Pathogens and Indicators in United States Class B Biosolids: National and Historic Distributions

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This paper reports on a major study of the incidence of indicator organisms and pathogens found within Class B biosolids within 21 samplings from 18 wastewater treatment plants across the United States. This is the first major study of its kind since the promulgation of the USEPA Part 503 Rule in 1993, and includes samples before and after the Part 503 Rule was promulgated. National distributions collected between 2005 and 2008 show that the incidence of bacterial and viral pathogens in Class B mesophilic, anaerobically digested biosolids were generally low with the exception of adenoviruses, which were more prevalent than enteric viruses. No *Ascaris* ova were detected in any sample. In contrast, indicator organism numbers were uniformly high, regardless of whether they were bacteria (fecal coliforms) or viruses (phage). Indicators were not correlated with pathogen loads. Historic distributions were collected between 1988 and 2006 at one location in Tucson, AZ. By comparing data collected before and after 1993, the influence of the USEPA Part 503 Rule on indicator and pathogen levels within Class B biosolids can be inferred. In general, the bacterial indicators total and fecal coliforms decreased from the 1980s to present. Enteric virus concentrations after 1993 are much lower than those reported in other studies in the 1980s, although our values from 1988 to 1993 are not significantly different from our values obtained from 1994 to 2006. Presumably this is due to better and more consistent treatment of the wastewater, illustrating that the Part 503 Rule has been effective in reducing public exposure to pathogens relative to 17 yr ago. The percent reduction of both indicators and pathogens during anaerobic mesophilic digestion was between 94 and 99% for all organisms, illustrating that such treatment is effective in reducing pathogen loads.

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Sewage sludge is defined as "the solid, semisolid, or liquid residue generated during the treatment of domestic sewage in a treatment works." In contrast, *biosolids,* as defined by a National Research Council Committee (2002) that addressed the health effects of biosolids, is the term given to the end product that results from treatment of sewage sludge to meet the land-application standards of the USEPA Part 503 Rule (USEPA, 1993).

Depending on the level of treatment, two classes of biosolids are produced: Class A biosolids (higher level of treatment-processes designed to further reduce pathogens), which contain no detectable levels of pathogens in 4 g of dry solids; or Class B biosolids (lower level of treatment-processes designed to reduce pathogens), which routinely contain bacterial, parasitic, and viral pathogens (Table 1). Approximately 5.5 billion kg (6 million dry tons) of biosolids are produced annually in the United States, of which 60% are used for land application, with the vast majority of it being Class B biosolids (NRC, 2002).

The greatest amount of uncertainty in quantitative microbial risk assessment is due to the lack of data on exposure to pathogens (Haas, 1996), and to properly assess the risk from land-applied Class B biosolids, it is important to know the number of pathogens in the biosolids after treatment and before land application. A previous framework has been developed for assessing the risks from land-applied biosolids, but data have been sparse on the occurrence of pathogens in biosolids (Eisenberg, 2006). There have been several published reviews on the potential hazards of human pathogens in biosolids. Of interest is the fact that one review (Straub et al., 1993) was published in 1993 before the promulgation of the USEPA Part 503 Rule (USEPA, 1993) that regulates sewage sludge treatment and land application of the resulting biosolids. A more recent study by Viau and Peccia (2009) quantified genome copies of several pathogens by quantitative polymerase chain reaction (qPCR), but this did not provide data on infectious pathogens (i.e.,

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Abbreviations: qPCR, quantitative polymerase chain reaction.

Table 1. Part 503 Rule pathogen density limits, adapted from USEPA (2000).

Pathogen or indicator	Standard density limits (dry wt.)†
Class A	
Salmonella	3 MPN per 4 g
Fecal coliforms	<1000 MPN per g
Enteric viruses	<1 PFU per 4 g
Viable helminth ova	<1 per 4 g
Class B	
Fecal coliforms	<2,000,000 MPN per g

† MPN, most probable number; PFU, plaque-forming unit.

