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Analyses of Livestock Production, Waste Storage, and Pathogen Levels and Prevalences in Farm Manures

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Survey results describing the levels and prevalences of zoonotic agents in 1,549 livestock waste samples were analyzed for significance with livestock husbandry and farm waste management practices. Statistical analyses of survey data showed that livestock groups containing calves of <3 months of age, piglets, or lambs had higher prevalences and levels of *Campylobacter* spp. and *Escherichia coli* O157 in their wastes. Younger calves that were still receiving milk, however, had significantly lower levels and prevalence of *E. coli* O157. Furthermore, when wastes contained any form of bedding, they had lowered prevalences and levels of both pathogenic *Listeria* spp. and *Campylobacter spp*. Livestock wastes generated by stock consuming a diet composed principally of grass were less likely to harbor *E. coli* O157 or *Salmonella* spp. Stocking density did not appear to influence either the levels or prevalences of bacterial pathogens. Significant seasonal differences in prevalences were detected in cattle wastes; *Listeria* spp. were more likely to be isolated in March to June, and *E. coli* O157 was more likely to be found in May and June. Factors such as livestock diet and age also had significant influence on the levels and prevalences of some zoonotic agents in livestock wastes. A number of the correlations identified could be used as the basis of a best-practice disposal document for farmers, thereby lowering the microbiological risks associated with applying manures of contaminated livestock to land.

Although cases of gastroenteritis and food-borne illness have decreased over the last 5 years in the United Kingdom, there are still an estimated 1.34 million cases annually in England and Wales (1). The infectious agents that cause most illness are *Campylobacter* spp., *Salmonella* spp., verotoxigenic *Escherichia coli* O157, *Listeria monocytogenes*, and *Cryptosporidium parvum* (1, 22, 23). A number of authors have commented on the fact that these organisms are zoonotic agents that can be shed into the fecal wastes generated by infected livestock (17, 22). In the United Kingdom livestock wastes are disposed of by spreading to agricultural land, where they provide a source of nutrients for crops grown in some soil types. The contribution to food-borne illness made by spreading livestock wastes contaminated with zoonotic agents to land used for the production of food is currently unclear (2, 22).

A number of surveys have characterized the prevalences of these zoonotic agents in individual animals on farms or at slaughter (4, 21, 27). Such studies have provided an excellent indication of the overall infection status of farmed livestock. However, in order to properly assess the risks to food safety posed by the fecal waste generated by infected livestock, information describing the levels of pathogens present in contaminated manure is required. The levels and incidence of zoonotic agents within British livestock wastes were determined for the first time by a recent national survey (14, 16). There are currently found in livestock wastes measurable prevalences (16) of the five pathogens responsible for most of the cases of gastroenteritis in the United Kingdom (1, 5, 17).

At the same time as wastes were collected from farms for microbiological and chemical analyses during the survey (16), additional information regarding waste management practices and stock husbandry was gathered. In this study we summarize the farming practices encountered on British farms between 2000 and 2003. In addition, we report the results of statistical analyses, which have revealed correlations between on-farm practices and the prevalences or levels of zoonotic agents. The results of these analyses provide useful information that could become the basis for guidelines aimed at assisting farmers. Large quantities of livestock waste are disposed of to agricultural land in the United Kingdom, and, thus, small reductions in either the prevalences or levels of zoonotic agents would significantly lower the risk of pathogen dissemination from the spreading of manures.

MATERIALS AND METHODS

Collection of livestock wastes. The selection and weighting criteria used to draw up the sampling plan have been previously reported (16). The majority of samples were collected during winter (November to March) when most animals were housed. Waste samples (n = 1,549) were collected almost continually between April 2000 and December 2002. A United Kingdom-wide outbreak of foot-and-mouth disease interrupted waste collections between February and July 2001. Each livestock waste sample was collected on farm and was derived from a group of animals living in a herd or flock. Samples were all statistically independent in the sense that only a single sample was collected from each waste store or livestock house on each farm that was visited.

