Burden and Transmission of Zoonotic Foodborne Disease in a Rural Community in Mexico

Mussaret B. Zaidi,^{1,2} Freddy D. Campos,^{1,2} Teresa Estrada-García,³ Flor Gutierrez,¹ Magda León,¹ Rodolfo Chim,^{1,2} and Juan J. Calva⁴

¹Microbiology Research Laboratory, Hospital General O'Horan, Mérida; ²Infectious Diseases Research Unit, Hospital Regional de Alta Especialidad de la Península de Yucatán, Mérida; ³Department of Molecular Biomedicine, CINVESTAV-IPN, Mexico City, and ⁴Clinical Epidemiology Unit, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

Background. The foodborne transmission and human health impact of Salmonella and Campylobacter infections have rarely been evaluated at the population level in highly endemic settings.

Methods. A prospective 15-month cohort study of 127 infants and 119 elderly people was combined with animal and food surveillance to determine the incidence and severity of *Salmonella* and *Campylobacter* gastroenteritis in a comparatively prosperous rural community in Mexico.

Results. Salmonella and Campylobacter were isolated in up to 75% and 57%, respectively, of raw retail meat and in up to 4.5% of ready-to-eat foods. Rates of acute gastroenteritis of any etiology in infants and elderly people were, respectively, 2.1 and 0.7 episodes per person per year. The annual incidence density rate of Salmonella gastroenteritis was 17.8 per 100 infants and 7.9 per 100 elderly people; the rate of Campylobacter gastroenteritis was 11.7 per 100 infants and 0 per 100 elderly people. Pulsed-field gel electrophoresis analysis yielded multiple clusters of human, meat, and/or animal Salmonella and Campylobacter isolates with indistinguishable patterns. On average, gastroenteritis episodes with these pathogens lasted 3 days in infants and 2 days in elderly people. Medical attention was sought in 44% of diarrheal episodes in infants and in 26% of diarrheal episodes in elderly people; none required hospitalization. Infants with multidrug-resistant Salmonella gastroenteritis had a higher frequency of bloody stools and medical visits (50% vs 11%; odds ratio, 8.5; P = .04) than those with more susceptible strains.

Conclusions. In this relatively advantaged Mexican rural community, the human health impact of a food chain heavily contaminated with *Salmonella* and *Campylobacter* was of low magnitude.

Foodborne disease results from the ingestion of contaminated food. *Salmonella* and *Campylobacter*, 2 zoonotic pathogens that are frequently harbored in the intestinal tract of food animals such as chicken, swine, and cattle, are leading causes of foodborne disease worldwide [1]. A recent study in the United States estimated that 9.4 million episodes of foodborne disease occur each

Clinical Infectious Diseases 2012;55(1):51–60

year; of these, 11% are caused by *Salmonella* and 9% by *Campylobacter*. *Salmonella* is a leading cause of hospitalization and death as well as gastroenteritis [2].

During the last 2 decades, industrialized countries have witnessed a rising incidence of sporadic foodborne disease and foodborne outbreaks while both *Salmonella* and *Campylobacter* have become increasingly resistant to clinically important antibiotics. Several studies have demonstrated an association between antimicrobial resistance and negative human health impacts such as increased duration of diarrhea, frequency of hospitalization, and mortality [3–6].

In recent years, experts have called for well-designed prospective studies on the burden of foodborne disease in developing countries [7]. In addition to the paucity of studies, the epidemiology of foodborne disease in

Received 1 December 2011; accepted 23 February 2012; electronically published 15 March 2012.

Correspondence: Mussaret Zaidi, MD, MSc, Departamento de Investigación, Hospital General O'Horan, Av Itzaes x Jacinto Canek, Mérida, Yucatán, Mexico (mbzaidi@prodigy.net.mx).

[©] The Author 2012. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com. DOI: 10.1093/cid/cis300

resource-limited settings differs from that in more affluent regions. Previous studies in Africa, Asia, and Latin America have shown that in these settings, infants and children are highly exposed to enteric pathogens. There is consequently an age-related decrease in the rates of clinically manifested infection, and asymptomatic colonization is a common phenomenon [8–10].

In this study, designed to measure the impact of contaminated food on a small rural community in southeast Mexico, we combined active and prospective surveillance of the food chain with concurrent clinical follow-up of infants and elderly people. The main objectives were to determine (1) prevalence of *Salmonella* and *Campylobacter* at different points throughout the food chain, (2) transmission of these pathogens to humans by molecular subtyping analysis, and (3) impact on the local population using incidence and severity of *Salmonella* and *Campylobacter* gastroenteritis in infants and elderly people as indicators.

METHODS

Study Setting

The study was conducted from March 2006 to May 2007 in Buctzotz, a rural community of 6752 inhabitants located 100 km northeast of Mérida, the capital city of Yucatán, Mexico. Buctzotz is an ideal site for studying the transmission of foodborne pathogens because it has a single municipal slaughterhouse, 1 municipal market, and 10 butcheries that supply retail meat for local consumption. The community is relatively prosperous, with an average monthly household income that is 3 times that of the state monthly minimum wage. More than 95% of the population has running water and electricity and all have access to healthcare; the median consumption of meat is 24 days (80%) per month (Table 1).