all of the genomes detected could represent viable or nonviable organisms). However, qPCR data could be used for a worst-case risk assessment, since culturable methods will not detect viable but nonculturable organisms. A recent review by Sidhu and Toze (2009) only contained recent data from countries other than the United States, except for one reference to indicator bacteria and *Salmonella* removal (Dahab and Surampalli, 2002). Pathogen loadings and removal processes vary in different countries and are unlikely to represent those found in the United States (Jimenez et al., 2000). Most of the existing studies on occurrence of viable pathogens in biosolids were undertaken before the Part 503 regulations for treatment of biosolids went into effect (Straub et al., 1993). Before this time there were no requirements or treatment standards, and there was a great deal of variability in treatment processes for biosolids at that time. As a result, data collected before these regulations went into effect may not reflect current levels of pathogens in biosolids in the United States. This study presents a large database on the incidence of pathogens and indicators in Class B biosolids, including national and historic distributions. For historic distributions, we monitored pathogens and indicators in Class B biosolids from the Ina Road Wastewater Treatment Plant in Tucson, AZ, on a monthly to quarterly basis for 18 yr (Pima County biosolids 1988–2006). For national distributions, we analyzed current levels of pathogens and indicators from 21 samplings from 18 wastewater treatment plants from all major regions of the United States. This is the first nationwide study of its kind since the USEPA Part 503 Regulations went into effect, and provides a comprehensive database on the current incidence of pathogens in Class B mesophilic, anaerobically digested biosolids. The databases presented here not only allow for a reevaluation of pathogen risks, but also document the variability of treatment for pathogen removal over time.

Materials and Methods

The data presented in this paper are the result of two separate studies: a historic data set from one location (Tucson, AZ; 1988–2006); and a national data set conducted during 2005 to 2008 across the United States.

Historic Data Set

Samples of biosolids from Pima County Ina Road Wastewater Treatment Plant were collected and analyzed during the period 1988 to 2006. Biosolids resulted from mesophilic anaerobic digestion with a solids content that in the 1980s was approximately 2%, compared with the higher solids content of the late 1990s of approximately 6 to 8%. Sampling frequency ranged from monthly to quarterly. The organisms assayed and the methods of analysis are outlined in Table 2. Of particular interest are the pre-1993 data, which allow for an evaluation of the impact of the Part 503 Rule on treatment efficiency.

National Data Set

Between 2005 and 2008 we conducted a national study on the incidence of human pathogens and indicators of pathogens in anaerobically digested mesophilic biosolids (Class B) produced within wastewater treatment facilities across the United States. Treatment plants generally served metropolitan areas, most of which had populations >0.5 million and were located in Arizona (two locations), California (five locations), Florida (one location), Michigan (one location), Minnesota (two locations), Nevada (one location), Oregon (one location), Washington (one location), Wisconsin (three locations), and Wyoming (one location). Most samples were "cake" (~20% solids), with some samples being a slurry (~8% solids). All samples were immediately shipped overnight on ice to the University of Arizona, where they were analyzed within 24 h.

The organisms assayed and the methods of analysis are shown in Table 3. Many of the pathogens assayed were known to exist before the Part 503 Rule and data on their incidence before 1993 are available. Other pathogens, such as *Escherichia coli* O157:H7, have emerged after the Part 503 Rule was promulgated.

Maximum likelihood methods were used to fit lognormal distributions to the data. The likelihood function of the data is given by Eq. [1]:

$$L = \prod_{i}^{N} f(\log Y_i \,|\mu,\sigma)^{\delta_i} F[\log(\mathrm{DL})|\mu,\sigma]^{1-\delta_i} \quad [1]$$

where Y_i denotes the organism concentration, and μ and σ^2 are the national mean and variance. The probability density distribution function of a normal distribution is denoted by $f(\log Y_i|\mu,\sigma)$ and the cumulative density function of a normal distribution evaluated at the censoring point of DL by $F[\log(\mathrm{DL})|\ \mu,\sigma]$. d_i is an indicator variable that is 0 for censored observations and 1 for measured observations.

Results and Discussion

The historic distributions are shown in Table 4, which illustrates the concentration of enteric organisms within raw sewage and treated biosolids obtained at the Pima County

Table 2. Organisms and methods of analysis for the temporal data set.