Wastes were categorized into two types by age, whether fresh or stored, according to criteria described previously (16). Samples were collected by taking a minimum of 30 handfuls, each of approximately 10-g mass, of farmyard manure from houses or farmyard manure storage heaps by using gloved hands. Handfuls were collected from different depths in the material. An identical number (n = 30) of slurry or dirty water subsamples was collected, again from different areas and depths of each slurry vessel, and combined to form a single liquid waste sample. Detailed protocols, conditions for shipping to the laboratory, and the laboratory analysis methodologies have been reported previously (16). Detections referred to as *Listeria* spp. were *L. monocytogenes* and *Listeria ivanovii*.

Additional information collected for samples. The purpose and design of the survey were to determine prevalences and levels of zoonotic agents in livestock

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TABLE 1. A summary of the levels and prevalences of zoonotic agents measured in British livestock manures

Zoonotic agent	Parameter	Prevalences (%) and levels (CFU g^{-1}) of zoonotic agents in fresh and stored livestock waste[a]							
		Cattle		Pig		Poultry		Sheep	
		Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
E. coli O157	P	13.2	9.1	11.9	15.5	ND	ND	20.8	22.2
	G	1.2×10^3	2.6×10^2	3.9×10^3	1.3×10^3			7.8×10^2	2.5×10^2
Salmonella spp.	P	7.7	10.0	7.9	5.2	17.9	11.5	8.3	11.1
	G	2.1×10^3	2.5×10^3	6.0×10^2	6.1×10^2	2.2×10^2	4.0×10^3	7.1×10^2	5.8×10^3
Listeria spp.	P	29.8	31.0	19.8	19.0	19.4	15.4	29.2	44.4
	G	1.1×10^3	1.1×10^3	3.1×10^3	6.1×10^2	8.3×10^2	3.3×10^2	2.0×10^2	3.0×10^2
Campylobacter spp.	P	12.8	9.8	13.5	10.3	19.4	7.7	20.8	11.1
	G	3.2×10^2	5.3×10^2	3.1×10^2	1.6×10^3	2.6×10^2	5.9×10^2	3.9×10^2	1.0×10^2
C. parvum	P	5.4	2.8	13.5	5.2	ND	ND	29.2	—
	G	1.9×10^1	1.0×10^1	5.8×10^1	3.3×10^1			1.0×10^1	—

[a] Data shown are the percentage of positive isolations (P) and the geometric mean (G) level of each zoonotic agent calculated from the positive samples only. Poultry wastes were not tested (ND) for *E. coli* O157 or *C. parvum.* No positive isolations (−) were made for *C. parvum* in stored sheep wastes.

manure. However, we attempted to maximize the usefulness of these farm visits by collecting information on the livestock and their husbandry by face-to-face interviews with farmers or farm managers at the same time as manure samplings were collected. The sampling staff that participated in this study are employed to collect statutory water and milk samples from farms and thus were well known to the majority of staff that were interviewed. The additional information gathered included livestock breed, gender, age, stocking density, diet, use of antimicrobial drugs, bedding materials, and age of oldest manure in a store or house at the time of sampling. In addition, information describing either the type of livestock housing (fresh samples) or store (stored samples) was noted.

Storage and analysis of questionnaire data. A custom-coded relational database (Microsoft Access 97) was constructed that allowed the data on the questionnaires to be entered into a computer for analysis. Questionnaire responses were entered into the database in a standardized and strictly defined manner. Laboratory analysis data (pathogen levels and chemical analysis results) were also entered into the database and linked with the corresponding questionnaire data. Data entry was validated by dual entry and direct comparison of the independently entered data sets. Custom queries were coded to identify and summarize individual subsets of the main data.

Statistical analysis of results. Subsets of data were exported from Microsoft Access and imported directly into a statistical analysis program (StatsDirect; Cheshire, United Kingdom). Mann-Whitney U tests for nonparametric data were used to compare pathogen levels in each subset of livestock wastes. Chi-squared or Fisher's exact tests were used, as appropriate, to test significance of presence-absence data. For normally distributed data, *t* tests, paired *t* tests, and analysis of variance were used. For all tests, a *P* value of <0.05 was used for significance. Correlation coefficients were calculated with Microsoft Excel by the least-squares method.