Epidemiologic and Household Surveillance

This study focused on the age groups with the highest gastroenteritis morbidity and mortality in Yucatán, namely, children <5 years and the elderly [11]. An initial population survey revealed that of the 1789 registered households, 436 had either a child <30 months (n = 219) or an adult >69 years (n = 217; hereafter referred to as "older adult") eligible for inclusion. Computer-driven lists of randomized numbers were used to select, in turn, house-blocks and 250 households; 127 households with infants and 119 with elderly people participated. Only 1 cohort child or older adult was selected per household.

Each cohort subject was visited in his or her home twice a week by a trained social worker. When a diarrheal episode (defined as \geq 3 loose stools in 24 hours) was detected, the social worker collected a fecal sample and administered a question-naire to the child's mother or to the elderly person regarding the severity and duration of disease. Fecal samples were transported

Table 1. Characteristics of Cohort Subjects and Noncohort Households at Recruitment

	Infants ≤30 Months	Elderly People ≥70 Years	Noncohort Households	
Characteristic	(n = 127)	(n = 119)	(n = 1543)	
Male, No. (%)	63 (49.6)	77 (64.7)	3233 (49.7)	
Median age (IQR)	14 (8, 18) months	80 (76, 85) years	15–29 years	
Median months of breastfeeding (IQR)	9 (4, 18)		NA	
Education level ^a				
Illiterate, %	8	52	18	
Primary school, %	54	48	60	
Secondary school or higher, %	38	0	22	
Household characteristics				
Median monthly income in USD (IQR)	\$190 (\$145, \$272)	\$109 (\$54, \$182)	\$180 (\$110, \$200	
Running water, No. (%)	125 (98)	115 (97)	1496 (97)	
Electricity, No. (%)	120 (94)	115 (97)	1481 (96)	
Toilets/latrines, No. (%)	74 (58)	71 (60)	1093 (71)	
Median number of household members (IQR) ^b	5 (4, 6)	2 (1, 3)	4 (3, 5)	
Food animals in household, ^c No. (%)	63 (50)	55 (46)	663 (43)	
Family consumption of chicken meat, median days per month (IQR)	13 (9, 13)	9 (9, 13)	9 (9, 13)	
Family consumption of pork meat, median days per month (IQR)	9 (9, 13)	9 (4, 9)	8 (4, 9)	
Family consumption of beef meat, median days per month (IQR)	2 (1, 4)	2 (1, 4)	2 (1, 4)	

Abbreviations: IQR, interquartile range (Q1, Q3); NA, not available; USD, US dollars.

^a For children, the education of the primary caregiver was considered. For noncohort households, refers to population >15 years of age.

^b Includes cohort subject.

^c Refers to chicken, turkey, swine, and, in lesser frequency, cattle.

3 times each week to the Hospital General O'Horan Research Laboratory, Mérida, in Cary-Blair medium. If the sample was positive for *Salmonella* and/or *Campylobacter*, household members were asked to submit a fecal sample within 14 days.

Food Chain Surveillance

Ten food-animal intestines (cecum) and 10 raw retail meat samples (5 chicken, 3 swine, and 2 cattle) were collected, respectively, from the municipal slaughterhouse and from the municipal market and butcheries; 10 samples of fresh, readyto-eat foods were obtained from households and small food establishments. All samples were collected in separate sterile plastic containers on a weekly basis; available establishments were sampled with equal frequency. Food and animal sample sizes were based on our previous studies that demonstrated animal/food-to-human transmission by pulsed-field gel electrophoresis (PFGE) [10, 12].

Microbiological Methods

Fecal samples from cohort subjects with gastroenteritis and their family members and samples from animal intestines and raw retail meat were tested for *Salmonella* and *Campylobacter*; ready-to-eat foods were only tested for *Salmonella*. For the purpose of comparability, identical methods [12, 13] were used for isolation and identification of *Salmonella* and *Campylobacter* from fecal samples, food, and animals. In brief, samples were placed in buffered peptone water (BPW) for 30 minutes and inoculated onto selective agars (brilliant green, XLT4, Cefex, and MacConkey) and broths (tetrathionate and Rappaport-Vassiliadis). *Salmonella* isolates were serotyped according to the Kaufmann-White scheme with commercial antisera (DIFCO, Sparks, Maryland). Human fecal samples were also processed for *Shigella* and pathotypes of diarrheagenic *Escherichia coli* according to standard methods [14, 15].

In a subset of randomly selected cohort households, hand cultures were collected without prior notice and tested for *Salmonella* and *E. coli*, as an indicator of fecal contamination. Hand cultures were performed by pouring 250 mL of BPW over hands while rubbing, and collecting the runoff in sterile plastic bags. The BPW was subcultured onto MacConkey agar and selective *Salmonella* broths and media.

Antimicrobial susceptibility testing for *Salmonella* isolates was first performed by disk diffusion for 12 antimicrobials; minimum inhibitory concentrations were determined for ceftriaxone, ceftazidime, and ciprofloxacin by agar dilution. *Campylobacter* isolates were tested for ciprofloxacin, gentamicin, erythromycin, and tetracycline by agar dilution [16, 17]. For *Salmonella*, PFGE analysis was performed on the 2 most frequent serotypes in the community (Agona and Meleagridis), the top 2 serotypes isolated from hospitalized Mexican children with diarrhea (Typhimurium and Enteritidis), and *Salmonella* enterica group II. For *Campylobacter*, PFGE was performed on all human, swine, and cattle *C. jejuni* isolates and every second *C. jejuni* isolate from chicken. *XbaI* and *SmaI* were used as restriction enzymes for *Salmonella* and *Campylobacter*, respectively; PFGE conditions were those specified in the PulseNet protocols developed by the Centers for Disease Control and Prevention [18, 19]. An external observer unaware of the objectives of the study analyzed the gels with BioNumerics software, version 5.10. Banding patterns were compared using Dice coefficients with a 1.5% band position tolerance.

