic Research*. Charlottetown: Prince Edward Island: VER, Inc., p. 338.
14. **Sanche R and Lonergan K** (2006) Variable reduction for predictive modeling with clustering. In *Proceedings of the Casualty Actuarial Society Forum*. Arlington, VA, pp. 89–100.
15. **Dohoo IR** (1997) An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. *Preventive Veterinary Medicine* **29**, 221.
16. **Ruple-Czerniak A *et al.*** (2014) Comparison of two sampling and culture systems for detection of *Salmonella enterica* in the environment of a large animal hospital. *Equine Veterinary Journal* **46**, 499–502.
17. **Burgess BA *et al.*** (2015) Rapid Salmonella detection in experiemtnally-inoculated equine feces and veterinary hospital environmental samples using commercially available lateral flow antigen detection systems. *Equine Veterinary Journal* **47**, 119–122.
18. **Palmer JP, Benson CE and Whitlock RH**. (1982) Subclinical salmonellosis in horses with colic. In *Proceedings of the Equine Colic Research Symposium*. Athens, GA: American Association of Avian Pathologists, University of Pennsylvania, pp. 161–164.