ORIGINAL ARTICLE

# Risk of Infectious Gastroenteritis in Young Children Living in Québec Rural Areas with Intensive Animal Farming: Results of a Case–Control Study (2004–2007)

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## Impacts

- The majority of the severe cases of gastroenteritis in young children living in Québec rural areas are of viral origin, predominantly due to rotavirus, which is not of zoonotic origin. However, among the few bacterial infection cases, there was some evidence of increased contact with animals (domestic, zoo or livestock).
- No link was observed between gastroenteritis incidence and drinking water microbiological quality (groundwater or treated surface water).
- Pathogenic bacteria isolated from children with gastroenteritis were not associated with drinking water, including groundwater in a farm environment.

**Keywords:**
Gastroenteritis; zoonotic; children; Québec; livestock; drinking water

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## Summary

This study was designed to evaluate the epidemiology of severe gastroenteritis in children living in Québec rural areas with intensive livestock activities. From September 2005 through June 2007, 165 cases of gastroenteritis in children aged from 6 months to 5 years, hospitalized or notified to the public health department were enrolled, and 326 eligible controls participated. The parents of cases and controls were asked questions about different gastroenteritis risk factors. The quality of the drinking water used by the participants was investigated for microbial indicators as well as for four zoonotic bacterial pathogens (*Campylobacter* spp, *Escherichia coli*, *Salmonella* spp and *Yersinia* spp) and two enteric parasites (*Cryptosporidium* spp and *Giardia* spp). From 134 stool specimen analysed, viruses were detected in 82 cases (61%), while 28 (21%) were found with at least one of the bacteria investigated, and five cases were infected by parasites. Campylobacteriosis was the main bacterial infection ($n = 15$), followed by *Salmonella* sp ($n = 7$) and *E. coli* O157: H7 ($n = 5$) among cases with bacterial gastroenteritis. No significant difference was found between cases and controls regarding the quality of water consumed; the frequency of faecal contamination of private wells was also similar between cases and controls. Considering the total cases (including those with a virus), no link was found between severe gastroenteritis and either being in contact with animals or living in a municipality with the highest animal density (4th quartile). However, when considering only cases with a bacterial or parasite infection ($n = 32$), there was a weak association with pig density that was not statistically significant after adjusting for potential confounders. Contact with domestic, zoo or farm animals were the only environmental factor associated with the disease.


 

## Introduction

Intensive livestock activities generate manure run-off, which can be an important source of microbial water contamination (Thurston-Enriquez et al., 2005). This could be a significant source of pathogenic microorganisms, if improper manure handling contaminates the water supply (Guan and Holley, 2003). As a consequence, drinking water in regions with intensive agricultural activities may be responsible for increasing the risk of acute enteric infections, especially from non-disinfected groundwater (Kapperud et al., 2003; Kuusi et al., 2003).

Agro-environmental activities represent a potential pressure on water quality and may lead to the presence of waterborne pathogenic microorganisms from animal sources (zoonotic agents) (Bigras-Poulin et al., 2004). This situation has been described in several studies in the province of Ontario (Canada), and cattle density was positively associated with notified gastrointestinal diseases caused by *Escherichia coli* O157:H7 (Michel et al., 1999) or Shiga toxin-producing *E. coli* (Valcour et al., 2002). In a review of waterborne outbreaks in Canada during the 1974–2001 period, Schuster et al. (2005) found eight outbreaks (of 288) attributed to livestock, and as reported by Guan and Holley (2003), several waterborne outbreaks in North America for the 1979–2001 period could have involved improper manure handling.

Children have been identified as a sensitive population for gastroenteritis morbidity (Balbus and Lang, 2001; Kosek et al., 2003; Olesen et al., 2005). Most studies have demonstrated that children are more prone to become ill from enteric pathogens than adults (Peterson and Calderon, 2003; Sinclair et al., 2005) with a disproportionate disease burden for those younger than 5 years (Koehler et al., 2006). Payment et al. (1997) reported that children 2–5 years old were the most affected by gastrointestinal illnesses during a prospective study. A recent survey also indicated a higher prevalence of gastroenteritis for children aged less than 5 years living in rural municipalities with an 'excess' of animal manure in the province of Québec (Febriani et al., 2009).