RESULTS AND DISCUSSION

Although questionnaires were specifically written for our survey, record keeping on some farms is poor, and the answers to some questions could not be obtained. For this reason, there were minor variations between the numbers of useable fields in seemingly identical record sets.

A comprehensive report of the levels and prevalences of zoonotic agents in British livestock manures and the physicochemical composition of the waste have been reported previously (16). An overview of this data is presented as Table 1.

Age of stored wastes. It has been widely reported that levels of zoonotic agents in stored livestock wastes decline over time (12, 13, 18, 32). The age of the oldest material in the waste stores sampled during the survey were grouped into one of five bins as follows: <1 month, 1 to 3 months, 3 to 6 months, 6 to 9 months, and >9 months. When we compared the pathogen levels between each of these manure groups, no significant differences were identified. A likely explanation for this apparent contradiction was that most farms do not manage their waste stores as batch operations. In reality, most stores are subject to constant additions of waste (Table 2) (25, 26) and, thus, potentially, continual additions of pathogens. Such practices have implications for food safety because there were no significant differences between the pathogen levels in stores containing wastes that were designated 1 to 3 months old and those of 6 to 9 months of age. Therefore, the age of the oldest manure in a store was not a reliable indicator of low levels or absence of zoonotic agents.

TABLE 2. Summary of the on-farm management of stored livestock wastes collected for this study

Type of stored waste	% of wastes from each animal species[a]			
	Cattle (n = 428)	Pigs (n = 58)	Poultry (n = 26)	Sheep (n = 9)
Stores to which fresh manures are continually added	69.3	81.0	53.8	0.0
Stored manures that contained bedding materials	84.0	75.0	50.0	100.0
Stores that are never turned, stirred, or otherwise aerated	90.1	91.4	88.5	88.9

[a] n is the number of samples analyzed for each set of data.

Significance of waste store type. It is likely that different types of waste stores allow different degrees of aeration of wastes, that different surface area-to-volume ratios will influence waste temperatures, and that other store-specific parameters could influence the levels or prevalences of zoonotic agents in livestock wastes. To determine if this was the case, analyses of pathogen levels and prevalences were undertaken on stored wastes (n = 522) grouped by store type (Table 3). Aboveground tanks and slurry lagoons, both of which tend to be uncovered, had significantly lower levels of pathogenic *Listeria* spp. in comparison to other store types. Weeping-wall stores are large slurry lagoons which have a wall constructed of poorly fitting concrete slabs built across one end. Liquid manure with a very low dry matter content strains through this wall, leaving behind an easily transportable mass of dry biosolids. Strainer boxes operate on a smaller scale than weeping-wall stores but according to a similar principle. Analyzed together, solid wastes from weeping-wall stores and strainer boxes had significantly lower levels of *Cryptosporidium*. The

TABLE 3. Types of storage most commonly used for wastes after removal from livestock housing for stored wastes collected during this study

Store type[a]	Percentage (%) of total no. of samples (n = 522)[b]
Heap on pad or yard	30.8
Field heap	27.2
Lagoon	15.1
Weeping-wall store	8.2
Below-ground tank	6.3
Above-ground tank	5.6
Strainer box	1.1
Covered shed	1.1

[a] Weeping-wall stores are large slurry lagoons that have a wall constructed of poorly fitting concrete slabs built across one end. Liquid manure with a very low dry matter content strains through this wall, leaving behind an easily transportable mass of dry biosolids. Strainer boxes, which use a similar principle, operate on a smaller scale than weeping-wall stores.

[b] n, total number of samples analyzed.

combined number of these stores was quite small (n = 50) compared with the remainder of the stored data set (n = 472), and thus the significance should be viewed cautiously. However, both of these store types allow liquid fractions from the waste to drain away from the solids. A possible explanation for this finding is that oocysts separate with the liquid waste fraction. Oocysts have been shown previously to partition and travel with moisture in solid matrices such as soil (6). There were no significant differences in pathogen prevalences between different store types.

