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Levels of zoonotic agents in British livestock manures

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ABSTRACT

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Aims: To determine the prevalence and levels of zoonotic agents in livestock wastes.

Methods and Results: A proportionally weighted survey was undertaken and livestock waste samples analysed quantitatively for *Escherichia coli* O157, pathogenic *Listeria*, *Salmonella*, *Campylobacter*, *Giardia* and *Cryptosporidium*. A significant proportion of wastes contained at least one zoonotic agent. Relationships were found between dry matter content and the presence and levels of some zoonotic agents.

Conclusions: British livestock wastes contain measurable levels of the zoonotic agents that cause most cases of gastroenteritis in the UK.

Significance and Impact of the Study: Animal wastes are disposed of by spreading to agricultural land used for the production of crops and livestock grazing. As British wastes are contaminated with significant levels of zoonotic agents, the practice may represent a way for pathogens to travel further up the food chain.

Keywords: *E. coli* O157, levels, livestock wastes, *Salmonella*, zoonotic agents.

INTRODUCTION

The infectious agents that contribute most to gastroenteritis and food-borne illnesses are *Salmonella*, *Campylobacter*, verotoxigenic *Escherichia coli* (VTEC) O157, pathogenic *Listeria* and *Cryptosporidium parvum* (Pell 1997; Plaut 2000; Adak *et al.* 2002). A significant number of cases of gastroenteritis have been linked with contaminated food (Steele *et al.* 1997; Hilborn *et al.* 1999) water (MacKenzie *et al.* 1994; Savill *et al.* 2001) and farm environments (Jackson *et al.* 1998; Jones 1999). Although reports of infectious food-borne disease fell by half between 1995 and 2000 in England and Wales, there are still an estimated 1·34 million cases annually (Adak *et al.* 2002).

The common agricultural practice of spreading livestock manures to land presents an obvious and well-described vector for the contamination of crops and watercourses at farm level if the wastes contain pathogenic micro-organisms (Pell 1997; Hilborn *et al.* 1999). Nearly 70 million tonnes of waste are spread to land each year in the UK (Hutchison *et al.* 2002). Although a number of studies have reported the prevalence of some zoonotic agents in localized regions (Chapman *et al.* 1997; Mechie *et al.* 1997; Stanley *et al.* 1998a,b), there has never been a national study undertaken in Great Britain (GB) of levels of pathogenic bacteria in livestock wastes. Furthermore, most previous studies derived only regional incidence and therefore, currently, there is a lack of information describing the levels of zoonotic agents present in livestock manures nationally. Until this information is obtained, it is difficult to make a quantitative assessment of any contribution that spreading manures to land may make towards the incidence food-borne illness.

In this study, we determined the prevalences and levels of *Salmonella*, *Campylobacter*, VTEC O157, *Listeria*, *Cryptosporidium* and *Giardia* in over 1500 livestock wastes collected from throughout GB. The results from this study enable, for the first time, a realistic assessment to be made regarding the risks to food safety posed by spreading livestock wastes to land used subsequently for food production.

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MATERIALS AND METHODS

Collection of livestock wastes

The number of wastes to be collected for each livestock species were calculated by simple proportion using the masses of manures generated in GB livestock housing determined previously (Hutchison *et al.* 2000; Smith *et al.* 2000, 2001). Proportional weighting to take account of geographical differences in the distribution of livestock for the wastes collected in England and Wales was also undertaken. A seasonal bias for collections was introduced with greater numbers of wastes collected during winter when animals are housed. The livestock wastes were gathered almost continuously between April 2000 and December 2002. A UK-wide outbreak of foot and mouth disease interrupted waste collections between February and July 2001. Each livestock waste was collected on-farm, and was derived from a group of animals living in a herd or flock.

