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Presence of zoonotic pathogens in physico-chemically characterized manures from hog finishing houses using different production systems

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ABSTRACT

Hog production has been intensified in Eastern Canada, by 50% over the last 20 years. Wastes are now managed with conventional production systems (slatted floor), litter systems or source separation systems. We studied the presence of total and fecal coliforms, *Campylobacter*, *Clostridium perfringens*, *Enterococcus*, *Escherichia coli*, *Salmonella*, *Yersinia enterocolitica*, *Giardia* and *Cryptosporidium* in the manure of all of these production systems. The concentrations of the studied zoonotic pathogens did not differ between the conventional and the litter systems investigated. The source separation system yielded separated solid and liquid fractions. Total and fecal coliforms, *C. perfringens*, *Enterococcus*, *E. coli*, and *Y. enterocolitica* were found in the solids and the liquid fractions of a source separation system. *Campylobacter* species were not detected in the liquid fractions. Zoonotic pathogens are therefore, found in the manure of Eastern Canadian hog finishing houses equipped either with conventional, sawdust litter or source separation systems. Pathogens inactivation will require changes in manure management practices or the use of manure disinfection methods.

1. Introduction

Outbreaks of foodborne or waterborne diseases associated with the consumption of meat, fruits, and vegetables, and/or water contaminated by zoonotic pathogens have raised serious concerns about current manure management practices in animal production. As an example, outbreaks of gastroenteritis were previously associated with the consumption of parsley contaminated by *Citrobacter freundii* from hog slurry (Tschape et al., 1995). The nature and concentration of zoonotic pathogens excreted by animals differ according to animal species and health, nutrition, age and housing environment (Bicudo and Goyal, 2003; Cliver, 2009). Many zoonotic pathogens are characterized by episodic outbreaks. The seasonal detection and spatial distribution of pathogens from farm-to-farm are therefore highly variable (Holzel and Bauer, 2008). In hog manure, bacteria and parasites persist for a time depending on geographic location of the farm, on the physicochemical composition of the manure (temperature, pH, free ammonia, and solids content), on aeration and on handling and storage management (Bicudo and Goyal, 2003; Forshell, 1993; Jones, 1980; Kearney et al., 1993; Strauch and Ballarini, 1994; Topp et al., 2009). Hogs are recognized as important hosts of zoonotic bacterial and parasitic pathogens. There are up to 125 different infectious agents found in hogs (D'Allaire et al., 1999).

Eastern Canadian hog production has been intensified by more than 50% over the last 20 years (Canadian Pork Council, 2009). Specialized/intensified Eastern Canadian hog finishing houses are totally enclosed, well-insulated buildings with mechanically controlled ventilation optimizing the temperature and the relative humidity according to animal weight. Currently, there are about 2500 buildings concentrated in a few rural areas, increasing potential for nuisance and environmental problems including odour annoyances, greenhouse gases, ammonia and hydrogen sulfide emissions, nitrogen (N) and phosphorous enrichment of waterways (eutrophication) and microbial contamination of surface and ground water (Imbeah, 1998). In Eastern Canada, large volumes of manure are managed using different systems:

In conventional or standard buildings, wastes composed of urine, feces, wash water and spilled feed are collected as slurry that has a solids content ranging between 3% and 10%. Slurries

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are left in the building for one to a few weeks and are thereafter transferred to external holding tanks or earthen lagoons (Bourque and Koroluk, 2003; Lu et al., 2005). Hog manures are stored for several months.

Currently, in Eastern Canada, there is growing interest in hog finishing houses equipped with source separation systems that separate the feces from the urine by mean of a mechanical scraper or conveyor located in the manure gutters under the concrete slats. The source separation of urine and feces is an effective way to separate nitrogen and phosphorous. More than 80% of the phosphorous may be concentrated in the solid fraction. Both the solid and liquid fractions are stored separately.

In deep and shallow litter systems, sawdust is usually used as a bedding material. The presence of sawdust enables the initiation of composting of hog manure inside finishing buildings. According to different studies, sawdust appears to be an ideal bulking agent for composting pig manure because of its ability to absorb moisture and its structure (Bhamidimarri and Pandey, 1996), which provides adequate porosity in the compost heap (Imbeah, 1998). Composting is an aerobic, biological process which uses naturally occurring microorganisms to convert biodegradable organic matter into a humus-like product (Imbeah, 1998). The process destroys pathogens. The extent of composting in the litter systems is influenced by factors such as temperature, aeration level, moisture content, pH, C:N ratio, particle size, and degree of compaction (Lau et al., 1993). Following removal from finishing houses after a production cycle, the litter is often stored 1–8 months prior land application.