Organism	Type	Source of method	Method
Total coliforms	Indicator	APHA (1985)	Method 908
Fecal coliforms	Indicator	APHA (1985)	Method 908
Fecal streptococcus	Indicator	APHA (1985)	Method 910
Salmonella	Pathogen	USEPA Standard Method	Method 9260
Enteric virus	Pathogen	American Society for Testing and Materials (ASTM)	Method D4994-89 (Goyal et al., 1984)

Wastewater Treatment Plant from 1988 to 2006. Indicator organisms assayed included total and fecal coliforms and fecal streptococci. Fecal coliform standards are used to define Class A or B biosolids, and values in the raw sewage were around 10^8 per gram dry weight throughout the study period. Numbers for all three indicator organisms in raw sewage were statistically similar for the two periods of 1988 to 1993 and 1994 to 2006 (as indicated in Table 4, $p > 0.05$ for t tests of means between the two periods). This is to be expected as long as water use per capita does not change significantly. In contrast, numbers of total and fecal coliforms in biosolids decreased significantly in the later period, indicating that treatment performance had improved. Treatment reduced total and fecal coliform removals by roughly an order of magnitude in the 1988–1993 period, but by approximately two orders of magnitude in the 1994–2006 period. The values for the 1988–1993 period are similar to those reported in 1993 (Straub et al., 1993) and the 1994–2006 values are similar to literature reports from 2006 to 2008 (Pepper et al., 2006; Sidhu and Toze, 2009). Of interest is the fact that fecal coliform numbers in biosolids after the Part 503 USEPA Rule (USEPA, 1993) were significantly lower than before the promulgation of the Rule, suggesting better treatment and increased treatment removal of coliforms.

Fecal streptococci numbers were not statistically different between 1988 and 1993 and 1994 to 2006 in either raw sewage or treated biosolids. However, numbers were approximately 10^6

Table 3. Organisms and methods of analysis for the national data set.

Organism	Type	*n*†	Source	Method
Heterotrophic plate count	Indicator	22	APHA (2005)	9215A
Total coliforms	Indicator	22	APHA (2005)	9221B
Fecal coliforms	Indicator	22	USEPA (1992) USEPA (2006)	Appendix F 1680 (since 2005)
E. coli 0157:H7	Pathogen	22	Blackburn and McCarthy (2000); Fratamico et al. (1995)	
Fecal streptococcus	Indicator	22	APHA (2005)	9230A
Clostridium perfringens	Indicator	22	Payment and Franco (1993)	
Shigella	Pathogen	22	APHA (2005)	9260E
F+ coliphage	Indicator	21	APHA (2005)	9224C
Somatic coliphage	Indicator	21	APHA (2005)	9224D
Campylobacter	Pathogen	15	APHA (2005)	9260G
Salmonella	Pathogen	22	USEPA (1992)	Appendix F 1682 (since 2007)
Enteric virus	Pathogen	22	USEPA (1992)	Appendix H
Adenovirus	Pathogen	5	USEPA (1992); Castro del Campo (2007); Van Heerden et al. (2003); Avellon et al. (2001)	Appendix H
Ascaris	Pathogen	22	USEPA (1992)	Appendix 1

† Number of samples.

Table 4. Historic concentrations of enteric organisms before and after anaerobic mesophilic digestion, from 1988 to 2006 (Pima County, Arizona, biosolids).

Organisms	Raw			Significance‡	Mesophilic			Significance‡
	Average†	*n*	SD		Average†	*n*	SD	
Total coliforms								
1988–1993	6.46×10^8	27	9.55×10^8	$t = 0.894$	1.83×10^7	33	3.09×10^7	$t = -3.019$§
1994–2006	4.58×10^8	45	6.74×10^8	df = 70 $p = 0.334$	4.13×10^6	45	5.52×10^6	df = 76 $p = 0.003$
Fecal coliforms								
1988–1993	1.30×10^8	27	1.48×10^8	$t = -0.328$	6.05×10^6	32	1.01×10^7	$t = 3.204$§
1994–2006	1.53×10^8	44	4.46×10^8	df = 57 $p = 0.744$	1.08×10^6	44	1.57×10^6	df = 74 $p = 0.002$
Fecal streptococcus								
1988–1993	3.50×10^6	28	3.44×10^6	$t = -0.919$	4.7×10^5	32	5.61×10^5	$t = -1.249$
1994–2006	1.71×10^7	45	7.78×10^7	df = 71 $p = 0.361$	1.51×10^6	45	5.55×10^6	df = 45 $p = 0.218$
Salmonella								
1988–1993	–	–	–	–	–	–	–	–
1994–2006	1884	35	3286	–	40.1	33	118.7	–
Enteric virus								
1988–1993	18.03	20	34.8	$t = -0.105$§	0.61	21	1.11	$t = -1.175$§
1994–2006	19.02	35	31.6	df = 37 $p = 0.917$	2.20	34	7.79	df = 35 $p = 0.248$

† Enteric virus and *Salmonella* values are per 4 g. All other values are per 1 g.