Statistical Analysis

Statistical analyses for the comparison of categorical data were performed with the χ^2 test and the 2-tailed Fisher exact test; the Mann-Whitney *U* test was used for continuous variables.

Ethical Considerations

The protocol was approved by the Hospital General O'Horan Internal Review Board. At the request of the Ethics Committee, health education on hand-washing and food-handling techniques and household management of gastroenteritis was given during household visits. Prior to initiating the study, written informed consent was obtained from the head of each participating household.

RESULTS

Of the 246 cohort subjects included in the study, 119 infants (94%) and 115 elderly people (97%) completed the 15-month

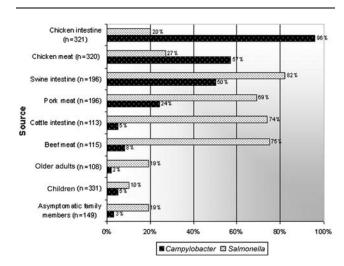


Figure 1. Prevalence of *Salmonella* and *Campylobacter* in food animals, raw retail meat, and diarrheal episodes in cohort subjects from Buctzotz, Yucatan. Animals and raw retail meats were commonly colonized/contaminated with *Salmonella* and *Campylobacter* species; *Salmonella* was a more frequent cause of gastroenteritis in both infants and elderly people. In elderly people, *Campylobacter* was isolated only from mixed infections. The total number of samples tested is indicated in parentheses.

0/ Cimilarity		Antibiogram				
% Similarity 응 우 응 응 은	PFGE-X bal	CHL GEN STR SSS SSS SSS SSS SSS	ID #	Source	Date Isolated	Serotype
		001000	yures06-57	BM	2006-03-04	Agona
			yurapus06-46-1	SI	2006-03-11	Agona
			yurapus06-52	SI	2006-03-18	Agona
			yurares06-54	BI	2006-03-18	Agona
			yupus06-63-1	PM	2006-03-11	Agona
			yuhs06-117	HD	2006-08-06	Agona
1			yurapols06-161	CI	2006-08-19	Agona
			yurapus06-189	SI	2006-09-30	Agona
			yupus06-178	PM	2006-08-26	Agona
			yupols06-124	CM	2006-06-17	Agona
			yures06-127	BM	2006-06-24	Agona
			yures06-213-1	BM	2006-10-07	Agona
			yuhs06-62 yuhs06-70	HD HA	2006-06-11 2006-06-19	Agona Agona
2			yurapus06-77	SI	2006-04-29	Agona
			yurapus07-51	SI	2007-03-24	Agona
1 11	inii i riin ï		yupus06-130	PM	2006-06-24	Agona
111			yuhs06-152	HD	2006-12-13	Agona
3			yurapus06-185	SI	2006-09-23	Agona
			yupus06-161	PM	2006-08-05	Agona
			yuhs06-153	HD	2006-12-26	Agona
			yuhs07-2	HA	2007-01-11	Agona
			yuhs07-3	HA	2007-01-11	Agona
		-	yupus06-225-1	PM	2006-10-14	Agona
			yuhs06-142	HD	2006-10-15	Agona
			yurapus06-210	SI BI	2006-10-21 2006-10-14	Agona
			yurares06-200 yurapus06-264-1		2006-12-30	Agona Agona
4			yurares06-257	BI	2006-12-23	Agona
4 1			yurapus07-25-1	SI	2007-02-10	Agona
1			yupols06-181	CM	2006-08-26	Agona
ii			yupus06-209-1	PM	2006-09-30	Agona
11		_	yupols06-218	CM	2006-10-07	Agona
11			yupols06-219	CM	2006-10-07	Agona
11			yupols06-220	CM	2006-10-07	Agona
11			yupols06-246-1	CM	2006-11-04	Agona
			yupols06-248	CM	2006-11-04	Agona
			yupols06-249	CM	2006-11-04	Agona
		-	yupols06-256	CM	2006-11-11 2006-09-22	Agona
		3 3 .	yuhs06-132 yuhs06-141-1	HD HD	2006-10-16	Agona Agona
5 11			yurapols06-169	CI	2006-08-26	Agona
	MULA		yurapus06-188	SI	2006-09-30	Agona
11			yurapols06-198	CI	2006-10-07	Agona
			yurapols06-196	CI	2006-10-07	Agona
			yurares06-211-1	BI	2006-10-28	Agona
			yurapols06-256	CI	2006-12-16	Agona
		_	yurapus06-213-1		2006-10-28	Agona
			yurapols06-228	CI	2006-11-11	Agona
		-	yurapols06-255	CI	2006-12-16	Agona
41			yupols06-58-1	CM	2006-03-04	Agona
6			yupols06-59-1	CM	2006-03-04	Agona
			yurapols06-44 yuhs06-136-1	CI HD	2006-03-04 2006-09-25	Agona Agona
			yunsuo-136-1 yuapbs07-7	FFB	2006-09-25	Agona
			yuapbs07-8	FFB	2007-05-12	Agona
			yupols07-61-1	CM	2007-05-12	Agona
			yures07-33	BM	2007-03-17	Agona
			yupols07-62-1	CM	2007-05-12	Agona
			yupus06-106-1	PM	2006-05-27	Agona
7			yures07-9	BM	2007-01-27	
			yuhs06-103-1	HD	2006-07-30	Agona
		_ _	yurapus07-46	SI	2007-03-17	-
8			yuhs06-139	HD SI	2006-10-02	Agona
111			yurapus06-105-1	31	2006-06-10	Agona