In Eastern Canada, the environmental concerns about these waste management practices have focused on odour and GHG emissions and the effects of N and P nutrients on water quality. In our study, we therefore wanted to evaluate and compare the prevalence of bacterial and parasitic zoonotic pathogens in eastern Canadian hog finishing houses using conventional systems or sawdust litter systems. The prevalence of bacterial and parasitic zoonotic pathogens was described as well in a building using a source separation system to manage hog manures. The following zoonotic pathogens were investigated: *Enterococcus* spp., *Escherichia coli*, *Clostridium perfringens*, *Yersinia enterocolitica*, *Campylobacter* spp., *Salmonella* spp., *Cryptosporidium* and *Giardia*.

2. Methods

2.1. Hog finishing houses

Representative housing was selected for each type of hog production system. The main characteristics of each system are described in Table 1. Conventional or standard buildings operated in an all-in/all-out mode and had slatted floor areas ranging from 30% to 100% of total floor area. Hog production systems with shallow and deep litter used sawdust. Sawdust was changed between each animal production cycle. The source separation operation had a slatted floor over 66% of its area. Under the slatted floor, the V-shaped gutter allowed the urine to flow by gravity in the downward direction and a scraper operated to move the feces in the opposite direction.

Hog manure from six conventional slatted floor buildings (Standard A–E, Source Separation Control), six buildings with litter (Sawdust A–F) and one Source Separation building were sampled between 2005 and 2007 (Table 1). The water supplies for the finishing operations were tested annually to ensure that they complied with Health Canada's guidelines on drinking water quality. One standard (B) and two buildings with sawdust (C and D) added a chlorine disinfectant to their drinking water. In all the operations under investigation, no animals were treated for respiratory infections or diarrhea. Usually, animals received low doses of antibiotics, tylosin and/or chlortetracycline, in feeds for prophylaxis or as growth promoters (Table 1).

2.2. Sample collection

Samples of hog manure were collected once, during the winter season, in Standard A–E and Sawdust A–F buildings whereas wastes from a Source Separation system and from a conventional system (Source Separation Control) were sampled at three intervals (June 2005, November 2005 and March 2006). The hog finishing houses were composed of rooms in which hogs were grouped in compartments. We sampled one room in each visited house. The manure of three randomly selected compartments was sampled in a room where there were more than 10 compartments and two compartments were sampled in a room where there were less than 10 compartments. For each compartment, manure samples were collected in 1 L sterile high-density polyethylene bottles at five locations (2–10 cm or 25–30 cm depth) in the slurry or urine transfer tanks, sawdust litters and feces piles. The samples were then mixed to form a composite sample representing each hog finishing house. Two litres of wastes were used for microbiological characterization and 1 L for physico-chemical analysis. The samples were kept on ice (4 °C) prior being analyzed. All samples have been analyzed within a 48-h period. Samples of water and feed were also taken in 1 L sterile high-density polyethylene bottles for pathogens detection. Feeds were sampled from storage containers.

2.3. Physico-chemical characterization of wastes

Temperatures and pH of the wastes were measured at the farm with a hand-held pH meter (model IQ150, IQ Scientific, Carlsbad, CA, USA) fitted with a stainless steel probe (PH77-SS, IQ Scientific, Carlsbad, CA, USA). Alkalinity, volatile fatty acids (VFAs), chemical oxygen demand (COD), total Kjehldahl nitrogen (TKN), ammoniacal nitrogen ($N\text{-}NH_3$), bacterial N and total and volatile solids were measured according to standard methods (American Public Health Association (APHA), 1992). VFAs (acetic, propionic, butyric, isobutyric, valeric, isovaleric and caproic acids) were analyzed with a gas chromatograph (GC Autosystem, Perkin Elmer, Norwalk, CT, USA) installed with a fused silica capillary column (#J1253232, J&W Scientific, Folson, CA, USA) using 2-ethyl butyric acid as an internal standard. The injection, the oven and the flame ionization detector (FID) temperatures were, respectively, programmed at 200, 135 and 250 °C. The COD of the wastes was analyzed by using the closed reflux colorimetric method (method 5220D, (American Public Health Association (APHA), 1992)) using a Spectronic 20D+ spectrophotometer (Spectronic Instruments Inc., Rochester, NY, USA) and TKN or $N\text{–}NH_3$ by selenous acid digestion at 400 °C (KjeltecTM 2400/2460 Autosampler System, Foss Tecator AB, Hoganas, Sweden). Ammoniacal-N was subtracted from TKN to determine organic N. Finally, total solids and volatile solids were determined by drying the samples for 16 h at 105 °C (Convection oven, CGA Precision Scientific Group, Chicago, IL, USA) followed by ashing for 2 h at 550 °C (Lindberg Blue M828 Incinerator, Lindberg Blue M, Asheville, NC, USA).