We conducted a population-based case–control survey of all children aged less than 5 years and living in a large rural area, with intensive animal farming, admitted to hospitals or notified to the public health department (as reportable diseases), with symptoms of acute diarrhoea during a period of 21 months. The specific objective of the study was to verify whether drinking water from private wells or public waterworks in rural or intensive animal farming was a risk factor for water contamination and gastroenteritis in a sensitive subpopulation. We also intended to evaluate more proximal factors such as contact with farming or domestic animals.

## Material and Methods

### Study area

The study was conducted from November 2004 through June 2007 in the administrative area of Chaudière-Appalaches (province of Québec, Canada), which had a population of approximately 400 000 inhabitants (5.2% of Québec's population) among 136 municipalities. This region was selected due to its importance in livestock agricultural production; the area includes 18% of the farms in Québec and is responsible for 29% and 20% of porcine and avian production, respectively (MAPAQ, 2008). The study was conducted with the collaboration of the paediatric departments of the five hospitals covering the entire area and where hospitalized cases were identified: three general care community hospitals and two tertiary university hospitals. Non-hospitalized cases (diagnosed in private clinics or at a doctor's office) notified to the region's public health department were reported to the research team. Because the notified cases were recruited only after August 2005 and the recruitment of the hospitalized patients was fully operating only after that date, the analysis only included data from 1 September 2005–31 June 2007.

### Cases and controls

We defined a case as a child who was (i) a resident of the study area; (ii) aged from 6 months to 5 years; (iii) either (a) hospitalized for diarrhoea in the absence of a diagnosed chronic gastrointestinal disease; or (b) notified to the public health department with a laboratory diagnosis of gastroenteritis for one of the microbial enteric pathogens of interest. Within hospitals, medical staff approached the parents or caretakers of eligible children to obtain consent to provide epidemiological and clinical information. Notified community cases (not hospitalized) were investigated by nurses from the public health department.

For each case, two controls in the same age category (Table 1), living in the study area, were selected at random from the Québec public health insurance plan (Régie de l'assurance-maladie du Québec) and contacted the following day after the case was discovered. Before being phoned, parents of potential controls had received a letter of information about the investigation. Criteria for exclusion of potential controls were (i) a history of diarrhoea during the preceding 2 months and (ii) being a sibling of a case.

### Interviews

Shortly after the children were discharged from the hospitals, data were gathered by asking parents or caretakers to complete a standardized questionnaire by telephone. The

**Table 1.** Descriptive information of young children participating to a case–control study of gastroenteritis in Québec rural areas (2004–2007)

| | Cases ($n$ = 165) No. (%) | Controls ($n$ = 326) No. (%) | Univariable model* | |
|---|---|---|---|---|
| | | | Crude OR (95% CI) | $P$ value |
| Socio-economic and demographic characteristics | | | | |
| Age | | | | |
| 6–12 months | 31 (18.8) | 63 (19.3) | 1.00 | 0.937 |
| 13–24 months | 61 (37.0) | 117 (35.9) | 1.07 (0.64–1.79) | |
| 25–42 months | 54 (32.7) | 106 (32.5) | 0.96 (0.56–1.65) | |
| 43–59 months | 19 (11.5) | 40 (12.3) | 0.90 (0.49–1.64) | |
| Sex | | | | |
| Male | 98 (59.4) | 154 (47.2) | 1.00 | 0.015 |
| Female | 67 (40.6) | 172 (52.8) | 0.63 (0.43–0.91) | |
| Education (no college or university diploma) | 74 (45.4) | 100 (30.7) | 1.67 (1.18–2.35) | 0.004 |
| Medical history | | | | |
| Breastfeeding | 120 (72.7) | 253 (77.6) | 0.81 (0.53–1.24) | 0.327 |
| Chronic diseases | 11 (6.7) | 7 (2.3) | 2.85 (1.21–6.72) | 0.016 |
| Premature child | 15 (9.1) | 19 (5.8) | 1.59 (0.73–3.45) | 0.240 |
| Low birth weight < 2500 g | 14 (8.6) | 15 (4.6) | 2.12 (1.15–3.90) | 0.017 |
| GI risk factors | | | | |
| Attendance of a child at a day care centre | 113 (77.9) | 234 (71.8) | 1.28 (0.78–2.11) | 0.336 |
| Swimming outdoors | 17 (10.7) | 63 (19.3) | 0.53 (0.32–0.89) | 0.017 |
| Travelled outside Québec province | 0 (0.0) | 6 (1.8) | – | – |
| Consumption of risky food | 89 (62.7) | 198 (61.1) | 1.06 (0.74–1.51) | 0.744 |
| A parent working as a day care provider | 22 (13.6) | 42 (12.9) | 1.19 (0.74–1.90) | 0.469 |
| A parent working in a pet shop, an animal clinic, a zoo or on a farm | 25 (15.4) | 66 (20.3) | 0.75 (0.49–1.17) | 0.210 |
| Child living on a farm | 23 (13.9) | 38 (11.7) | 1.15 (0.60–2.19) | 0.675 |