‡ The difference between the two time periods was tested using a *t*-test statistic: the associated two-tailed significance is shown (*p*) with degrees of freedom (df) and *t*-test values (*t*).

§ Equal variances not assumed (tested with Levine's test for variance).

to 10^7 per gram dry weight in sewage and were reduced by one order of magnitude following treatment. Fecal streptococci are of interest because they are utilized as indicators of water quality in recreational surface waters (Bartram and Rees, 2000).

Table 4 also gives historic data on the incidence of bacterial pathogens (*Salmonella* 1994–2006) and enteric viral pathogens (1988–1993 and 1994–2006). These pathogens are important because they are used as part of the criteria for Class A vs. Class B biosolids. *Salmonella* numbers were reduced by two orders of magnitude by anaerobic digestion compared with one order of magnitude for enteric viruses. Virus numbers, in raw sewage sludge and in treated biosolids, were not significantly different in samples obtained before and after 1993. However, earlier studies have reported higher numbers of enteric viruses in anaerobically digested biosolids, before the 503 regulations went into effect. For example, Ward et al. (1984) gave a range of 0.2 to 210 enteric viruses per gram. The wide range is probably reflects the lack of standard treatment processes at the time.

Removal of the study organisms obtained during anaerobic mesophilic digestion are shown in Table 5. Overall, the percent reduction of both pathogens and indicator organisms averaged over all years (1988–2006) was between 94 and 99%. The increase in indicator reductions following treatment (Table 4) is also reflected in the higher percent reductions seen in 1994 to 2006 as compared with 1988 to 1993. Table 6 shows more recent data collected between 2005 and 2008 on the national occurrence of indicator organisms and pathogens in mesophilic anaerobically digested biosolids. Analyses for most organisms reflect data collected from 21 samples from 18 wastewater treatment plants from around the United States. Thus, these data reflect pathogen and indicator organism incidence distributions from across the nation.

The high numbers of heterotrophic bacteria found within all biosolids are due to the presence of large amounts of biodegradable organic matter in biosolids. Arithmetic means of fecal and total coliforms across the United States in the 2005–2008 period are similar to the values found between 1988 and 1993 in Tucson, but one order of magnitude greater than corresponding values between 1994 and 2006. Interestingly, fecal streptococci values from the historic data set at one location are much higher than the more recently collected national data set. *Clostridium perfringens* have also been considered as an indicator of fecal contamination (Bisson and Cabelli, 1980). Mean *Clostridium* numbers within the national data set averaged ~10^7 per gram.

Bacteriophage are often considered to be suitable indicators of enteric viruses, and generally adsorb onto solids and concentrate in the biosolids (Chetochine et al., 2006; Sidhu and Toze, 2009). Somatic coliphage have been reported to be the most abundant in wastewater sludge followed by F+ coliphage (Mignotle-Cadiergues et al., 2002). Our data reported here are similar, with somatic coliphage averaging 10^5 per gram compared with 10^4 per gram for F+ coliphage. Sidhu and Toze (2009) reported values of 10^4 per gram for somatic coliphage compared with 10^3 per gram for F-specific phage.

Bacterial pathogen numbers within biosolids were also evaluated nationally across the United States. *Salmonella* numbers were lower than corresponding values from the Tucson data set, but agree with more recent analyses taken in 2008 to 2009 from Tucson (data not shown). Likewise, *E.coli* O157:H7, *Shigella*, and *Campylobacter* numbers were also low. Similar trends are reported by Sidhu and Toze (2009).

Table 5. Percent reduction by anaerobic mesophilic digestion from 1988 to 2006 (Pima County, AZ, biosolids).

Year	Enteric virus	Fecal coliform	Fecal streptococcus	Total coliform	*Salmonella*
1988	96.17	96.84	93.56	97.60	
1989	ND†	92.31	82.52	89.46	
1990	99.52	97.16	94.85	99.63	
1991	97.89	97.63	0.00	99.02	
1992	92.83	ND	52.61	0.00	
1993	ND	97.60	83.74	97.52	
1994	24.00	99.29	94.34	99.54	
1995	87.38	96.55	91.57	98.19	94.22
1996	98.50	98.40	ND	99.04	99.55
1997	95.67	99.20	58.56	98.73	99.56
1998	97.13	95.20	89.98	95.93	67.72
1999	97.18	94.40	91.11	95.39	99.63
2000	89.03	97.80	46.59	99.29	98.93
2001	ND	99.08	89.49	99.10	97.32
2002	100.00	99.77	66.46	99.54	99.14
2003	46.17	98.39	94.27	98.55	99.78
2004	100.00	98.89	65.83	99.28	99.63
2005	99.96	99.40	80.77	98.97	92.72
2006	96.17	ND	99.95	99.96	
1988–1993‡	97.49	95.40	80.42	97.71	
1994–2006‡	92.39	99.64	97.50	99.37	97.59
All years‡	94.37	98.93	97.01	98.76	97.59

† ND = no data. Gaps also represent no data.