2.4. Isolation and identification of indicator bacteria and zoonotic bacterial pathogens

For total and fecal coliforms, *Enterococcus*, *E. coli*, *C. perfringens* and *Y. enterocolitica* isolation, feed, water and manure samples were prepared by diluting 1:10 (either as 10 g per 90 mL or as 10 mL per 90 mL) in sterile sodium metaphosphate buffer (2 g/L). From the original dilution, 10-fold dilutions (1:10 to 1:10,000) were prepared for enumeration of bacteria. The pathogens in water

Table 1
Hog housing characteristics with conventional, sawdust litter or with a source separation system.

Houses	m^2/hog	Days in finishing house	Feed texture	Antibiotic diet[a]	Waste age (days since elimination)	Litter removal	Disinfection
Standard A	0.73	45	Pelleted	Tylosin	1	NA[b]	Yes
Standard B	0.65	38	Finely ground	Chlortetracycline, penicillin, tylosin	217	NA	No
Standard C	1.14	67	Finely ground	Tylosin	16	NA	Yes
Standard D	1.12	91	Finely and coarsely ground	Chlortetraclycine	21	NA	Yes
Standard E	0.83	43	Pelleted	None	1	NA	Yes
Sawdust A	ND[c]	ND	Pelleted	ND	NA	Between batches[e]	Yes
Sawdust B	1.45	80	Pelleted	None	NA	Each 2 batches	No
Sawdust C	1.10	84	Finely ground	Chlortetracycline	NA	Between batches	Yes
Sawdust D	1.34	64	Pelleted	Fenbendazole, piperazine, chlortetracycline, tylosin	NA	Between batches	Yes
Sawdust E	1.12	78	Pelleted	Fenbendazole, tylosin	NA	Between batches	Yes
Sawdust F	1.19	79	Pelleted	Fenbendazole, chlortetracycline, tylosin	NA	Between batches	Yes
Source separation control (standard)	0.83	92	Pelleted	Chlortetracycline	2	NA	Yes
Source separation	0.37	87	Pelleted	None[d]	1	NA	Yes

[a] Subtherapeutic doses of antibiotics for growth promotion or prophylaxis.
[b] NA = not applicable.
[c] ND = not determined.
[d] Use of therapeutic doses of chlortetracycline.
[e] A batch represents a production cycle of growing/finishing pigs.

samples were enumerated by membrane filtration on the media indicated below. Membrane filtration involved filtration of the sample (0.1, 1 and 10 mL volumes) through a sterile GN-6 membrane (Pall-Gelman, VWR, Mississauga, ON, Canada), and subsequent plating of the membrane on the chosen media. Total coliforms were enumerated by direct plating on mEndo-LES agar (Difco, Mississauga, ON, Canada) and incubated at 37 °C for 18–20 h. Colonies which produced a distinctive metallic green sheen were counted as total coliforms. Fecal coliforms were quantified by direct plating on mFC agar (Difco, Mississauga, ON, Canada) and incubated for 18–20 h at 44.5 °C. Colonies producing a distinctive indigo blue color were enumerated as fecal coliforms. *Enterococcus* spp. were counted by direct plating onto mEnterococcus agar (Difco, Mississauga, ON, Canada) and incubated at 37 °C for 48 h. Colonies producing a burgundy color were counted as *Enterococcus* sp. *E. coli* were enumerated by direct plating on mFC basal medium (Difco, Mississauga, ON, Canada) supplemented with 3-bromo-4-chloro-5-indolyl-β -D-glucuronide (100 mg/L, Med-Ox Diagnostics, Ottawa, ON, Canada) and incubated for 18–24 h at 44.5 °C. Colonies producing the characteristic blue color indicative of β-glucuronidase activity were enumerated as *E. coli*. *C. perfringens* was quantified on mCP agar (Med-Ox Diagnostics, Ottawa, ON, Canada) incubated at 44.5 °C for 24 h. Yellow colonies with a yellow halo and which turned magenta after exposure to ammonium hydroxide fumes were expected to be *C. perfringens*. Presumptive colonies were confirmed by inoculation into skim milk broth. Stormy fermentation of the broth after 24 h was considered a positive confirmation for *C. perfringens*. *Y. enterocolitica* were enumerated by direct plating onto Cefsulodin-Irgasan-Novobiocin agar (CIN, Difco, Mississauga, ON, Canada) and incubated for 18 h at 30 °C. Colonies that were less than 2 mm in diameter and formed a red bulls-eye were considered presumptive colonies for *Y. enterocolitica*. Five presumptive colonies were picked and inoculated into lysine arginine iron agar slants (LAIA) and considered positive if LAIA results were alkaline slant, acid butt, H_2S negative and gas negative.