Wastes ($n = 1549$) were derived from cattle, pigs, poultry or sheep, and were classified into two groups by waste dry matter content. Liquid wastes that are commonly referred to as slurries, were composed of faeces and urine and typically had a dry matter content of <9% (w/w). Dirty waters of <2% (w/w) dry matter were composed of faeces, urine, dairy parlour washings and rainwater. Farmyard manures (FYM) typically had a dry matter content of >10% (w/w) and were solid wastes that typically contained faecal material, hay, straw or other bedding materials.

Wastes were also categorized into two types by age. Wastes were defined as fresh if they were collected from the house, pen or yard in which they had been deposited by stock. Wastes were termed stored when they were moved from the location of deposition to a secondary store such as a slurry lagoon or FYM heap. Fresh and stored slurry samples comprised a minimum of five combined subsamples collected from different depths and areas of tanks, lagoons or storage pits. Where appropriate slurry samples were also collected as a minimum of 20 handfuls of waste material directly deposited onto hard standings. Fresh solid waste samples also comprised a minimum of 20 handfuls of faeces and bedding material. Stored solid manure samples were collected by systematically digging to a depth of up to 1 m at 20 points of the waste heap with a sterilized spade or fork.

Wastes were refrigerated at 2°C and shipped from farms to laboratories so that analyses commenced within 24 h (England and Wales) or 36 h (Scotland).

Microbiological methods

Poultry-derived wastes were not tested for *E. coli* O157 or protozoa. *E. coli* O157, *Salmonella*, *Campylobacter*, and *Listeria* were enumerated using a general filter resuscitation method with different selective media (Table 1) used for each organism. Briefly, subsamples (25 g) of wastes were mixed with 225 ml of initial diluent. Samples were homogenized in mesh filter bags (6041/STR; Seward, Thetford,

Table 1 Selective media and organism-specific variations used for enumeration of bacterial zoonotic agents from livestock wastes. All media sourced from Oxoid (Basingstoke, UK) unless otherwise specified

	E. coli O157	*Salmonella*	*Listeria*	*Campylobacter*
Initial diluent	TSB, 80 μg ml^{-1} novobiocin	TSB, 40 μg ml^{-1} novobiocin	LSEB	PSW (boiled to remove O_2)
Cellulose nitrate filter pore size	0·45 μm	0·45 μm	0·45 μm	0·1 μm
Resuscitation media	TSB, 40 μg ml^{-1} novobiocin	TSB, 20 μg ml^{-1} novobiocin; 1% (w/v) Iodine	LSEB	BFEB microaerophilic conditions
Resuscitation time (h)	6	16	24	24
Counting media	CHROMagar O157 (Becton Dickinson 264105, Oxford, UK)	Rambach agar	BCM Listeria agar (Biosynth C0608 and C0610, Staad, Switzerland)	CCDA microaerophilic conditions
Incubation conditions prior to counting	16 h at 41°C	28 h at 37°C	28 h at 37°C	28 h at 37°C
Colour/morphology of presumptive positive colonies	Purple	Pinkish red	Blue/convex	Grey/moist/flat
Confirmation by	Latex agglutination (Oxoid DR120M)	API 20E (BioMérieux, Basingstoke, UK)	Listeria confirmation agar (Biosynth C0612)	API Campylobacter (BioMérieux)

TSB, tryptone soya broth; LSEB, Listeria selective enrichment broth; PSW, peptone salt water; BFEB, blood free enrichment broth; CCDA, Campylobacter agar with charcoal and deoxycholate.

UK) in a stomacher (Colworth 400, Seward) for 1 min before centrifugation (300 *g*, 5 min) and filtration of the supernatant through a glass fibre filter (Sartorius 13430–0475) to remove particulates. Filtrates were diluted decimally, 10^{-1} to 10^{-5} in initial diluent. Each dilution was vacuum-filtered through a cellulose nitrate filter with an organism-specific pore size (Table 1). Filters containing bacteria were placed on felt pads soaked in resuscitation media at 37°C for different periods dependent on organism (Table 1). Filters were transferred to counting media plates and incubated as shown in (Table 1). Confirmation of presumptive-positive, coloured colonies was as described in (Table 1). Colony counts were converted to CFU g^{-1} waste according to the criteria specified by the International Standards Organisation (ISO 4833 1991; ISO 6887, 1999).