Figure 2. Pulsed-field gel electrophoresis (PFGE) analysis of 179 *Salmonella* ser Agona isolates yielded 8 clusters of 65 human and food/animal isolates with indistinguishable PFGE patterns (the complete dendrogram can be viewed as an online supplement). In clusters 2 and 4, strains recovered from ill and asymptomatic humans are indistinguishable from strains isolated from swine intestine and beef meat. In cluster 2, strain yuhs 06-62, recovered from an ill child, is indistinguishable from yuhs 06-70, recovered from his asymptomatic father (*box*). In cluster 4, strain yuhs 06-153 isolated

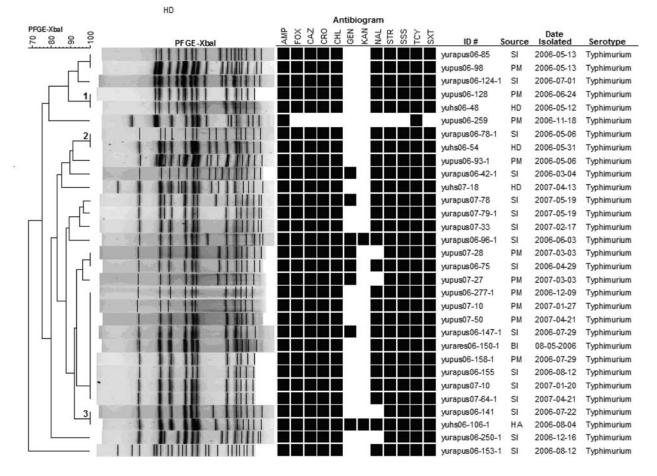


Figure 3. Pulsed-field gel electrophoresis (PFGE) analysis of 31 *Salmonella* ser Typhimurium isolates yielded 3 clusters of 6 human and food/animal isolates with indistinguishable PFGE patterns. In all clusters, strains from ill and asymptomatic humans (yuhs 06-48, 06-54, and 06-106-1, respectively) are indistinguishable from strains recovered from pork meat (yupus 06-128) and swine intestine (yurapus 06-78-1 and 06-141). Black squares indicate resistance to the corresponding antibiotic. Codes for antibiotics: AMP, ampicillin; CAZ, ceftazidime; CHL, chloramphenicol; CRO, ceftriaxone; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; SSS, sulfisoxazole; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; TCY, tetracycline. Source codes: BI, bovine intestine; HA, human asymptomatic case; HD, human diarrheal case; PM, pork meat; SI, swine intestine.

follow-up period. The characteristics of study subjects at recruitment were very similar to those of noncohort households (Table 1).

Prevalence of *Salmonella* and *Campylobacter* Throughout the Food Chain

The number of samples collected and the recovery rates for *Salmonella* and *Campylobacter* in food-animal intestines, raw retail meat, and cohort diarrheal episodes are shown in Figure 1. Cattle and swine had the highest rates of *Salmonella*

contamination, whereas *Campylobacter* was highest in chicken intestines. Of the 522 ready-to-eat food samples, *Salmonella* was most frequently found in cooked pork (5 of 111 samples, 4.5%), cooked chicken (4 of 109, 3.7%), and fresh fruit beverages (4 of 100, 4%); it was not found in fresh fruits, vegetables, or cooked beef. A total of 100 hand cultures were collected from 40 households (50 from children and adolescents, 50 from adults). Only 1 sample was positive for *Salmonella* ser Poona; 31 (16 adults and 15 children) were positive for *E. coli*.

Figure 2 continued. from another ill child is indistinguishable from strains yuhs 07-2 and yuhs 07-3, isolated from her asymptomatic mother and 11-year-old sister, respectively (*box*). Black squares indicate resistance to the corresponding antibiotic. Codes for antibiotics: CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; SSS, sulfisoxazole; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; TCY, tetracycline. Source codes: BI, bovine intestine; BM, beef meat; CI, chicken intestine; CM, chicken meat; FFB, fresh fruit beverage; HA, human asymptomatic case; HD, human diarrheal case; PM, pork meat; SI, swine intestine.