For *Campylobacter* spp. isolation, 25 g of manure, sawdust bedding or feed and 20 mL of water were added to 50 and 20 mL, respectively, of Bolton broth (Oxoid, Nepean, ON, Canada) supplemented with 0.02 mg/mL of cefoperazone, 0.02 mg/mL of vancomycin, 0.02 mg/mL of trimethoprim, 0.05 mg/mL of cycloheximide and 5% (v/v) of Lake Horse Blood (Oxoid, Nepean, ON, Canada) for selective enrichment and incubated for 18–24 h at 42 °C in microaerophilic conditions in an anaerobic jar with GasPakTM EZ Campy (6–16% O_2, 2–10% CO_2, BD, Oakville, ON, Canada). Then, one loop of enriched cultures was stroked on the blood-free selective media plates of Charcoal Cefoperazone Desoxycholate Agar (CCDA, Oxoid, Nepean, ON, Canada) supplemented with 0.032 mg/mL of cefoperazone and 0.01 mg/mL of amphotericin B (Oxoid, Nepean, ON, Canada). Plates were incubated at 42 °C in microaerophilic conditions for 48 h in an anaerobic jar with GasPakTM EZ Campy (6–16% O_2, 2–10% CO_2, BD, Oakville, Ontario, Canada). *Campylobacter*-like colonies (translucent/metallic and irregular colonies) were confirmed with Gram coloration, oxydase and catalase tests, indoxyle acetate hydrolysis (Sigma–Aldrich, Oakville, ON, Canada), hyppurate hydrolysis (Sigma–Aldrich, Oakville, ON, Canada) and API Campy (BioMérieux, Ville Saint-Laurent, QC, Canada).

Salmonella spp. was isolated from 10 g of manure/sawdust, 100 g of feed and 20 mL of water to which were added 90, 900 and 20 mL of Nutrient Broth, respectively (EMD, Gibbstown, NJ, USA). Following 18–24 h of incubation at 37 °C, 1 mL of pre-enriched cultures was added to 9 mL of Tetrathionate Brilliant Green (TBG) broth (BD, Oakville, ON, Canada) containing 20 μL/mL of iode. Tubes were incubated for 18–24 h at 37 °C and then selective enriched cultures were stroked onto Brilliant Green Sulfa (BGS) agar (BD, Oakville, ON, Canada) plates containing 20 μg/mL of novobiocine. Following 18–24 h of incubation at 37 °C, typical *Salmonella* colonies, lactose-negative colonies, were further tested on Triple Sugar Iron agar (TSI, BD, Oakville, ON, Canada), Christensen's urea (BD, Oakville, ON, Canada), by agglutination against polyvalent rabbit O-antisera (Difco, Mississauga, ON, Canada), and with

API20E (BioMérieux, Ville Saint-Laurent, QC, Canada). Serotyping of isolates was done by LEAQ (MAPAQ, St-Hyacinthe).

Cryptosporidium and *Giardia* identification and enumeration were performed using an adapted version of the United States Environmental Protection Agency (USEPA, 2005) method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/UIMS/FA. Briefly, following homogenization of wastes, approximately 10 g were added to 40 mL sterile solution of Tris–Tween 80 0.5%, agitated for 15 min and then filtered through sterile 10 × 10 cm 8-ply gauze (All Gauze, A.R. Medicom Inc, Lachine, QC, Canada). About 10 mL of filtrate were diluted 1:5 in sterile filtrated water, vortexed for 15 min, and centrifuged at 1500*g* for 15 min. Pellets were suspended in sterile filtrated water and oocysts/cysts were concentrated by adding *Cryptosporidium*/*Giardia* specific antibodies-labeled magnetic beads (Dynabeads® GC-Combo, Dynal® Biotech Inc, Lake Success, NY, USA). Briefly, using a magnetized device against *Cryptosporidium*/*Giardia*-beads complexes, solutions were washed twice and acidified with hydrochloric acid (HCl) 0.1 N to dissociate parasites and magnetic beads. Finally, solutions were neutralized by adding sodium hydroxide (NaOH) 1 N. *Cryptosporidium* oocysts and *Giardia* cysts were detected in the resulting solutions using specific fluorescein isocyanate (FITC) labeled monoclonal antibodies against cell wall antigens (MeriFluor® *Cryptosporidium*/*Giardia*, Meridian Bioscience, Inc., Cincinnati, OH) and Eriochrome Black solution as a counterstain. Marked samples were observed with an epifluorescence microscope at 200× and 400× magnifications [Leica DM LB2 microscope equipped with a blue filter (450–490 nm-excitation) for the detection of FITC-mAb labeled cysts/oocysts and an ultraviolet (UV) filter block (340–380 nm excitation) for 4′-6-diamidino-2-phenylindole (DAPI)]. Presence of stained oocysts/cysts was identified according to morphology. Positive and negative controls (MeriFluor® *Cryptosporidium*/*Giardia*, Meridian Bioscience, Inc., Cincinnati, OH, USA) were run with samples. *Cryptosporidium*/*Giardia* viability was determined by using the fluorochrome 4′,6-diamidino-2-phenylindole (DAPI).