‡ Calculated from averages of the number of organisms before and after treatment.

Viral pathogen numbers are also low in the national data set, with enteric viruses averaging 0.19 per 4 g. This value is much lower than corresponding values reported in the 1980s (Ward et al., 1984). Methods used for the detection of enteric viruses largely select for the enteroviruses, and overall enteroviruses and adenoviruses appear to be the most common viruses found in wastewater, along with perhaps norovirus. There are currently believed to be >100 types of enteroviruses (Yamashita et al., 2010) and at least 52 types of adenoviruses (Mena and Gerba, 2009). However, adenoviruses are generally reported to occur in higher numbers in wastewater than enteroviruses (Mena and Gerba, 2009). Although only a limited number of samples could be tested for adenoviruses, they were found in greater numbers in the treated biosolids than the method currently used for enteric viruses, probably because the method largely selects for the isolation of enteroviruses. These data are among the first to be reported in the United States on viable adenoviruses in biosolids, and show that adenovirus numbers are in fact higher than enteric viral numbers in biosolids. However, more data are needed on the incidence of adenovirus in treated biosolids. Finally note that no viable *Ascaris* ova were detected in any biosolid samples collected anywhere in the country. While *Ascaris* infections still occur in the United States, the incidence of infections is so low that they are not typically detected in the 4-g dry-solid amount of biosolid that is required for analysis.

The Spearman (nonparametric) correlation coefficients (Table 7) indicate that, in general, pathogen occurrence is not well correlated with indicator organism occurrence. Neither of the process variables, retention time and percent solids, were correlated with microbial quality, except for a modest negative correlation between retention time and *Clostridium* ($r = -0.49$, $p = 0.027$). The only significant correlation of a pathogen and indicator was between *Shigella* and total coliforms, and this was only a moderate correlation ($r = 0.48$) that was only modestly significant ($p = 0.026$). The closest correlations are among similar indicators (total and fecal coliforms: $r = 0.74$; F+ and somatic coliphage: $r = 0.71$). These data indicate the need for better indicators of pathogens within biosolids or direct detection of pathogens themselves.

Conclusions

This study suggests that pathogen levels of enteric viruses, *Salmonella*, and *Ascaris* ova in mesophilic anaerobically digested Class B biosolids are fairly low in the United States, and oftentimes meet Class A requirements. No viable *Ascaris* ova were detected, indicating Class A performance. However, this is more likely a reflection of the low incidence of infection in the population in the United States at the current time. Emerging pathogens such as *Campylobacter* and *E. coli* 0157:H7 were never detected, and *Shigella* was only detected occasionally. In contrast, adenoviruses may be more commonly present in greater numbers than enteroviruses, suggesting that additional data on adenovirus may be useful in future risk assessments.

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Table 6. National occurrence of indicator and pathogenic organisms in mesophilic anaerobic digested biosolids from 21 samples collected from 18 different locations around the United States.†