Age of fresh wastes. Analyses of fresh wastes collected from the pen or house where they were deposited were subject to similar problems as those encountered for stored samples regarding the age of the waste samples. In keeping with the classification established by Smith et al. (25, 26), samples were designated as fresh if they had not been moved from the housing where they were deposited. We were able to obtain accurate records of when the livestock housing was last cleared of all fecal material (16). Although it was not always the case, most fresh waste samples were of mixed age because the wastes were continually deposited by housed stock. However, some fresh wastes were also taken from housing after stock had been turned out to pasture after winter but before the farmer had cleaned out the housing. Such material was not subject to the potential addition of fresh zoonotic agents. Furthermore, some fresh wastes were collected from housing where stock had the choice of being indoors or outdoors, and as such the wastes from these farms would have fresh additions made at a lower frequency than when stock were exclusively housed. For these reasons, there were few significant correlations between fresh waste pathogen levels and the age of the waste. *Salmonella* levels, however, were significantly lower in fresh wastes older than 31 days in comparison to levels in wastes collected from houses that had been cleaned out within the previous month. Mean log levels were 1.65 log and 2.03 log CFU/g of waste, respectively.

Stock age. There were a number of relationships identified if the age of the fresh material was not considered and the fresh wastes were treated as a single data set and analyzed en masse. Previously, other authors have reported higher prevalences for *E. coli* O157 among groups of cattle containing calves (10, 21, 30, 33). Comparison of pathogen levels in fresh wastes generated by groups of stock containing either calves (<3 months), lambs, or piglets (n = 158) and groups without younger animals (n = 869) showed that the levels of *Campylobacter* and *E. coli* O157 were significantly higher when young animals were present. Also, both *Campylobacter* and *E. coli* O157 were significantly more prevalent when wastes were generated by groups of stock containing young animals. A potential intervention measure to help lower pathogen loadings in wastes generated from livestock groups containing young animals could be to manage them separately by using an extended storage period before disposal to land.

Although younger stock generally generated wastes with increased levels and prevalences of *E. coli* O157, this was not true when young stock were still receiving milk (n = 24). We did not isolate *E. coli* O157 from any of the 24 farms where calves were suckling. The influence of milk feeding has been investigated previously and a number of publications (10, 21, 33) have compared the prevalence of *E. coli* O157 in weaned and unweaned calves with similar results.

Stock gender and pathogen prevalence. No significance between levels of zoonotic agents in fresh wastes and male (n = 148), female (n = 477), or groups of mixed gender stock (n = 280), with or without inclusion of stock of unknown status (n = 122) in the comparison data, was identified. Although cattle of unknown gender had a significantly elevated prevalence of *Listeria* compared with the other groups, no meaningful correlations between prevalence and gender were found. Previously, it has been reported that in year-old cattle, verotoxigenic *E. coli* prevalence was the same in steers and heifers (31).

Influence of breed on pathogen prevalence. A large amount of information concerning livestock breed was gathered as part of the study with a view to determining if pathogen shedding was influenced by this parameter. A large number of breeds of pig, sheep, and poultry were encountered, and the data set was too widely spread across these breeds for analysis to be attempted. Although the range of cattle breeds encountered was less broad, lack of standardized farming practices outside of small localities made analysis challenging. Cattle (n = 146) were grouped according to whether they were purebreds or crosses or whether they were purebreds that shared housing with other purebreds (i.e., mixtures of purebreds). Crossbreeds were sorted by paternal breed, and mixtures were sorted alphabetically. A summary of the main groups is shown as Table 4. Due to small numbers in some groups, only prevalence-based analyses were undertaken. *Salmonella* prevalence in

TABLE 4. Main cattle breed groupings and the numbers of fresh waste samples collected for each group

Cattle breed	Relationship of animals in the group	No. of samples
Angus	Crossed with other breed(s)	10
Belgian Blue	Crossed with other breed(s)	15
Charolais	Pure breed	23
Charolais	Crossed with other breed(s)	37
Continental	Crossed with other breed(s)	12
Friesian	Pure breed	62
Friesian	Penned with different pure breeds	39
Friesian	Crossed with other breed(s)	48
Hereford	Crossed with other breed(s)	23

[a] Groups composed of fewer than 10 samples were not included as individual groups in the analyses.