2.5. Statistical analysis

Continuous data from all studied hog finishing houses were expressed using mean ± standard deviation or as percentage for categorical data. The analyses of categorical variables from Standard (A–E) and Sawdust (A–F) production systems were performed using the Fisher's exact test. Student's *t*-test was used to compare continuous data from Standard (A–E) and Sawdust (A−F) production systems. The analysis of the data from the Source separation control (standard) and the Source separation system is descriptive. The Pearson's correlation coefficients were used to measure the relationship between the variables. All data were normally distributed as confirmed by the Shapiro–Wilk test. The results were considered significant with *p*-values $\leqslant 0.05$. The data were analyzed using the statistical package program SAS v9.1.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of hog finishing houses with or without sawdust litters

The characteristics of the conventional hog finishing houses and the buildings with sawdust litters that were studied are reported in Table 1. As mentioned in the Table 1, in conventional houses, there was on average less area per animal than in buildings using sawdust, 0.89 and 1.24 m^2/hog, respectively ($p = 0.02$). The fenbendazole, an anthelmintic, was only added in the diet of hogs fed on sawdust litters. No other differences have been observed in the studied characteristics of hog finishing houses with or without sawdust litters.

3.2. Feed and water sampled from hog finishing houses with or without sawdust litters

Unusual high concentrations of total and fecal coliforms were recovered from feeds of Standard B, 90,000 CFU/g and 15,000 CFU/g, respectively (data not shown). Fecal coliforms were also detected at low concentrations in feeds of Standard C. *Enterococcus* spp. concentrations frequently reached 10^2–10^3 CFU/g of feeds (data not shown). Standard A–C and Sawdust A, B, E and F were all positive for *Enterococcus* spp. Standard B and C showed the highest *Enterococcus* concentrations with 60,000 and 6200 CFU/g, respectively. No detectable *E. coli*, *C. perfringens* and *Campylobacter* spp. were found in the analyzed feeds while 1800 CFU/g of *Y. enterocolitica* were found in feeds from Standard B. *Salmonella* spp. was detected in the feeds of Sawdust E and F. Feeds from Sawdust E were contaminated by *Salmonella* group C1 (*Salmonella* serotype Braenderup; data not shown).

Water samples from the eleven hog houses investigated with or without sawdust litters contained undetectable total and fecal coliforms (data not shown). *E. coli*, *Enterococcus* spp., *C. perfringens*, *Y. enterocolitica* and *Salmonella* spp. were not detected in water supplies of the sampled buildings. Only one water sample from Standard C was positive for *Campylobacter*, specifically *C. coli* (data not shown). The presence of *Cryptosporidium* and *Giardia* was not evaluated in feeds and water samples.

3.3. Physico-chemical properties of manures from hog finishing houses with or without sawdust litters

With a few exceptions, there were significant differences ($p < 0.05$) between the physico-chemical characteristics of manures collected from conventional houses and those from operations using sawdust litters. Slurries from standard systems reached average temperatures of 15 °C and pH of 7.1 (data not shown) while litters showed a mean temperature of 27 °C (up to 30 °C for Sawdust C) and an average pH of 7.5 (data not shown). Acetic, propionic, butyric, isobutyric, and isovaleric acids were in higher concentrations in conventional slurries than in litters ($p < 0.05$; Fig. 1). For example, manure from standard buildings contained 8024 mg/L of acetic acid while wastes from systems with sawdust litters contained only 2060 mg/L. Wastes from conventional systems were also characterized by lower concentrations of total COD and higher quantities of soluble COD than litter samples ($p = 0.004$ and 0.0005, respectively). While TKN concentrations were not statistically different in wastes from the two production systems, $N–NH_3$ showed a mean concentration of 4591 mg/L in conventional wastes compared to 2644 mg/L in bedding materials ($p = 0.0008$). Finally, as expected due to the presence of sawdust, the total solid concentrations in systems with litters were higher than the solids contents in waste from conventional operations (34.5% and 7.2%, respectively, $p < 0.0001$; data not shown).

3.4. Presence of zoonotic pathogens in manures from hog finishing houses with or without sawdust litters

Total and fecal coliforms, *E. coli* and *Enterococcus* spp. were found in concentrations between 10^4 and 10^6 CFU/g of wastes (Fig. 2). *C. perfringens* and *Y. enterocolitica* were detected in lower concentrations (10^2–10^5 CFU/g) and were both absent in wastes of Standard C. *Y. enterocolitica* were also not detected in Sawdust A. Out of eleven houses, five showed hog wastes contaminated with *Campylobacter* spp. (two with sawdust) and six showed wastes contaminated with *Salmonella* spp. (four with sawdust).