*Models obtained using generalized linear models by taking into account the possible correlation between individuals in the same municipality.

same protocol was applied to non-hospitalized children after the nurse from the public health department enrolled the case. Enrolled children were further excluded if the parent(s) refused to complete the telephone follow-up. Parents or caretakers of controls were also contacted by telephone to be interviewed with a similar standardized questionnaire. There was a mean delay of 2 days between interviewing the parents of cases and those of controls. The interviewers were not blinded to the case/control status of the study subjects; however, they were given training so that they interviewed parents of cases and controls in the same systematic manner.

### Questionnaire

The questionnaire covered personal, demographic and socio-economic data, as well as specific exposures occurring in the previous weeks of the reference date (the beginning of symptoms for the cases and the time of the interview for controls). Among the included exposures were travel abroad (previous month), eating outside of the home, eating meat products or milk products (previous week), drinking water not from waterworks or from bottles and contact with domestic, zoo or farm animals (previous 2 weeks). Information describing the quality and supply of the household's drinking water was also collected. All study subjects were also asked about chronic gastrointestinal illness.

### Faecal specimen collection

*For hospitalized cases*

Three faecal samples were collected from the cases within 48 h of admission to hospital. Samples for bacteriological culture were placed in an Enteric Pathogen Transport medium (Quelab laboratories, Québec, Canada), preserved at 4°C and transported to the hospital laboratory. Samples for protozoan evaluation were collected in a parasitology medium (sodium acetate, acetic acid and formalin – SAF) and stored at room temperature. Samples for virus detection were collected in a transport tube, frozen at −20 to −80°C (depending on hospital facilities).

*For non-hospitalized cases*

Stools of non-hospitalized cases were collected wether at home or at clinics or in doctors' offices and sent to hospitals where they were analysed for bacteria in the

  

same way as specimens from hospitalized cases. However, protozoa and virus detection was not systematically carried out for these cases because it is not a usual practice for family doctors to request those tests for a child having gastroenteritis.

### Microbiological analysis

*Bacteriology*

Fresh faecal samples were checked for *Campylobacter* sp, *E. coli* O157:H7, *Salmonella* sp and *Yersinia* sp according to general methods described in Murray et al. (1999) and Gilligan et al. (1992). *Campylobacter* species were identify using agar plates (Skirrow medium) incubated microaerophilically at 42°C. Isolated colonies were sent to Laboratoire de santé publique du Québec (LSPQ) for species identification according to Barrett et al. (1988) and Morris and Patton (1985). *Escherichia coli* O157 was identified by plating specimens on sorbitol-MacConkey selective medium, followed by latex agglutination test, according to Sowers et al. (1996). Selected colonies were sent to LSPQ for flagellar antigen H7 identification by monoclonal antibodies techniques (He et al., 1996). Salmonella–Shigella, MacConkey or xylose–lysine media, followed by biochemical test (Kliger slant culture, ONPG-PAM test, TSA plates) were used *Salmonella* sp and *Yersinia* sp identification. Serovars of *Salmonella* sp. were determined by LSPQ using anti-sera agglutination tests according to Kauffmann–White classification scheme.

*Parasitology*

Parasites were first detected with combined *Giardia*/*Cryptosporidium* antigen detection EIA assay (IVD Research Inc., Carlsbad, CA, USA). A positive detection led to a second EIA assay with individual antigen detection assays for both protozoa (IVD Research Inc.). A positive result was further submitted to microscopy with iron-haematoxylin stain to detect cysts or oocysts in the concentrated specimens; analyses were carried out at the McGill University Centre for Tropical Diseases (Montréal, Québec).

*Virology*

Specimens were investigated for the presence of enteric viruses (adenovirus, calicivirus-like, coronavirus, parvovirus, picornavirus, reovirus, rotavirus and Norwalk virus) by negative-staining electron microscopy (EM) (Berthiaume et al., 1981; Palmer and Martin, 1988) at the electron microscopy laboratory of INRS-Institut Armand-Frappier (Institut national de la recherche scientifique, Laval, Quebec, Canada).