TABLE 5. Most commonly encountered bedding materials found in fresh and stored livestock wastes collected during this study

Bedding type	Percentage of total no. of samples analyzed (n = 1,549)
Straw	73.8
None	16.7
Sawdust	4.1
Woodchips	1.7
Sand	1.3

purebred Charolais cattle was significantly elevated in comparison to the rest of the fresh cattle samples. Charolais and Friesian purebreds and Belgian Blue crosses had significantly higher prevalences of *Listeria* in their wastes. Belgian Blue crosses and purebred Angus also had higher prevalences of *Cryptosporidium*. However, the overall *Cryptosporidium* prevalence for the entire data set (5%) combined with the low numbers of positive detections (Angus, 2 of 10 or 20%; Belgian Blue, 4 of 15 or 26.7%) for these breeds means that these results should be interpreted cautiously. No breed-specific significant differences in *E. coli* O157 or *Campylobacter* prevalences were identified.

Bedding materials. The influence of bedding materials on pathogen levels in stored wastes was investigated. It had previously been reported that the intensification of agriculture over the last 50 years in the United Kingdom had resulted in a greater proportion of liquid livestock wastes (29). Traditionally, animal wastes had included bedding materials such as straw and wood shavings. A significant proportion of the manures we sampled still contained bedding materials (Tables 2 and 5). Although the presence of bedding materials would be expected to help aerate the wastes, there were no significant differences when overall pathogen levels were compared for stored wastes containing and lacking bedding. When *Listeria* and *Campylobacter* were analyzed individually, however, they were found at significantly lower levels when any type of bedding was present in the wastes. Although the most commonly encountered type of bedding material for stored samples was straw (n = 391), only *Campylobacter* levels were significantly lower when bedding containing straw was used. Testing for the absence of each of the zoonotic agents and inclusion of bedding material in stored wastes again showed significance only for *Listeria* and *Campylobacter*.

Stock diet. Animal diet and fodder change have previously been shown to influence the prevalence of pathogen shedding in fresh manures (20). Increasing the fiber content of sheep diets by feeding grass hay also caused an increase in the times of *E. coli* O157 shedding into feces (20). More generally, a positive correlation between the cereal content of cattle diets and the levels of generic *E. coli* shed into wastes has been reported (7).

For fresh manures, there were no significant differences in pathogen levels when the stock diet consisted mainly of cereal concentrates (n = 295), compared to pathogen levels in stock that was not eating cereal concentrates. However, animals feeding mostly on cereal solids (grain processed to remove starch; n = 115) shed significantly higher levels of *Cryptosporidium* and *Listeria*. Significant influences were also found when stock consumed grass as a principal feed. Although both *E. coli* O157 and *Salmonella* levels in fresh wastes were significantly lower when grass was consumed (n = 60), *Campylobacter* levels in fresh wastes were significantly higher. Although the differences were identified when the diet of livestock was composed mainly of grass, there are significant husbandry differences when stock are eating grass compared with other diets. Most significant is that grass-fed livestock have access to an outdoor pasture normally during warmer months, whereas for most other diets, stock can be fed exclusively indoors. Thus, the influence of such different environmental conditions cannot be excluded. Previously, it has been reported that housed and grazed stock do not have significantly different prevalences for *E. coli* O157 (11, 19). Livestock eating grass silage (n = 309), hay (n = 60), or pellets (n = 29) as the main diet did not shed significantly different levels of pathogens, although livestock that were fed maize silage (n = 72) had significantly higher levels of *Listeria* in their wastes. Feeds were not tested as part of this study, but other investigators have shown that *Listeria* can survive an inadequate silage fermentation (8, 9, 24).

The influence of antibiotics or feed growth promoters on prevalences and levels of zoonotic agents in wastes was examined. Levels of *Cryptosporidium* and *Campylobacter* were significantly higher when antimicrobials were given. Although it is possible that the antibiotics could selectively clear microbial populations, allowing *Campylobacter* to take advantage of reduced competition, there was a disparate ratio for the two sample subsets (931:104). Such a small population of animals being fed antimicrobials made drawing firm conclusions difficult.