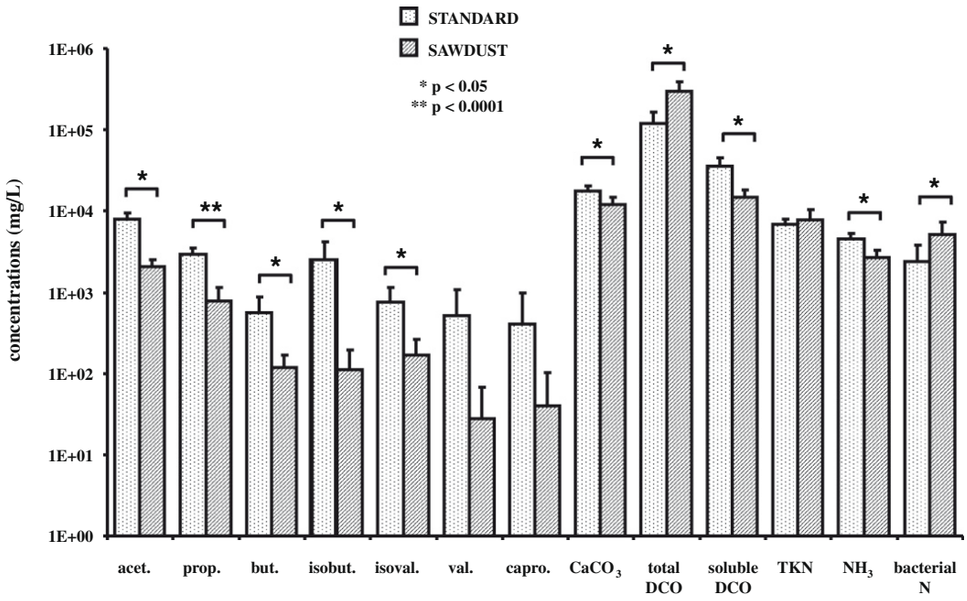

Fig. 1. Physico-chemical properties of manures from standard hog finishing houses and buildings with sawdust litters (Mean ± SD).

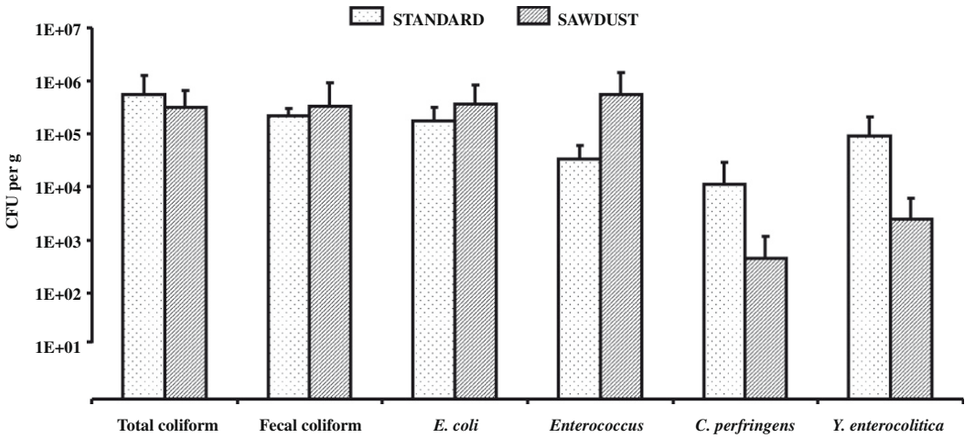

Fig. 2. Concentrations of bacterial zoonotic pathogens in stored manures of standard hog finishing houses and buildings with sawdust litters (Mean ± SD; detection limit, 100 CFU/g).

Cryptosporidium oocysts were detected in four of the 11 farms (three with sawdust) and cysts of *Giardia* in seven of the 11 hog finishing houses (four conventional). Total coliform concentrations were significantly correlated to the presence of fecal coliforms, to *Enterococcus* spp. and to *Y. enterocolitica* ($p < 0.05$). There were also statistical associations between *E. coli* and fecal coliforms and between *E. coli* and *Enterococcus* spp. ($p < 0.05$).

There was no statistical difference in the presence or in the concentrations of zoonotic pathogens between manures from conventional hog finishing houses and from systems with sawdust litters ($p > 0.05$, Fig. 2).

3.5. Characterization of the solids and the liquid fraction of a source separation system

The Separation source control, a hog finishing house with a conventional production system, and the Source separation building had equivalent building management practices, animal diets and production cycles (Table 1). Hogs were therapeutically treated with chlortetracycline in the barn equipped with a source separation system while subtherapeutic doses of chlortetracycline were added in feeds of the conventional building for growth promotion (Table 1). Feeds of the source separation building were positive for *Enterococcus* spp. *E. coli*, *C. perfringens*, *Y. enterocolitica*, *Campylobacter* spp. and *Salmonella* spp. were not found in the analyzed feeds of the two houses (data not shown). Water samples from both houses were free of coliforms and zoonotic bacterial pathogens (data not shown).