### Drinking water quality evaluation of private wells

Water samples from each household were collected from the kitchen tap in sterile bacteriological containers. Samples were preserved at 4°C and processed within 48 h of collection at the laboratory of the Quebec Ministry of Agriculture (pathogenic bacteria) and at the laboratory of the Ministry of Environment (indicator microorganisms).

### Indicator organisms in drinking water

*Escherichia coli* was detected in a two-step procedure, the first being part of standard method 9222D (Fecal Coliform Membrane Filter procedure) (APHA-AWWA-WEF, 1998) adapted by the Quebec Ministry of Environment laboratory. Enterococci were identified by a two-step procedure, based on method 9230C (APHA-AWWA-WEF, 1998). F-specific (male specific, $F^+$) coliphages virus (bacteriophages) was detected using the USEPA 1601 presence/absence method (EPA 821-R-01-030).

### Bacterial pathogens in drinking water

*Campylobacter* species were identified according to the method of Giesendorf et al. (1992), based on gene amplification coding for 16S rRNA. Identification of *E. coli* O157:H7 was carried out by a two-step procedure: identification of verotoxin type 1 and 2, then identification of the O157:H7 serotype (Tyler et al., 1991). *Salmonella* spp detection was carried out according to Trkov et al. (1999), using non-selective and selective enrichment media. PCR primers ST11 and ST15, which have previously been shown to be highly specific for *Salmonella* spp (Aabo et al., 1993; Trkov et al., 1999), were used. *Yersinia enterocolitica* detection was carried out according to the method developed by Wannet et al. (2001), based on targeting the chromosomal factor *ail*, an attachment invasion gene present in pathogenic serotypes.

### Water distribution systems

All water distribution networks that served the residences of participants were assessed to evaluate their vulnerability to microbiological contamination. This was carried out using a systematic evaluation based on the following criteria: (i) source susceptibility, (ii) water treatment efficiency, (iii) distribution system management and (iv) overall management (Cool et al., 2010). An index of vulnerability to microbiological contamination was then calculated for each participating network.

### Livestock density

Livestock density data of each municipality were obtained from the Ministère de l'Agriculture du Québec (MAPAQ); data available as of January 2006. Livestock density was defined as the number of animal units relative to total cultivated area (a.u./ha) within a municipality.

### Statistical analysis

Collected data were analysed using the SAS software, version 9.1 (SAS. *User's Guide*. SAS Institute Inc., Cary, NC, USA). Distribution of possible confounding factors (demographic factors, medical history, GI risk factors and water exposure) between cases and controls was compared with odds ratios (ORs), their 95% confidence intervals (CIs) and *P* value. The animal exposure was evaluated by contact with domestic or farm or zoo animal in the previous weeks and by livestock density in the residence area. The categorization of livestock density exposure was first based on quartiles of exposure of the control groups; however, the fourth quartile was further considered the exposed category and the pooling of the other quartiles, the unexposed category. Odds ratios and their 95% CIs for association with the various exposures were determined using unconditional logistic regression models while controlling for possible covariates and matching variables. No collinearity was observed between independent variables retained. All variables associated in univariable analysis with gastrointestinal illness (with a higher proportion among cases than controls and *P* value <0.15) were included in the multivariable analyses. To take into account the municipality level of the livestock density variables, all ORs were obtained by the generalized estimating equation (GEE) approach, with link logit and binomial distribution, and the municipality was entered as a repeated effect. The statistical significance level was 5%.

### Ethics

The study received approval from standing committees on ethics in research within each of the participating hospitals and from a provincial ethics committee (Comité central d'éthique du Ministère de la Santé et des Services sociaux du Québec). Informed written consent was obtained in the hospitals from the caretakers of the children enrolled in the study, before collecting stool specimens.

## Results

A total of 165 cases were included in this study: 142 hospitalized and 23 non-hospitalized and recruited by the regional public health department. For each case, two controls were found; 330 controls were enrolled, but four of them were excluded *a posteriori* (because they were retrospectively found to be ineligible). Participation among eligible hospitalized was about 90% and 87% among notified cases. Participation among eligible controls was about 92%. Characteristics of cases and controls are presented in Table 1. Due to the matching design, cases and controls had a similar age distribution. However, males were more represented in cases than in controls ($P = 0.015$), and parents of cases had a lower education than parents of controls ($P = 0.004$). A history of chronic disease was also more frequent in cases ($P = 0.016$). Risk factors for gastrointestinal disease did not appear more frequent statistically in cases than in controls. Moreover, swimming outdoors was more frequent in controls.