Antibody capture was used to determine the levels of protozoa. Aliquots of wastes (1 g) were vortexed with 10 ml of water containing 0·01% (v/v) Tween-20 and overlaid onto 40 ml of 1·09 g ml^{-1} sucrose solution. The sample was centrifuged at 5000 *g* for 10 min without braking. The top 25 ml of the supernatant was mixed with 25 ml of deionized water and recentrifuged as before. *Cryptosporidium* and *Giardia* oocysts were enumerated from the pellet using the GC-combo IMS kit (Dynal, Bromborough, UK) according to manufacturer's instructions. Assessment of viability was by staining using 4′,6′-diamidino-2-phenylindole (DAPI) and epifluorescence microscopy as described previously (Pepperell *et al.* 2003).

Chemical methods

The pH and conductivity of liquid and solid wastes were determined directly on the samples without dilution (slurry) or with decimal dilution (FYM), respectively, using conductivity (Philips PW9526, Reigate, Surrey, UK) and pH meters (WPA Model CD620, Cambridge, UK). Dry matter was assessed by weighing the wastes and drying in an oven at 100°C for 16 h; remaining weight was expressed as a percentage of initial weight. Ammonium-N was extracted from wastes with 2 M KCl. The pH of an aliquot of each extract was raised by addition of NaOH and the released gaseous ammonia recovered by distillation and condensation. The concentration of ammonia was determined by titration with 0·05 M sulphuric acid using methyl red/bromo cresol green as indicators.

Analysis of results

Raw pathogen counts were normalized by transforming to $\log_{10}$ CFU g^{-1}. Mean values and associated standard deviations for pathogen levels and manure ages were calculated for each category of livestock wastes using Access (Microsoft Access 97, Microsoft Corporation, 1997). Mann–Whitney *U*-tests for nonparametric data were used to compare pathogen levels in each class of livestock wastes (SPSS 11·5, SPSS Inc., Chicago, IL, USA). The chi-squared test was used to test significance between incidences of contamination (StatsDirect, Cheshire, UK).

RESULTS

The waste sample numbers and geographical collection regions calculated proportionally as described in Materials and methods are shown in Table 2. The data reflects the relative contributions made to the total mass of wastes generated in GB and also the differences in livestock densities in different areas of the country.

There were measurable prevalences of the six pathogens responsible for most UK cases of gastroenteritis (Cole *et al.* 1999; Jones 1999) and food-borne illness in GB livestock wastes (Table 3). Of the 1456 wastes collected from cattle, sheep or pigs, 50·4% contained a measurable level of at least one bacterial pathogen. A significant percentage (45·2%) of the 93 poultry samples collected, were positive for at least one of *Campylobacter*, *Salmonella* or *Listeria*. Compared with bacteria, both the mean prevalences and levels of protozoa were lower in pig (Table 3) and cattle wastes (Table 4).

The bacterial levels isolated from the wastes surveyed were not distributed evenly (results not shown). Thus, average levels of zoonotic agents were subject to disproportionate influence from small numbers of high counts. To take account of the skewed distribution, summary data are presented in Table 4 as both arithmetic and geometric averages.

Pathogens were isolated at levels of up to $2{\cdot}6 \times 10^8$ g^{-1} of waste and generally, higher levels of pathogens were observed in fresh wastes (Table 3). However this was not the case for all waste types and may have been an artefact of the manure age classification we adopted (Smith *et al.* 2001).