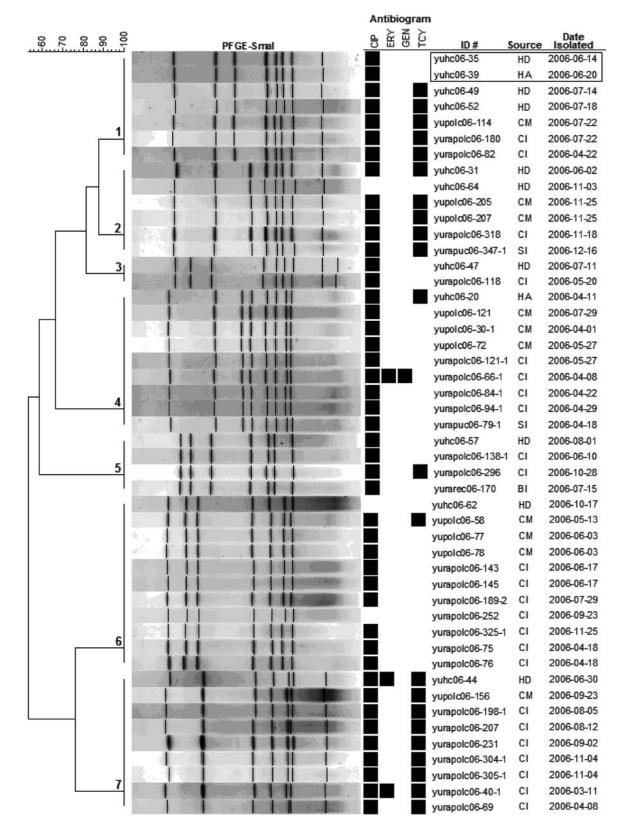


Figure 4. Pulsed-field gel electrophoresis (PFGE) analysis of 138 typable *Campylobacter jejuni* isolates yielded 7 clusters of 48 human and food/ animal strains with indistinguishable PFGE patterns (the complete dendrogram can be viewed as an online supplement). In cluster 1, strain yuhc 06-35, recovered from an ill child, has the same antibiogram and indistinguishable PFGE pattern as yuhc 06-39, isolated from his asymptomatic father (*box*). In the same cluster, strains yuhc 06-49 and 06-52, isolated from 2 other ill children, are indistinguishable (by PFGE and antibiogram) from strains yupolc

A total of 906 *Salmonella* and 944 *Campylobacter* isolates were recovered from humans, food, and animals. Overall, *Salmonella* ser Agona was the most common serotype (19.3% from all sources); the two 2 virulent serotypes, Typhimurium and Enteritidis, comprised 3.5%. Among *Campylobacter* isolates, chicken intestine and meat more frequently contained *C. jejuni* (64% and 53%, respectively), whereas swine and cattle mostly contained *C. coli* (swine and cattle intestine: 95% and 85%; pork and beef meat: 97% and 100%, respectively). Human isolates were equally divided among *C. jejuni* and *C. coli*.

Food Chain and Intrahousehold Transmission

Fifty-four *Salmonella* gastroenteritis episodes occurred in infants and elderly people. We were able to obtain stool cultures from 108 of 151 (72%) familial contacts. Eight of these had diarrhea during the same week as the index case, but only 1 stool culture was positive for *Salmonella*, albeit with a different serotype than that of the cohort subject. Of the 100 samples from asymptomatic family members, 23 (23%) were positive.

In 5 households, 7 asymptomatic family members carried strains with PFGE patterns indistinguishable from their corresponding cohort subject with gastroenteritis (for *Salmonella* Agona and *Salmonella* Typhimurium; Figures 2 and 3, and Supplementary Data). In all 5 serotypes analyzed by PFGE, we found clusters of human, food, and/or animal isolates with indistinguishable patterns.

Of the 20 *Campylobacter* episodes in cohort subjects, stool samples were collected from 52 (49%) of 106 familial contacts. Three of these had diarrhea during the same week, but none of the stool cultures yielded *Campylobacter*. Of the 49 asymptomatic family members, 4 (8%) were positive for *Campylobacter*; 1 *C. jejuni* isolate was indistinguishable by PFGE. One hundred forty-three *C. jejuni* isolates were subjected to PFGE, which yielded 7 clusters with human isolates that were indistinguishable from those collected from retail meat and animal intestines (Figure 4). In both the *Salmonella* and *Campylobacter* dendrograms, several clusters of human, meat, and/or animal isolates were recovered within a 2-week period and had the same antibiogram.

Incidence and Severity of *Salmonella* and *Campylobacter* Gastroenteritis

One hundred twenty-seven infants were surveyed for a total of 7896.2 weeks of follow-up, during which 331 diarrheal episodes were detected in 109 (86%). Eighty-nine (70%) of the infants had \leq 3 episodes during the study period. The 119 elderly

people were surveyed for a total of 7549.6 weeks of follow-up, during which 108 diarrheal episodes occurred in 60 (50%). One hundred twelve (94%) had \leq 3 episodes and 7 (6%) had >3 episodes during the study period. Annual incidence density rate for diarrhea of any etiology was 2.1 episodes per child and 0.7 episodes per elderly people. In infants, diarrheagenic *E. coli* was the most frequently recovered pathogen (10.3%), followed by *Salmonella* (8.2%), *Shigella* (4.5%), *Campylobacter* (3.6%), and mixed infections (3.3%). In elderly people, *Salmonella* was isolated from 15.7% of episodes, followed by *Shigella* (6.5%), diarrheagenic *E. coli* (4.6%), and mixed infections (3.7%).

There were 34 *Salmonella* gastroenteritis episodes in infants (*Salmonella* only, 27; mixed infection, 7) and 20 episodes in elderly people (*Salmonella* only, 17; mixed infection, 3). The annual incidence density rate of *Salmonella* gastroenteritis (those with *Salmonella* as the sole etiology) was 17.8 episodes per 100 infants and 11.7 episodes per 100 elderly people. Eighteen episodes were positive for *Campylobacter* in infants (*Campylobacter* only, 12; mixed infection, 6). In elderly people, the only 2 episodes positive for *Campylobacter* occurred as mixed infections. The annual incidence density rate for *Campylobacter* gastroenteritis (sole etiology) in infants was 7.9 episodes per 100, and 0 per 100 in elderly people.