Organism	Arithmetic mean	MLE median	95% confidence interval median	SD	Median of the observed values	Min.	Max.	No. of samples below detection limit
HPC‡§	3.89×10^{11}	7.63×10^{8}	1.81×10^{8}–3.21×10^{9}	1.74×10^{12}	8.57×10^{8}	1.35×10^{6}	8.00×10^{12}	0
Total coliforms§	1.45×10^{7}	7.64×10^{5}	1.72×10^{5}–3.39×10^{6}	3.58×10^{7}	1.62×10^{6}	545	1.58×10^{8}	0
Fecal coliforms§	1.27×10^{7}	9.61×10^{4}	1.57×10^{4}–5.88×10^{5}	3.62×10^{7}	2.49×10^{5}	51.7	1.58×10^{8}	0
E. coli 0157:H7¶	<1	<1		<1	<1	<1	<1	21
Fecal streptococcus#	1.27×10^{4}	510	170–1600	3.2×10^{5}	545	<1	3.15×10^{5}	2
Clostridium perfringens§	4.16×10^{7}	6.76×10^{5}	2.70×10^{5}–1.69×10^{6}	1.86×10^{8}	9.47×10^{5}	3.98×10^{4}	8.53×10^{8}	0
Shigella#	4.49	0.37	0.053–2.63	53.7	7.6	<1	9.2	16
F+ coliphage#	6.36×10^{7}	7.48×10^{4}	15,500–361,000	5.40×10^{10}	2.4×10^{5}	<1	3.79×10^{6}	1
Somatic coliphage#	8.40×10^{8}	2.09×10^{5}	3650–1,200,000	3.38×10^{12}	8.8×10^{5}	<1	1.92×10^{7}	1
Campylobacter¶	<1	<1		<1	<1	<1	<1	21
Salmonella (4 g)†#	3.24	0.96	0.299–3.09	10.4	6.1	<1	13.4	15
Enteric virus (4 g)†#	0.419	0.194	0.020–1.90	0.801	1	<1	3.2	19
Adenovirus	17.6	14.1	7.8–25.4	13.3	18.7	3.7	22.6	0
Ascaris (4 g)†¶	<1	<1		<1	<1	<1	<1	21

† All units are shown as organisms per dry gram of biosolids unless noted as per 4 g of biosolids.

‡ Heterotrophic plate count bacteria.

§ Sample arithmetic mean and standard deviation shown rather than maximum likelihood estimation (MLE) estimates.

¶ All values less than detection limit and no MLE method possible.

The MLE method used for arithmetic mean, geometric mean, and standard deviation because some values are below detection limit.

Table 7. Spearman correlation coefficients for organisms analyzed in the national study.

		RT	%SS	HPC	TC	FC	EHEC	FS	Clo	Shi	F+	Som	Cam	Sal	Ent
Retention time (RT)	CC†	1.00													
	Sig.														
Percent solids, dry wt. (%SS)	CC	−0.22													
	Sig.	0.36													
Heterotrophic plate count	CC	0.15	−0.405	1.000											
(HPC)	Sig.	0.53	0.068												
Total coliform (TC)	CC	0.04	−0.162	0.374	1.000										
	Sig.	0.88	0.482	0.095											
Fecal coliform (FC)	CC	0.07	−0.199	0.158	*0.742*‡	1.000									
	Sig.	0.76	0.388	0.493	*0.000*										
E. coli O157:H7 (EHEC)	CC														
	Sig.														
Fecal streptococcus (FS)	CC	0.198	−0.305	−0.053	0.073	0.075		1.000							
	Sig.	0.402	0.178	0.821	0.754	0.748									
Clostridium perfringens (Clo)	CC	*−0.493*	−0.178	0.040	*−0.462*	−0.266		−0.171	1.000						
	Sig.	*0.027*	0.440	0.862	*0.035*	0.243		0.459							
Shigella (Shi)	CC	−0.212	−0.121	0.210	*0.484*	0.191		0.011	0.069	1.000					
	Sig.	0.370	0.602	0.360	*0.026*	0.406		0.961	0.767						
F+ coliphage (F+)	CC	0.203	−0.294	*0.503*	*0.439*	0.231		0.076	−0.025	0.322	1.000				
	Sig.	0.392	0.197	*0.020*	*0.047*	0.313		0.743	0.915	0.154					
Somatic coliphage (Som)	CC	0.351	−0.295	*0.594*	*0.614*	0.192		0.008	−0.343	0.145	*0.709*	1.000			
	Sig.	0.129	0.195	*0.005*	*0.003*	0.404		0.973	0.128	0.530	*0.000*				
Campylobacter (Cam)	CC														
	Sig.														
Salmonella (Sal)	CC	−0.051	0.339	0.042	0.379	0.020		−0.044	−0.207	0.231	0.081	0.228		1.000	
	Sig.	0.831	0.133	0.855	0.090	0.933		0.850	0.369	0.314	0.726	0.320			
Enteric virus (Ent)	CC	0.282	−0.160	−0.264	0.302	0.231		0.396	−0.215	0.377	−0.038	0.043		0.052	1.000
	Sig.	0.228	0.489	0.247	0.184	0.313		0.076	0.348	0.092	0.869	0.854		0.822	

† CC = correlation coefficient; Sig. = significance.

‡ Statistically significant correlation coefficients are in italics.

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