Table 2 Number of wastes from each livestock type collected from each geographical region of GB

	Livestock type			
Region of GB	Cattle	Pig	Poultry	Sheep
East Anglia	70	42	19	2
East Midlands	120	27	11	3
NE England	43	40	2	1
North England	128	8	2	4
Scotland	75	10	11	5
South England	93	10	13	3
SW England	374	35	22	4
Wales	247	4	6	9
West Midlands	89	8	7	2
Total	1239	184	93	33

Table 3 Percentage of GB livestock wastes that contained zoonotic agents

	Livestock and waste category (%)							
	Cattle		Pig		Poultry		Sheep	
Zoonotic agent	Fresh ($n = 810$)	Stored ($n = 429$)	Fresh ($n = 126$)	Stored ($n = 58$)	Fresh ($n = 67$)	Stored ($n = 26$)	Fresh ($n = 24$)	Stored ($n = 9$)
Escherichia coli O157	13·2	9·1	11·9	15·5	ND	ND	20·8	22·2
Salmonella	7·7	10·0	7·9	5·2	17·9	11·5	8·3	11·1
Listeria	29·8	31·0	19·8	19	19·4	15·4	29·2	44·4
Campylobacter	12·8	9·8	13·5	10·3	19·4	7·7	20·8	11·1
Cryptosporidium parvum	5·4	2·8	13·5	5·2	ND	ND	29·2	0
Giardia intestinalis	3·6	2·6	2·4	1·7	ND	ND	20·8	0

ND = not determined.

Table 4 A summary of the levels of zoonotic pathogens observed in British livestock manures containing zoonotic agents. Data shown are calculated as both arithmetic (A) and geometric (G) means for positive isolations only. Highest levels observed for each pathogen and manure type are also shown (M). The number of positive isolations used to calculate each mean is shown (n)

	Levels of pathogens (CFU g^{-1}) found in positive livestock waste types							
	Cattle		Pig		Poultry		Sheep	
Zoonotic agent	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
E. coli O157								
A	$2·9 \times 10^6$	$8·6 \times 10^3$	$6·9 \times 10^4$	$4·5 \times 10^3$			$1·1 \times 10^4$	$2·5 \times 10^3$
G	$1·2 \times 10^3$	$2·6 \times 10^2$	$3·9 \times 10^3$	$1·3 \times 10^3$	ND	ND	$7·8 \times 10^2$	$2·5 \times 10^2$
M	$2·6 \times 10^8$	$7·5 \times 10^4$	$7·5 \times 10^5$	$1·8 \times 10^4$			$4·9 \times 10^4$	$5·0 \times 10^3$
n	107	39	15	9			5	2
Salmonella								
A	$3·9 \times 10^4$	$1·9 \times 10^5$	$9·6 \times 10^3$	$8·9 \times 10^2$	$5·0 \times 10^3$	$4·7 \times 10^3$	$1·1 \times 10^3$	$5·8 \times 10^3$
G	$2·1 \times 10^3$	$2·5 \times 10^3$	$6·0 \times 10^2$	$6·1 \times 10^2$	$2·2 \times 10^2$	$4·0 \times 10^3$	$7·1 \times 10^2$	$5·8 \times 10^3$
M	$5·8 \times 10^5$	$7·2 \times 10^6$	$7·8 \times 10^4$	$2·0 \times 10^3$	$2·2 \times 10^4$	$8·0 \times 10^3$	$2·0 \times 10^3$	$5·8 \times 10^3$
n	62	43	10	3	12	3	2	1
Listeria								
A	$1·5 \times 10^4$	$2·2 \times 10^4$	$4·6 \times 10^4$	$1·6 \times 10^4$	$3·2 \times 10^4$	$5·6 \times 10^2$	$4·5 \times 10^2$	$2·1 \times 10^3$
G	$1·1 \times 10^3$	$1·1 \times 10^3$	$3·1 \times 10^3$	$6·1 \times 10^2$	$8·3 \times 10^2$	$3·3 \times 10^2$	$2·0 \times 10^2$	$3·0 \times 10^2$
M	$4·2 \times 10^5$	$9·8 \times 10^5$	$9·7 \times 10^5$	$1·5 \times 10^5$	$1·9 \times 10^5$	$1·3 \times 10^3$	$1·7 \times 10^3$	$8·1 \times 10^3$
n	241	133	25	11	13	4	7	4
Campylobacter								
A	$7·6 \times 10^3$	$1·1 \times 10^4$	$1·9 \times 10^3$	$2·6 \times 10^4$	$4·2 \times 10^3$	$6·4 \times 10^2$	$8·6 \times 10^2$	$1·0 \times 10^2$
G	$3·2 \times 10^2$	$5·3 \times 10^2$	$3·1 \times 10^2$	$1·6 \times 10^3$	$2·6 \times 10^2$	$5·9 \times 10^2$	$3·9 \times 10^2$	$1·0 \times 10^2$
M	$1·5 \times 10^5$	$1·5 \times 10^5$	$1·5 \times 10^4$	$1·0 \times 10^5$	$2·9 \times 10^4$	$8·7 \times 10^2$	$2·1 \times 10^3$	$1·0 \times 10^2$
n	104	42	17	6	13	2	5	1
Cryptosporidium parvum								
A	$2·7 \times 10^2$	$6·6 \times 10^1$	$3·0 \times 10^2$	$1·4 \times 10^2$			$5·3 \times 101$	
G	$1·9 \times 10^1$	$1·0 \times 10^1$	$5·8 \times 10^1$	$3·3 \times 10^1$	ND	ND	$1·0 \times 10^1$	–
M	$3·5 \times 10^3$	$4·8 \times 10^2$	$3·6 \times 10^3$	$3·1 \times 10^2$			$2·5 \times 10^2$	
n	44	12	17	3			7	0
Giardia intestinalis								
A	$2·2 \times 10^2$	$5·9 \times 10^0$	$5·3 \times 10^4$	$1·2 \times 10^1$			$3·8 \times 10^2$	
G	$1·0 \times 10^1$	3	$6·8 \times 10^1$	$1·2 \times 10^1$	ND	ND	$2·0 \times 10^1$	–
M	$5·0 \times 10^3$	$3·6 \times 10^1$	$1·6 \times 10^5$	$1·2 \times 10^1$			$1·2 \times 10^3$	
n	29	11	3	1			5	0