Salmonella and Campylobacter gastroenteritis episodes in infants had similar numbers of stools per day, bloody stools, vomiting, and days of duration. Fever was slightly more common in Campylobacter episodes (33% vs 19%), and infants with a Salmonella episode more frequently required medical attention (22% vs 8%). Compared to infants, Salmonella episodes in elderly people less frequently produced bloody stools (22% vs 6%) and lasted 1 day less (3 days vs 2 days), but these differences were not statistically significant (Table 2). No infants or elderly people required hospitalization and no diarrhea-related deaths occurred during the study period.

Susceptibility results for *Salmonella* isolates are shown in Table 3. Multidrug resistance to \geq 3 antibiotics was present in 15.9%, 15.2%, and 13.7%, respectively, of isolates from humans, food, and animals and was mainly limited to serotypes Typhimurium and Anatum (resistance phenotypes for Typhimurium are shown in Figure 2). When comparing cohort subjects with resistant and susceptible strains, infants with multidrug-resistant (MDR) *Salmonella* (\geq 3 antibiotics) had a significantly higher frequency of bloody stools and visits to the physician (50% vs 11%; odds ratio, 8.5; *P* = .04). No differences could be identified for MDR *Salmonella* in elderly people.

Figure 4 continued. 06-114 and yurapolc 06-180 and 06-82, from chicken meat and chicken intestines, respectively. Clusters 2–7 show other human isolates that are indistinguishable from retail meat and/or animal intestines. Black squares indicate resistance to the corresponding antibiotic. Codes for antibiotics: CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; TCY, tetracycline. Source codes: CI, chicken intestine; CM, chicken meat; HA, human asymptomatic case; HD, human diarrheal case; SI, swine intestine.

Table 2. Severity of Salmonella and Campylobacter Diarrheal Episodes in Infants and Elderly People^a

	S	almonella	c i i i i b
	Infants (n = 27)	Elderly People (n = 17)	Campylobacter ^b Infants (n = 12)
Annual incidence density rate	17.8/100	11.7/100	7.9/100
No. stools per 24 hours, median (IQR)	5 (4, 6)	4 (4, 6)	4 (3, 6)
Fever, No. (%)	5 (19)	3 (18)	4 (33)
Vomiting, No. (%)	3 (11)	3 (18)	1 (8)
Bloody stools, No. (%)	6 (22)	1 (6)	2 (17)
Days duration, median (IQR)	3 (2, 8)	2 (2, 4)	3 (2, 5)
Sought medical attention, No. (%)	6 (22)	5 (29)	1 (8)
Hospitalization, No. (%)	0 (0)	0 (0)	0 (0)

Abbreviation: IQR, interquartile range (Q1, Q3).

^a Includes diarrheal episodes in which a sole etiologic agent was isolated.

^b No elderly people presented a diarrheal episode with *Campylobacter* as the sole etiologic agent.

Resistance phenotypes for *C. jejuni* strains are shown in Figure 3. In *C. coli*, resistance to ciprofloxacin and erythromycin was highest in swine (90.8% and 55%, respectively) and cattle (91.6% and 33.3%, respectively). In humans, resistance rates were 47.6% and 9.5%, respectively.

DISCUSSION

To our knowledge, this is the first cohort study to assess the human health impact of a heavily contaminated food chain in a Mexican rural community. As this is not a markedly impoverished community, most homes have electricity, running water, and adequate protein intake, as evidenced by the monthly consumption of meat (approximately 24 days per month). Despite the fact that *Salmonella* and *Campylobacter* raw meat contamination reached 75%, 50% of the households had food animals, and 40% had no toilets, the overall impact on human health was surprisingly low.

Although the incidence of gastroenteritis in our cohort infants (2.1 episodes per person-year) was higher than in

industrialized countries (0.1–0.9 episodes per person-year) [20, 21], it is lower than the current worldwide estimate for childhood diarrhea (approximately 4 episodes per person-year) [22]. The incidence rate in our elderly population (0.7 episodes per year) was very similar to a recent estimate for the general population in the United States [2], a striking finding if one considers the food chain contamination in this rural community.

Although both pathogens were present in high frequencies in animals and food, *Salmonella* more frequently caused gastroenteritis, including coinfections (10% of the gastroenteritis episodes in infants and 18.5% of the episodes in elderly people), than did *Campylobacter* (5.4% in infants and 1.9% in elderly people). The near absence of *Campylobacter* gastroenteritis in our elderly people population concurs with previous longitudinal studies [9, 23] in which *Campylobacter* diarrhea episodes markedly decrease after 5 years of age. The lower incidence and severity of gastroenteritis in elderly people reinforces the notion postulated by others [9, 24, 25] that persistent stimulation by early and intense exposure to *Campylobacter* and *Salmonella* results in the development of

Table 3.	Antimicrobial	Susceptibility in	Salmonella Isolate	s From Humans,	, Animals, a	and Raw	Retail Meat,	Buctzotz, I	Mexico, Percen-
tage of Re	esistance ^a								