Stocking density. In-house stocking density (Table 6) was also investigated for fresh manure deposited within a month of emptying the housing. There were a limited range of stocking densities encountered for sheep (0.25 to 1.06 animals m^{-2}) and only a small number of samples that fitted the selection criteria (n = 14). For similar reasons no firm conclusions could be drawn for poultry wastes (n = 27). Analyses of fresh pig samples (n = 106) grouped by range showed that, on average, pigs were stocked at a density of 0.4 animals m^{-2}. Pigs stocked at densities lower than this mean had significantly lower levels of *Cryptosporidium* oocysts in their wastes. Fresh cattle wastes that had been deposited within the previous month (n = 500) showed no significant differences in pathogen levels when

TABLE 6. Summary of stock-related farming practices for all fresh wastes collected during this study

Parameter	Value by livestock type[a]			
	Cattle (n = 805)	Pigs (n = 126)	Poultry (n = 65)	Sheep (n = 24)
Mean stocking density (animals m^{-2})	0.32	0.79	5.04	0.67
Percentage of farms operated organically	4.8%	7.9%	12.3%	8.3%
Type of manure generated[b]				
FYM	64%	54%	100%	100%
Slurry	36%	46%	—	—

[a] n, number of samples analyzed for each set of data; −, indicated type of waste not generated by livestock type.

[b] FYM, farmyard manure (>15% dry matter [wt/vol]). Slurry is <10% dry matter (wt/vol).

TABLE 7. Types of livestock housing and environments most commonly encountered during collection of freshly deposited livestock waste samples

Sample collection environment[a]	Percentage of total no. of samples (n = 1,020)
Straw-bedded yard	43.4
Cubicle	17.4
Cowshed	7.5
Straw yard	7.1
Loose-straw yard	4.9
Free range	2.9
Kennels	1.8
Part bedded	1.5

[a] Straw-bedded yards have 50% of their area covered in straw bedding with the remainder of the yard covered in concrete around feed and water troughs areas. Loose-straw yards have a similar organizational arrangement but use packed soil or small loose pebbles instead of concrete.

stocking densities above or below the mean of 0.29 animals m^{-2} were compared. There were no significant differences in pathogen prevalences for either cattle or pigs when above- or below-mean stocking density groups were compared. A possible explanation for these findings may lie in the fact that the epidemiological relationships between housing type, housing floor space, and stocking density are poorly understood. Although attempts were made to classify and characterize housing for this study (Table 7), the reality of the situation was that a diverse range of livestock housing was encountered, and it was not possible to undertake reliable analyses to determine if housing type influences the prevalence or levels of zoonotic agents shed. It is possible that any significance of the stocking density was masked by different housing types and floor space areas. Such a diverse range of housing makes it unlikely that survey data will be able to define any influence of housing and stocking densities on pathogen levels in wastes and that controlled experiments will be required.

Seasonal effects on pathogens in bovines. Seasonal effects in the prevalences and levels of a number of zoonotic agents have been reported previously (4, 28). Only the fresh cattle-derived data set was large enough to analyze for monthly prevalence (Table 8). Although several hundred samples were collected during September and October, it was not possible to predict waste age before visiting a farm, and the number of samples that met the selection criteria (fresh waste, <32 days old) for September and October are low (n = 13 for both). The data for these months have been included for information, but the number of samples for these months were too low for statistical analysis. Significant seasonal differences were detected for *Listeria*, which was more likely to be isolated in March to June. We found that *E. coli* O157 was most likely to be isolated in May and June (Table 8), a finding in broad agreement with a previously published extensive regional study (4). Levels of *Campylobacter* in cattle have been shown previously to rise in November and December in the United Kingdom (28). Although we did not observe an elevated incidence in November, we isolated *Campylobacter* from 44.8% of cattle samples in December (Table 8). Despite a comprehensive investigation of possible factors which could cause increased levels of *Campylobacter* in wastes (28), the reasons for the seasonal effect are presently unknown. In our study, *Cryptosporidium* was significantly more prevalent in June and December. We identified no seasonally dependent changes in prevalence for *Salmonella*.