One hundred and thirty-four cases (81.2%) had a microbiological analysis of their stools (132 for bacteriological analysis, 112 for parasites and 104 for viruses). Bacteria were found in 21.2% of cases, parasites in 4.5% and viruses in 78.9%, with these percentages calculated on the basis of analysed samples for each group of microorganisms. Only one child had a concomitant infection: parasitic and bacterial infections. The list for each bacterial species detected is presented in Table 2. *Campylobacter* spp was detected in 15 cases, *Salmonella* spp in nine cases and *E. coli* in six cases. Rotavirus was detected in 68 cases. Other species were rarely detected.

No major difference was found between cases and controls regarding the type of water consumed (Table 3). In particular, the microbiological vulnerability indicator of the consumed water serving residences from public waterworks was similar between cases and controls ($P = 0.254$). Moreover, children living in houses supplied by community surface or groundwater waterworks were distributed equally between the two groups ($P = 0.648$). Frequency of private wells as the source of drinking water was similar between cases and controls, but drilled wells were more frequent in cases than surface wells. However, the frequency of faecal contamination of private wells (presence of *E. coli* or enterococci) was similar for cases and controls: 7.6% versus 7.3%, respectively ($P = 0.941$) (data not shown). No coliphage virus was found in these wells.

The logistic regression analysis estimated the odds ratios associated with animal exposure for univariable and multivariable models (Tables 4 and 5). When all the cases (mostly viral cases) were considered globally, no link was found between severe gastroenteritis and either contact with animals or living in a municipality with animal density in the 4th quartile. However, when considering only the cases with a bacterial or a parasitic infection (the so-called 'potential zoonotic pathogens'), the crude analysis revealed an association between hog density and disease occurrence ($P = 0.015$). However, when adjustment was carried out


 

**Table 2.** Microbiological stool sample analysis results of cases of children gastroenteritis in Québec rural areas ($n$ = 165)

| | Cases $n$ (%) |
|---|---|
| No specimen collected | 34 (18.8) |
| Number of stool specimens analysed | 134 (81.2) |
| Samples analysed for bacteria ($n$ = 132) | |
| *Campylobacter* species | 15 |
| *jejuni* | 9 |
| *jejuni jejuni* | 2 |
| *upsaliensis* | 1 |
| Species not identified | 3 |
| *E. coli* species | 6 |
| O157:H7 | 5 |
| Serotype not identified | 1 |
| *Salmonella* species | 7 (5.3) |
| *enterica* (no serotype identified) | 1 |
| serovar Choleraesuis | 4 |
| Species not identified | 2 |
| *Yersinia* species | 0 |
| Cases with at least one bacterium | 28 (21.2) |
| Samples analysed for parasites ($n$ = 112) | |
| *Cryptosporidium* sp. | 3 (2.7) |
| *Giardia* sp. | 2 (1.8) |
| Cases with at least one parasite | 5 (4.5) |
| Samples analysed for viruses ($n$ = 104) | |
| Rotavirus | 68 |
| Picornavirus | 2 |
| Adenovirus | 10 |
| Coronavirus-like | 2 |
| Parvovirus | 2 |
| Viral particles not identified | 1 |
| Cases with at least one virus | 82 (78.9) |

for important co-variates, this increase was reduced and became statistically non-significant. However, bacterial or parasitical infection was found significantly associated with contact with domestic, farm or zoo animals (adjusted OR = 2.57; 95% CI: 1.21–5.47). A sensitivity analysis demonstrated that, when adjustment was not provided for contact with animals, the association with swine density was slightly stronger but remains not statistically significant (Table 5).

## Discussion

This study confirms the importance of viral aetiology in severe gastroenteritis among children living in rural areas and the lower rate of bacterial and parasitical infections (Table 2). The results do not support an impact of animal farming activities on the incidence of viral as well as bacterial or parasitical infections. However, a link with contact with either domestic, zoo or farm animals was found for gastroenteritis cases.