Source	AMP	CAZ	CHL	CIP	CRO	GEN	KAN	NAL	SSS	STR	SXT	TET
Human ^b (n = 92)	9.8	3.3	13.0	0.0	3.3	1.0	1.0	18.5	30.4	32.6	18.5	29.3
Chicken ^c (n = 170)	4.1	0	6.5	0.0	0.0	2.4	2.4	16.5	24.1	31.2	10.6	27.7
Swine ^c (n = 411)	17.0	6.3	16.6	0.0	6.3	3.9	4.1	21.7	43.6	34.6	21.4	42.1
Bovine ^c (n = 233)	5.2	1.3	6.9	0	1.3	1.3	2.2	10.7	28.3	19.3	8.2	29.6

Abbreviations: AMP, ampicillin; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; SSS, sulfisoxazole; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

^a Includes resistant and intermediate strains.

^b Includes strains from cohort subjects and their family members. Some individuals had 2 Salmonella serotypes.

^c Includes isolates from food-animal intestines and retail meat.

acquired immunity. We postulate that the greater susceptibility to *Salmonella* during old age may be due to a greater decline in the immune responses required to eliminate intracellular pathogens [26], a subject that requires future investigation.

Our PFGE analyses revealed multiple clusters of isolates from animals, foods, and humans with indistinguishable subtyping patterns. Frequently, animal and food strains had been isolated shortly before the human ones, suggesting that animal and food strains are continuously being transmitted to humans, causing symptomatic and asymptomatic infections. Many of the PFGE subtypes circulated for months, consistent with our previous studies [27, 28] showing that the same subtypes may circulate for years in the same region. Our findings provide evidence that in communities such as the one we studied, foodborne pathogens are continuously transmitted to humans, either through the commercial food chain or through direct contact with food animals. Such continuous and intense exposure to foodborne pathogens raises the possibility that PFGE clusters of indistinguishable isolates reflect hyperendemic infections rather than epidemics, the favored interpretation of public health specialists in industrialized countries [29, 30].

In this cohort, the overall incidence and severity of Salmonella gastroenteritis in infants was greater than that for Campylobacter, and episodes caused by Salmonella strains resistant to ≥ 3 antibiotics tended to be more severe than episodes caused by more susceptible strains. MDR Salmonella strains were limited to specific serotypes that are also virulent, such as Typhimurium [31, 32]. Thus, greater severity of disease may be a function more of virulence than of antimicrobial resistance. MDR Salmonella in elderly people and ciprofloxacinresistant Campylobacter strains in infants were not associated with greater severity. Although antimicrobial-resistant Salmonella and Campylobacter circulate in this community, the burden on the population as a whole appears to below, conceivably because most of the episodes were relatively mild and self-limiting. Medical attention was sought in 44% of episodes in infants and 26% in elderly people; none required hospitalization.

Possible factors contributing to the low incidence and severity of gastroenteritis in this particular study were adequate food intake, acquired immunity, and the provision of health education by our social workers. Several studies have shown that even modest reductions in hand contamination can reduce the risk of pathogen transmission [33, 34]. Policy makers need to identify simple and cost-effective interventions to reduce the burden of foodborne disease.

There were several limitations to our study. The exclusive focus on infants and elderly people precluded the comparison of different age groups. Insofar as our study focused on the commercial food chain, the design was not optimal for studying household transmission. Family members were cultured only if a cohort subject was positive for *Salmonella* or *Campylobacter*. Moreover, we made no attempt to culture food animals within the household. It is likely that household transmission occurred more frequently than we could detect. Finally, the comparison of diarrheal episodes caused by resistant and susceptible strains was hampered by the small sample sizes. Nevertheless, we were still able to demonstrate a greater burden for MDR *Salmonella* in infants.

In conclusion, in this relatively advantaged Mexican rural community, the human health impact of a food chain heavily contaminated with *Salmonella* and *Campylobacter*, including MDR strains, was of low magnitude. The findings raise the possibility that the combination of adequate food intake, access to water and medical care, and provision of relevant health education may be sufficient to mitigate the human health risks of foodborne pathogens in highly endemic, low-resource settings.

Notes

Acknowledgments. The authors are indebted to Jason Abbott for assistance with the dendrogram analyses.

Financial support. This work was supported by the National Council for Science and Technology, Mexico (CONACyT), Grant SALUD-2004-CO1-159, and funds from the Mexican Foundation for Health, Peninsular Chapter.

Potential conflicts of interest. M. B. Z. has received an honorarium from Wyeth for participating in a clinical trial. J. J. C. has received travel support from Bristol-Myers Squibb and has served as a board member for Pfizer. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed in the Acknowledgements section.

References

- Acha PN, Szyfres B, eds. Zoonoses and communicable diseases common to man and animals. PAHO scientific and technical publications no. 580; 2001.
- Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 2011; 17:7–15.
- Varma JK, Molbak M, Barrett TJ, et al. Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. J Infect Dis 2005; 191:554–61.
- Martin LJ, Fyfe M, Dore K, et al. Increased burden of illness associated with antimicrobial-resistant *Salmonella enterica* serotype Typhimurium infections. J Infect Dis 2004; 189:377–84.
- Engberg J, Neimann J, Nielsen EM, Aarestrup FM, Fussing V. Quinolone-resistant *Campylobacter* infections in Denmark: risk factors and clinical consequences. Emerg Infect Dis 2004; 10:1056–63.
- Nelson JM, Smith KE, Vugia DJ, et al. Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. J Infect Dis 2004; 190: 1150–7.
- WHO Initiative to Estimate the Global Burden of Foodborne Diseases. First formal meeting of the Foodborne Disease Burden Epidemiology Reference Group (FERG): implementing strategy, setting priorities and assigning the tasks. Geneva, Switzerland: World Health Organization, 2008.
- Georges-Courbot MC, Bernard-Cassel AM, Gouandjika I, Georges AJ. Prospective study of enteric *Campylobacter* infections in children from

birth to 6 months in the Central African Republic. J Clin Microbiol 1987; 25:836–9.