Manure temperatures in the Source separation building were equivalent to the temperatures observed in the standard hog finishing houses (data not shown). The pH of the solid and the liquid fractions were both alkaline (pH 8.0 and 8.2, respectively) while slurries from the Source separation control had a pH of 7.4. The other investigated physico-chemical properties of slurries from the Source separation control and those of the solids and the liquid fraction of the Source separation system are described in Table 2.

Table 2
Physico-chemical properties of slurry from a conventional hog finishing house and of the solid and the liquid fractions from a hog finishing house with a source separation system.

Houses	°C	pH	Volatile fatty acids (mg/L)[b]						$CaCO_3$ (mg/L)	DCO (mg/L)		TKN (mg/L)	N–NH_3 (mg/L)	Bacterial N (mg/L)	Total solids (%)	Volatile solids (%)
			Acet	Prop	But	Isobut	Isoval	Val	Capro		Total	Soluble					
Control (standard)	17.6[a]	7.4	7434	2134	340	1413	573	186	128	20,300	143,532	30,855	8321	5116	3204	10.1	8.1
Liquid fraction	15.4	8.2	5868	1,047	153	813	276	70	92	20,022	56,575	20,423	6753	5509	1245	5.2	3.6
Solids	16.8	8.0	9885	2,567	331	4,870	537	283	177	20,614	385,645	58,506	15,756	5727	10,028	28.8	24.6

[a] Mean from data collected in June 2005, November 2005 and March 2006.
[b] Acet: acetic acid; Prop: propionic acid; But: butyric acid; Isobut: isobutyric acid; Isoval: isovaleric acid; Val: valeric acid; Capro: caproic acid.

Results are expressed as mean of the data collected in June 2005, November 2005, and March 2006. Total and fecal coliforms, *C. perfringens, Enterococcus* spp., *E. coli*, and *Y. enterocolitica* were found in slurries, in the solids and in the liquid fraction of the manure (Fig. 3). Interestingly, *Campylobacter* were only detected in the solids while *Salmonella* were recovered from the slurries of the Source separation control and in both fractions of the Source separation system (data not shown). *Giardia* and *Cryptosporidium* were only determined in the solids. Samples were positive for both parasites in the March 2006 sample (data not shown). Source separation control slurries were contaminated by *Cryptosporidium*.

4. Discussion

The recent industrialization of hog finishing houses has been associated with the development of production systems that properly collect, handle and stored animal wastes prior its agronomic valorization as an organic fertilizer. Destruction of zoonotic pathogens in hog manure has never been a priority even though bacterial and parasitic zoonotic pathogens may be responsible for diseases with extensive debilitating effects like arthritis and meningitis (Public Health Agency of Canada (PHAC), 2001, 2003).

The presence of zoonotic bacterial and parasitic pathogens was evaluated in hog manures from conventional (standard) liquid handling systems and from operations with sawdust litters. First, we investigated if the feed and water supplies could be an important source of contamination. One of the 11 studied hog finishing houses with or without litters had more than 10^4 CFU/g of total and fecal coliforms and *Enterococcus* (Standard B) while the others had none or less than 10^3 CFU/g of zoonotic bacterial pathogens in feeds. None of the houses had contaminated water.

There were significant differences in temperatures, VFAs concentrations, and chemical oxygen demand in manure from conventional houses and from the operations with sawdust litters. However, there was no significant difference in zoonotic pathogen concentrations between the two systems (Fig. 2). One would have expected lower concentrations of bacterial pathogens in the litters than in the slurries due to composting activities since the persistence of bacterial zoonotic pathogens in manure is reduced with time, elevated temperatures, and aeration (Cliver, 2009; Derbyshire and Brown, 1979; Hutchison et al., 2005; Ros et al., 2006). The temperature is the most important indicator of the efficiency of the composting process and is dependent on aeration rate (Lau et al., 1992). A maximum temperature of 55–65 °C is necessary to destroy pathogens in composting hog manure, but mesophilic temperatures of 45–55 °C must be maintained for maximum biodegradation (Imbeah, 1998). The aeration promotes the growth and the action of microorganisms that adversely affect the pathogens. In our study, the low temperature (26.7 ± 3.1 °C) in the litter systems and the presence of VFAs indicated that there was a low microbial activity in the mixture of sawdust and manure. Composting is a oxidative biological process that is influenced by several factors such as aeration level, moisture, pH and nutritional requirements, and C/N ratio (Forshell, 1993). Aeration may have been insufficient in bedding materials. All litter operations investigated have only used passive aeration. Sawdust was added once before the beginning of the production cycle or before the arrival of the hogs, and there was no subsequent addition of sawdust during the production cycle. The mixing of the feces and urine with the sawdust was only done by the movement of the animals. Particles size also influences the aeration and a too finely cut sawdust may also have adversely affected the aeration of the litters (Forshell, 1993). However, Lau et al. reported similar data from composts which were not aerated and the aerated composts suggesting that passive aeration would be suitable for hog manure composting