Geographical influences. There were significant differences when prevalences were compared for grouped Ordnance Survey map grid references. The real-world significance of such analyses should be viewed cautiously because livestock distribution in the United Kingdom is not even. Cattle are more prevalent in the western part of Britain, where higher rainfall provides better conditions for the growth of grass pasture. Poultry and pigs tend to be distributed more toward the center and eastern side of the country, respectively. Furthermore, a large proportion of the samples collected were of cattle manure because cattle generate over 77% (wt/wt) of the livestock waste in the United Kingdom (15, 25). Prevalences and levels of *E. coli* O157 and *Listeria* showed significant differences between different subsets of grouped ranges of easterly coordinates. Data for *E. coli* O157 and *Campylobacter* also showed significant differences between different subsets of the northerly coordinates groups. In all cases, however, the trends were nonlinear, indicating that it was not the case that samples collected further east or north were more or less likely to harbor pathogens. Compared with samples from England and Wales, samples collected from Scotland were no more, or less, likely to contain zoonotic agents.

TABLE 8. Monthly prevalences of zoonotic agents present in samples of fresh cattle feces collected between 2000 and 2002

Month	No. of samples collected	Prevalences (%) of zoonotic agents[a]				
		E. coli O157	*Salmonella*	*Campylobacter*	*Listeria*	*Cryptosporidium*
January	33	0.0 ↓	12.1	9.1	0.0 ↓	0.0
February	44	11.4	0.0	0.0 ↓	31.8	9.1
March	23	17.4	0.0	8.7	56.5 ↑	4.3
April	55	15.2	0.0	0.0 ↓	42.4 ↑	12.1
May	50	18.2 ↑	7.3	23.6 ↑	43.6 ↑	9.1
June	34	35.3 ↑	11.8	5.9	47.1 ↑	14.7 ↑
July	102	2.9	5.9	1.0 ↓	11.8 ↓	1.0
August	57	10.5	10.5	0.0 ↓	22.8	5.3
September	13	0.0	7.7	15.4	23.1	0.0
October	13	23.1	7.7	7.7	38.5	0.0
November	46	6.5	2.2	10.9	0.0 ↓	6.5
December	29	41.4 ↑	6.9	44.8 ↑	69.0 ↑	20.7 ↑

[a] All samples were from housed cattle and collected within one month of deposition. Prevalences that are significantly lower or higher than those calculated for the same organism from the other 11 months are denoted by ↓ and ↑, respectively.

Genera-specific correlations. An interesting and unexpected correlation was highlighted when undertaking en masse multiple correlations between the numeric data collected during our survey. When we analyzed the combined fresh and stored data sets (n = 1,549) for all livestock types, we found a significant correlation (r = 0.596) between levels of *Salmonella* and *Listeria*. The reasons for this are unclear. This finding may indicate that infection or colonization of livestock by one of these bacteria predisposes the animal to infection or colonization by the other zoonotic agent. The relationship was only between levels of the two zoonotic agents; the prevalences of *Listeria* and *Salmonella* in the entire set of manures surveyed were significantly different.

The analyses reported in this study indicate that on-farm stock and waste management practices may influence the levels and prevalences of pathogens, confirming the hypothesis proposed originally by Hancock et al. (11). A number of the relationships identified in this study (e.g., use of straw bedding material) could form the basis of farm-level intervention measures that are simple and cheap to implement and that reduce the levels or prevalences of zoonotic agents in manures. Before spreading wastes to land, farmers could give consideration to the season in which the waste was generated to assess the likely microbiological risks associated with the material. Around 150 million tons of livestock waste are spread or directly deposited to land annually in the United Kingdom (3). Since such large quantities of wastes are involved, a small reduction in the levels or prevalences of zoonotic agents in this material would substantially lower the total numbers of pathogens released to the environment. Whether reduced levels of zoonotic agents in wastes spread to land would result in a lowered incidence of food-borne disease is currently unknown and an area that requires further study.

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