- Calva JJ, Ruiz PG, López VA, Ramos A, Bojalil R. Cohort study of intestinal infection with *Campylobacter* in Mexican children. Lancet 1988; 1:503–6.
- Zaidi MB, McDermott PF, Fedorka-Cray PJ, et al. Nontyphoidal Salmonella from human clinical cases, asymptomatic children and raw retail meats in Yucatan, Mexico. Clin Infect Dis 2006; 42:21–8.
- Reporte de Casos Nuevos de Enfermedad y Defunciones para Programas Prioritarios por Grupos de Edad, Sexo y Municipio. Servicios de Salud de Yucatan. Subdireccion de Informacion y Evaluacion. 2005.
- Zaidi MB, Calva JJ, Estrada-Garcia MT, et al. Integrated food chain surveillance system for *Salmonella* spp. in Mexico. Emerg Infectious Dis 2008; 14:429–35.
- 13. On SL. Identification methods for *Campylobacters*, *Helicobacters* and related organisms. Clin Microbiol Rev **1996**; 9:405–22.
- 14. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds.Manual of clinical microbiology. 8th ed. Washington, DC: ASM Press, **2003**.
- 15. Lopez-Saucedo C, Cerna JF, Villegas-Sepulveda N, et al. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. Emerg Infect Dis **2003**; 9:127–31.
- CLSI. Performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI document M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.
- CLSI. Performance standards for antimicrobial disk susceptibility tests: approved standard–10th edition. CLSI document M02-A10. Wayne, PA: Clinical and Laboratory Standards Institute, 2009.
- Ribot EM, Fair MA, Gautom K, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella* and *Shigella* for PulseNet. Foodborne Pathog Dis 2006; 3:59–67.
- Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J Clin Microbiol **2001**; 39:1889–94.
- De Wit MAS, Koopmans MPG, Kortbeek LM, et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: Incidence and etiology. Am J Epidemiol 2001; 154:666–74.
- Tam CC, Rodrigues LC, Viviani L, et al. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut 2012; 61:69–77.
- Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ 2003; 81:197–204.

- 23. Oberhelman RA, Gilman RH, Sheen P, et al. *Campylobacter* transmission in a Peruvian shantytown: a longitudinal study using strain typing of *Campylobacter* isolates from chickens and humans in household clusters. J Infect Dis **2003**; 187:260–9.
- Sarasombath S, Banchuin N, Sukosol T, Rungpitarangsi B, Manasatit S. Systemic and intestinal immunities after natural typhoid infection. J Clin Microbiol 1987; 25:1088–93.
- Taylor DN, Echeverria P, Pitarangsi C, Seriwatan J, Bodhidatta L, Blaser MJ. Influence of strain characteristics and immunity on the epidemiology of *Campylobacter* infections in Thailand. J Clin Microbiol 1988; 26:863–8.
- Castle SC. Clinical relevance of age-related immune dysfunction. Clin Infect Dis 2000; 31:578–85.
- Zaidi MB, Leon V, Canche C, et al. Rapid and widespread dissemination of multidrug-resistant blaCMY-2 Salmonella Typhimurium in Mexico. J Antimicrob Chemother 2007; 60:398–401.
- 28. Zaidi MB, Estrada-Garcia MT. A perspective on the role of pulsed-field gel electrophoresis for *Salmonella* surveillance in Mexico. In: Calva JJ, Calva E, eds. Molecular biology and molecular epidemiology of *Salmonella* infections. Kerala: Research Signpost, **2009**.
- Riley LW. Molecular epidemiology of infectious diseases: principles and practices. Washington, DC: American Society for Microbiology Press, 2004.
- Barrett TJ, Gerner-Smidt P, Swaminathan B. Interpretation of pulsedfield gel electrophoresis patterns in foodborne disease investigations and surveillance. Foodborne Pathog Dis 2006; 3:20–31.
- 31. Crump JA, Medalla FM, Joyce KW, et al. Emerging Infections Program NARMS Working Group. Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. Antimicrob Agents Chemother **2011**; 55:1148–54.
- 32. Hendriksen RS, Vieira AR, Karlsmose S, et al. Global monitoring of Salmonella serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis 2011; 8:887–900. Epub 2011 Apr 14.
- Luby SP, Agboatwalla M, Painter J, Altaf A, Billhimer WL, Hoekstra RM. Effect of intensive handwashing promotion on childhood diarrhea in high-risk communities in Pakistan. JAMA 2004; 291:2547–54.
- 34. Luby SP, Halder AK, Huda T, Unicomb L, Johnston RB. The effect of handwashing at recommended times with water alone and with soap on child diarrhea in rural Bangladesh: an observational study. PLoS Med **2011**; 8(6):e1001052.