In hospital settings, most diagnosed infectious gastroenteritis is caused by viruses, with bacteria and protozoan parasites usually accounting for less than 10% (Barnes et al., 1998; Friesema et al., 2012; Wiegering et al., 2011), while some studies report a higher rate (Denno et al., 2012). In our study, 82 cases (78.9%) were infected by a virus, mainly rotavirus, a reported leading cause of gastroenteritis among children (Bettinger et al., 2011; Fischer et al., 2011), including those living in rural areas (Bessell et al., 2010). Twenty-eight cases (21.2% of analysed samples) had a bacterial aetiology, which is a higher percentage

**Table 3.** Sources and treatment of drinking water of children cases of gastroenteritis and their controls, in Québec rural areas

| | Cases ($n$ = 165) $n$ (%) | Controls ($n$ = 326) $n$ (%) | Univariable model* | |
|---|---|---|---|---|
| | | | Crude OR (95% CI) | $P$ value |
| Type of water consumed | | | | |
| Boiled tap water | 8 (4.9) | 9 (2.8) | 1.72 (0.67–4.39) | 0.258 |
| Unboiled and unfiltered tap water | 79 (48.2) | 178 (54.6) | 0.81 (0.54–1.19) | 0.280 |
| Filtered tap water | 15 (9.2) | 42 (12.9) | 0.84 (0.49–1.45) | 0.533 |
| Water provided to household | | | | |
| Community waterworks | 95 (57.9) | 199 (61.2) | 1.00 | 0.713 |
| Domestic (private) wells | 69 (42.1) | 126 (38.8) | 1.08 (0.71–1.65) | |
| Water provided to household for community waterworks | | | | |
| Community groundwater | 46 (49.5) | 118 (61.5) | 1.00 | 0.648 |
| Community surface water | 47 (50.5) | 74 (38.5) | 1.13 (0.68–1.87) | |
| Water provided to household for domestic wells | | | | |
| Drilled wells | 58 (84.1) | 89 (75.4) | 1.00 | 0.201 |
| Surface wells | 11 (15.9) | 29 (24.6) | 0.62 (0.29–1.29) | |
| Microbiological vulnerability to contamination | | | | |
| Low | 96 (66.7) | 201 (69.6) | 1.00 | 0.254 |
| High | 48 (33.3) | 88 (30.4) | 1.38 (0.79–2.40) | |

*Models obtained using generalized linear models by taking into account the possible correlation between individuals in the same municipality.

**Table 4.** Prevalence and odds ratios (crude) of animal exposure for acute children gastroenteritis in Québec rural areas (2004–2007)

| Livestock density variables | Cases *n* (%) | Controls *n* (%) | Univariable model* | |
|---|---|---|---|---|
| | | | Crude OR (95% CI) | *P* value |
| All gastrointestinal cases | 165 | 326 | | |
| Contact with domestic, zoo or farm animals | 114 (71.7) | 216 (66.5) | 1.16 (0.82–1.64) | 0.407 |
| Swine density† ($\geq$0.79 AU/ha (Q4) versus <0.79) | 46 (27.9) | 67 (20.6) | 1.55 (0.86–2.79) | 0.149 |
| Cattle density† ($\geq$0.96 AU/ha (Q4) versus <0.96) | 45 (27.3) | 87 (26.7) | 0.54 (0.27–1.09) | 0.087 |
| Poultry density† ($\geq$0.10 AU/ha (Q4) versus <0.10) | 43 (26.1) | 83 (25.5) | 1.10 (0.61–2.00) | 0.752 |
| Only cases with a bacterial or a parasite infection | 32 | 326 | | |
| Contact with domestic, zoo or farm animals | 22 (81.5) | 216 (66.5) | 2.17 (0.91–5.21) | 0.082 |
| Swine density† ($\geq$0.79 AU/ha (Q4) versus <0.79) | 14 (43.8) | 67 (20.6) | 2.99 (1.24–7.22) | 0.015 |
| Cattle density† ($\geq$0.96 AU/ha (Q4) versus <0.96) | 6 (18.8) | 87 (26.7) | 0.49 (0.13–1.87) | 0.296 |
| Poultry density† ($\geq$0.10 AU/ha (Q4) versus <0.10) | 14 (43.8) | 83 (25.5) | 2.38 (0.98–5.80) | 0.056 |

*Models obtained using generalized linear models by taking into account the possible correlation between individuals in the same municipality.
†Density by cultivated area for the municipality.