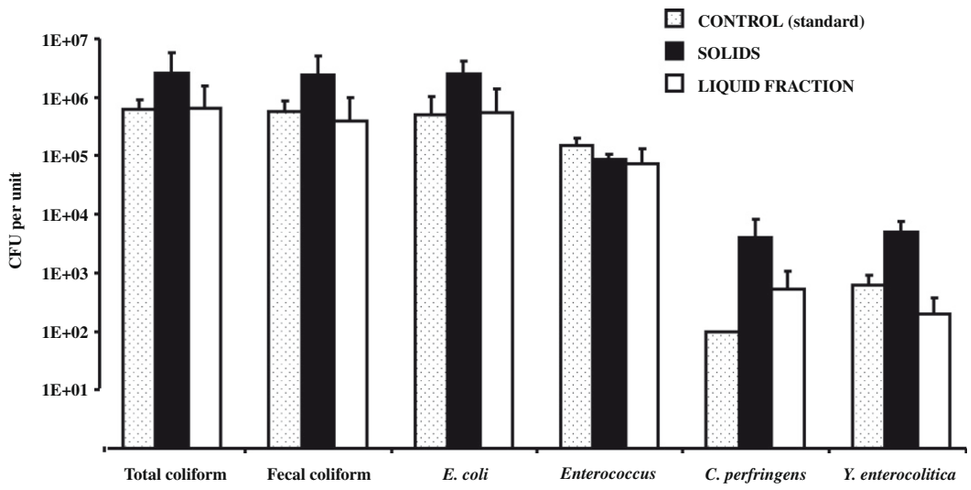

Fig. 3. Concentrations of bacterial zoonotic pathogens in stored manures of one standard hog finishing house and in solids and liquid fraction of one building with a source separation system (Mean ± SD; detection limit, 100 CFU/g and 4 CFU per 100 mL; unit = g or mL).

(Lau et al., 1992). Furthermore, composting may have not happened in litters since manure, a liquid mixture of 7.1 ± 3.8% of total solids, were directly mixed with the sawdust. Indeed, it has been shown that composting substrates with moisture content of above 65% is not feasible (Kashmanian and Rynk, 1995). A separation of the solids would have reduced moisture content and improve composting. Optimal pH for the composting is 5.5–8 with extremes of 3–11. Nevertheless, control of pH is normally not necessary (Forshell, 1993). The mean pH of the litter investigated in this study was weakly alkaline (pH 7.5 ± 0.3) and highly suitable for composting. The low level of composting activity might also be due to a too high C/N ratio that is when the animals are on fresh litter or to a too low C/N ratio when there were areas saturated by excrements. An excess of sawdust or hog manures would certainly slow down the composting process. Liao et al. successfully composted hog manure, containing 79% moisture, with sawdust, as a bulking agent, in a ratio of 5:1 (manure: sawdust, w/w) (Liao et al., 1993). Therefore, the litter operations investigated did not reach optimal conditions for the elimination of pathogens. A few authors recommended a density of fecal coliforms for compost sanitation of 500–1000 CFU/g (Ros et al., 2006). In our study, we detected on average $3.4 \pm 5.7 \times 10^5$ CFU/g of fecal coliforms in hog finishing houses with litter.

The physical separation of feces and urine by the source separation system resulted in solids having TS and VS concentrations of 29% and 25%, respectively. Total and fecal coliforms, *C. perfringens Enterococcus, E. coli*, and *Y. enterocolitica* have been found in the solids and in the liquid fractions. However, *Campylobacter* species, an oxygen sensitive organism, were only detected in the solids. Pathogen reduction in the solids might be more difficult than in slurries of conventional hog finishing houses as bacteria survival is increased in manure with a higher solid content (Strauch and Ballarini, 1994).

5. Conclusions

The concentrations of zoonotic pathogens in manures did not differ between conventional and sawdust litter. Therefore, manures from conventional and litter systems will both have to be stored or adequately disinfected prior land application. Zoonotic pathogens have been found as well in the solids and the liquid fractions of a source separation system. Compared to conventional slurries, solids resulting from manure fractioning may have to be stored a longer time before disposal on agricultural lands since a high content in solids may promote survival of zoonotic pathogens associated with foodborne and waterborne illnesses. Further studies must to be designed to evaluate the impact of the fractioning method on pathogens fate after the use of the solids as an organic fertilizer.

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