ND = Not determined.

In this study, fresh and stored are arbitrary classifications based on waste collection location. Depending on how farms are managed it is possible that for some samples, manure labelled as stored could be newer than fresh material. The use of a standard classification system was confounded because on farm waste stores tend not to be operated as batch units (Smith *et al.* 2000, 2001). Wastes are added to stores on a continual basis with little or no record of additional inputs being kept. Thus manure is a mixture of old material with numerous additions of newly deposited waste. As most farmers keep accurate records of when stores and housing were last completely emptied, the age used for both the fresh and stored manures reported in this study is the age of the oldest material in the house or store. To provide a summary overview of how the terms fresh and stored relate to the age of each waste material, the range and average storage time is provided in Table 5. Although pathogen levels are normally expected to decline with time, measuring the age of wastes found on farms is not precise and therefore cannot be used as an indicator of likely levels of zoonotic agents.

Chemical analyses were undertaken for all wastes examined during this study (Table 6) and analyses were undertaken to determine if there was any relationship between waste chemistry and pathogen presence or levels. Prevalences and levels of *Listeria monocytogenes* and *L. ivanovii* were higher in low dry matter content slurries than in slurries with higher dry matter content. The trend was significant for both bovine and porcine-derived liquid wastes when *Listeria* levels in low (≤1·99%, w/w) and high (>1·99%, w/w) dry matter contents were compared ($P < 0{\cdot}05$). *Salmonella* levels were not significantly greater in either slurries or FYMs with higher dry matter content. Although it has been reported previously that dry matter content of waste can influence survival of *Salmonella* (Jones 1976), their potential for longer survival did not give rise to

Table 5 Summary of the range and mean age (days) of livestock wastes found on GB farms

	Age of waste sampled (days)							
	Cattle		Pigs		Poultry		Sheep	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
FYM								
Min	<1	<30	<1	<30	3	<30	<1	30–90
Max	400	>365	200	>270	378	>270	>999	>270
Mean	71·9		22·7		71·2		91·4	
Slurry								
Min	<1	<30	<1	<30	N/A	N/A	N/A	N/A
Max	420	>270	120	>270				
Mean	50·7		26·2					

N/A = Waste not generated by livestock type.