**Table 5.** Adjusted odds ratios of animal exposure for acute children gastroenteritis in Québec rural areas (2004–2007)

| Livestock density variables | Adjusted OR* (95% CI) | *P* value | Adjusted OR* (95% CI) | *P* value |
|---|---|---|---|---|
| All gastrointestinal cases | | | | |
| Contact with domestic, zoo or farm animals | 1.30 (0.90–1.89) | 0.158 | – | – |
| Swine density† ($\geq$0.79 AU/ha (Q4) versus <0.79) | 1.92 (0.89–4.12) | 0.096 | 1.93 (0.91–4.09) | 0.085 |
| Cattle density† ($\geq$0.96 AU/ha (Q4) versus <0.96) | 0.51 (0.23–1.16) | 0.110 | 0.50 (0.22–1.14) | 0.098 |
| Poultry density† ($\geq$0.10 AU/ha (Q4) versus <0.10) | 0.69 (0.32–1.50) | 0.350 | 0.68 (0.32–1.43) | 0.310 |
| Only cases with a bacterial or a parasite infection | | | | |
| Contact with domestic, zoo or farm animals | 2.57 (1.21–5.47) | 0.014 | – | – |
| Swine density† ($\geq$0.79 AU/ha (Q4) versus <0.79) | 2.03 (0.77–5.34) | 0.151 | 2.46 (0.94–6.45) | 0.068 |
| Cattle density† ($\geq$0.96 AU/ha (Q4) versus <0.96) | 1.10 (0.30–4.02) | 0.889 | 1.14 (0.33–3.97) | 0.835 |
| Poultry density† ($\geq$0.10 AU/ha (Q4) versus <0.10) | 2.56 (0.95–6.95) | 0.064 | 2.28 (0.87–5.99) | 0.095 |

*Model obtained using generalized linear models by taking into account the possible correlation between individuals in the same municipality, and OR adjusted for season, age group, sex, education, chronic diseases, low birth weight, swimming outdoors and for all of the variables included in the table. Swimming outdoors was not included in model for bacterial or parasite infection.
†Density by cultivated area for the municipality.

than the one reported by a retrospective study conducted in a Parisian hospital (6.8%) (Lorrot et al., 2010), but lower than the incidence of 32% reported by Friesema et al. (2012) in children requiring hospitalization in the Netherlands. However, given that our bacterial cases are a mix of hospitalized and not hospitalized patients, comparison with hospital data might be difficult.

In this study, the main bacterial genus involved was *Campylobacter* sp (53% of bacterial infections). Several studies have suggested that farm animals might be a source of zoonotic gastroenteritis associated with *Campylobacter* bacteria. Green et al. (2001) showed that the incidence of *Campylobacter* infections was significantly higher in populations living in rural and agricultural areas, with the highest rates occurring in people living in proximity of high-density animal farming. In a retrospective ecological study using reported cases in the province of Québec, Arsenault et al. (2012) demonstrated a higher incidence of campylobacteriosis in areas with high ruminant density. *Campylobacter* species are carried by dairy cows (Gilpin et al., 2008) but are also present in swine (Varela et al., 2007; Guévremont et al., 2004); however, as reported by these authors and others (Boes et al., 2005; Nielsen et al., 1997), the dominant species in swine is *C. coli*, accounting for more than 95–99% of *Campylobacter* species, while species like *C. jejuni* is almost absent. In our study, *Campylobacter* sp was the most frequent bacterium found in faeces of cases (15 cases), but the most often identified strain was *C. jejuni* (11 cases), while *C. coli* was never recovered from stools samples.

*Escherichia coli* O157:H7 was identified in five children (Table 2). This serotype is usually reported in bovine herds (Oporto et al., 2008), and contamination is usually through food consumption (Karmali et al., 2010), but drinking water contaminated by cattle farm operations has also been involved (Krewski et al., 2002). Outbreaks associated with petting zoo have been described, but food or water exposures were not involved (Centers for Disease Control and

Prevention, 2005; Goode et al., 2009). Cases infected by *E. coli* O157:H7 could have been contaminated from cattle strains (by drinking water, food or following direct contact with animals on a farm), but this scenario has not been verified. Jackson et al. (1998) identified an *E. coli* O157:H7 infection in a young child resulting from well water contamination by infected cattle on an Ontario farm. However, in the present study, no *E. coli* O157:H7 was recovered from cases' drinking water.