Table 6 Range and mean physiochemical properties of livestock wastes analysed during this study. Values for slurries include the low dry matter wastes commonly referred to as dirty waters

	Cattle FYM	Cattle slurry	Pig FYM	Pig slurry	Poultry FYM	Sheep FYM
pH						
Min	3·5	4·9	5·6	6	4·9	7·6
Max	9·5	9·3	9·4	8·5	9·3	9·2
Mean	7·8	7·2	7·8	7·53	7·84	8·42
Dry matter (% w/w)						
Min	6·6	0·6	10	0·3	3·2	2·6
Max	67·6	6·20	69·9	6·8	8·73	9·06
Mean	22·7	1·13	22·7	0·975	4·16	3·25
Ammonium N (mg NH_4–N kg waste^{-1})						
Min	0	8	12	308	25	19
Max	8100	26 480	7040	46 000	11 800	3590
Mean	580	929	1658	2272	3586	1053
Conductivity (μSi cm^{-1})						
Min	79	40	358	195	200	634
Max	45 800	38 400	48 300	32 800	69 700	33 100
Mean	3760	4223	6335	10 270	12 511	6723

higher prevalences or levels in the wastes that we analysed. In addition, levels of both *Campylobacter* and *Giardia* were significantly higher in low dry matter (≤0·99%, w/w) wastes than in the high dry matter wastes (>0·99%, w/w) ($P < 0·05$).

DISCUSSION

The results of this study provide an up-to-date snapshot of pathogen levels in livestock wastes collected nationally throughout GB. The data presented builds on previous studies which have tended to concentrate on smaller regions of the UK.

Stanley *et al.* (1998b) have previously reported the incidence of *Campylobacter* carriage in beef cattle as 89·4% in an abattoir over a 3-year period from 1993. Although differences in methodologies mean direct comparisons between studies may not be possible, the incidence reported (89·4%) is significantly higher than the 12·8% incidence for fresh cattle (dairy and beef combined) that we observed. It seems unlikely that such a significant decrease in *Campylobacter* infections could have occurred in cattle without intervention. A possible explanation for the difference is that livestock which have been subjected to stress are more likely to shed campylobacters. A similar effect has been reported for poultry during transport (Whyte *et al.* 2001). The Stanley *et al.* (1998b) study also determined mean levels of *Campylobacter* as $6·1 \times 10^2$ most probable number (MPN) g^{-1} which agrees closely with the mean levels observed by this study in fresh cattle waste.

Dairy and beef cattle are important reservoirs of *E. coli* O157 and a comprehensive study estimated that up to 36·8% of cattle in Northern England are infected (Chapman *et al.* 1997). Seasonally dependent variations in prevalence were observed, although overall Chapman and coworkers found that 15·7% of cattle, 2·2% of sheep and 0·4% of pigs tested immediately after slaughter contained *E. coli* O157. By comparison, we observed 13·2, 20·9, 11·8% for fresh wastes on-farm from cattle, sheep and pigs respectively. Thus there was an obvious difference in the recorded incidence over the 6 years between the two studies in pigs and sheep. Whether this increase is significant is difficult to determine because the Chapman study evaluated the infection status of individual animals, whereas our samples determined the status of herds or flocks. It is unclear what the effect of transport stress is for *E. coli* O157. A recent publication determined a lower incidence of EHEC O157 shedding after slaughter when compared with on-farm (Barham *et al.* 2002). Conversely a Danish study showed an increased risk of *E. coli* O157 infection if young animals had been moved within the previous month (Rugbjerg *et al.* 2003).