Among the seven cases infected by *Salmonella* species, four were by the Choleraesuis serotype, the most frequently isolated from swine. It is an infrequent serotype isolated from human sources in North America, but the epidemiological pattern differed greatly in Asian countries (Chiu et al., 2004) where it can be of particular concern (Chiu et al., 2006). Jones et al. (2008) identified only 55 human cases of Choleraesuis serotype during the 1996–2006 time frame in the United States (about five cases/year). One study suggested that groundwater consumption could be an independent risk factor of Salmonella choleraesuis infection in Taiwan (Li et al., 2009). The origin of these infections may be the environmental impact of swine herds (three of six cases infected with this serotype lived in areas with a swine density $\geq 0.78$ AU); however, it could result from food or direct contact with animals. In our study, there was an association of children having a bacterial gastroenteritis resulting from contact with animals; however, most of the contacts (97%) were related to domestic animals, and when contact with animals was not considered in the model, the association was not significantly modified. Therefore, we do not consider that contact with farm animals and particularly pigs was a credible explanation for cases illness in this study.

No link was found between occurrence of these infections and the microbiological quality of the drinking water served in the participants' residences. Based on what is known about rotavirus, it is not possible at this time to implicate this virus as a source of zoonotic transmission. However, it is worthwhile to mention that despite the fact that rotavirus infects particular species, heterologous infections may occur under natural and experimental conditions. It has been shown that human and animal strains of rotavirus may share a high degree of genetic similarities. Reassortants between heterologous strains of porcine and human origin may have occurred and spread among populations (Martella et al., 2010). However, rotavirus transmission through water consumption has not been demonstrated.

Taken globally, our study does not support the potential environmental impact of farming activities on the occurrence of severe gastroenteritis. Several studies have reported associations between livestock farming activities and acute gastroenteritis incidence. Drinking water from private wells is usually considered as a risk factor (Kuusi et al., 2003; Denno et al., 2009), but a study in Vancouver area revealed that private well water was not associated with an increase risk of intestinal infectious diseases (Teschke et al., 2010). In Québec, an ecological study found an increasing risk of children's hospitalization associated with higher animal farming activities, especially poultry (Febriani et al., 2009). Studying the notified cases of gastroenteritis in Québec's children 0–4 years of age living in small municipalities, Kaboré et al. (2010) found an association with cattle density, but no association with poultry or swine density. However, a cross-sectional survey, also carried out in Québec, resulted in a negative association between intensive farming activities and acute gastrointestinal illness (Febriani et al., 2010). In Alberta, Pearl et al. (2009) in a multilevel analysis reported that cattle density was not associated with acute gastroenteritis.

Our study has some important strength that should be underlined. In particular, it is a population-based study, with all cases and controls derived from the same population base. Given the accessibility of all the population to hospital and medical consultation in all the areas of the study, we do consider that all severe cases of gastroenteritis in young children were gathered within the study area, the collaboration of all hospitals in the sector was very effective, and we are confident that few cases could have been missed by our recruitment procedures. The evaluation of bacteria, viruses and parasites at the same time for most of the cases should be underlined, as well as the important characterization of the quality of the water serving the participants' residences. Finally, our rather extensive questionnaire permitted us to consider in the analysis most of the factors commonly associated with gastroenteritis.

Some limitations should be considered when interpreting the results. Unexpectedly, very few cases of bacterial and parasitical infection occurred, reducing the power to detect some statistically meaningful results. This limits our ability to answer to the research question. Also, the evaluation of viral and parasitical infection for community cases was frequently lacking, but it did not have any impact on the bacterial findings that were the most preeminent infection among potential zoonotic cases. We nevertheless acknowledge that community cases were only a selection of incident cases (that consulted a physician and had faecal sample). Our measure of animal farming intensity was quite crude, which may reduce our ability to detect an association with animal farming activity. Finally, this study was conducted only in rural areas, which have precluded a formal evaluation between rural and urban settings. However, the variety of environmental exposure, especially to animal burden, was important across the study areas, which makes it very adequate to

study the potential effect of over exposure to animal farming activities.

In conclusion, despite an important number of cases of infantile gastroenteritis in this rural population, no environmental factors, except contact with different animals, could be linked to the occurrence of the disease. In particular, no link was found between the microbiological quality of drinking water and illness. However, the low rate of potentially zoonotic diseases (bacterial and parasitic) limits the statistical power of the study.

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