There has been a statutory requirement for the reporting of *Salmonella* isolations in UK livestock since 1975. Thus reliable figures are available for infections which cause clinical symptoms in farmed animals. In the UK in 2002, there were 1000 isolations from cattle, 200 from both sheep and pigs, and 1300 from poultry (VLA 2002). These reported isolations are likely to grossly underestimate the true incidence of livestock by *Salmonella* because low-level infections may not generate obvious clinical symptoms in stock (Jones 1976). An investigation of 20 cattle farms in England assessed the prevalence of *S. typhimurium* DT104 (Davies 1997). The purpose of the study was not to measure local or national prevalence, although it did report an initial prevalence of 89%, calculated from individual animals. Prevalence fell to 25% over the course of the study as intervention measures aimed at improving farm hygiene were implemented. An earlier study examined 187 cattle slurries and found *Salmonella* present in 11% of samples, with low numbers, typically less than one organism per gram of slurry (Jones and Mathews 1975). Our measured prevalence was 7·7% from fresh waste which is lower than the 1975 incidence (Jones and Mathews 1975) and may indicate the effect of veterinary advice for contaminated farms as a consequence of zoonoses order. However, although our measured prevalence was lower, we typically isolated *Salmonella* at levels of 10^3–10^4 CFU g^{-1} of cattle wastes which is significantly higher than that measured in 1975 (Jones and Mathews 1975).

Poor record-keeping on farms and continuous addition of fresh wastes to stores meant that the age of waste could not be reliably used to predict pathogen levels. However, we did observe apparent differences in incidence for both *Campylobacter* and *Cryptosporidium* when fresh and stored wastes were compared (Table 3). For *Campylobacter*, chi-squared testing revealed that differences between the fresh and stored wastes were not significant ($P > 0·05$) when each livestock type was evaluated individually. However, if the isolations were grouped and evaluated *en masse* then the decreased *Campylobacter* isolations in stored wastes were significant ($P < 0·05$). Those overall, stored wastes are less likely to contain *Campylobacter* which is not surprising given the apparent fragility of this organism (Solomon and Hoover 1999).

Statistical analyses (chi-squared) for *Cryptosporidium* showed that the differences between prevalences in fresh and stored wastes in cattle were significant ($P < 0·05$) as were prevalences when data from all species was grouped and analysed *en masse* ($P < 0·05$). Thus *Cryptosporidium* was also less likely to be found in stored wastes. Differences between pig and sheep isolations were not significant ($P > 0·05$). Possible explanations for these differences may be linked with physical changes which occur in stored manure heaps. *Cryptosporidium* survival in water is well-documented (Robertson *et al.* 1992; Suwa and Suzuki 2003) as is the water-assisted transport of oocysts through solid

matrices such as soil (Darnault *et al.* 2003). A possible contributing factor for the lower incidence of *Cryptosporidium* in stored wastes could be that oocysts are removed from heaps of waste in the leachate that drains from these ad-hoc stores. Currently however, there does not appear to be any published information on this potential route for the dissemination of oocysts to the wider environment.

The data reported in this study allows, for the first time, a realistic assessment to be made of the likely risks posed by land spreading of livestock wastes generated on GB farms. Taken collectively, over 30% of the livestock wastes examined contained at least one microbial pathogen. Livestock wastes are likely therefore to be a significant hazard to the on-farm section of the food chain. However, further studies are necessary before a full assessment of the risks that livestock waste poses to human health and the food chain can be undertaken. It is difficult to compare the findings of our study with those published previously because of differences in analyses methodologies, the timeframes in which studies were undertaken and how and where samples were collected. This study attempts to address such problems by providing a raw national baseline which can be used to measure the future effects of intervention measures and nationally issued guidance on levels of zoonotic agents in livestock wastes.

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