AN INTERVENTION-CONTROL STUDY OF CORROLLING OF FREE-RANGING CHICKENS TO CONTROL CAMPYLOBACTER INFECTIONS AMONG CHILDREN IN A PERUVIAN PERIURBAN SHANTYTOWN

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Abstract. Campylobacter jejuni is a major cause of diarrhea among children in developing countries. Since free-ranging chickens are a major source of Campylobacter infections, we hypothesized that corolling of these chickens would result in decreased rates of Campylobacter infections and Campylobacter-related diarrhea. We tested this hypothesis in Peruvian families in a periurban shantytown with free-ranging chickens and randomized by household using a (corolling) intervention versus control study design. Samples from participants and chickens were cultured for Campylobacter at the start of surveillance, and samples from children less than six years of age with diarrhea episodes and two sentinel chickens were cultured for Campylobacter monthly. Overall, 4,257 human stool specimens and 3,950 avian stool specimens were cultured over a 17-month period. Rates of Campylobacter-related diarrhea in children were significantly higher in the corral group, which demonstrated twice the incidence of Campylobacter diarrhea compared with controls overall, and seven times the rate of Campylobacter diarrhea versus controls in the subset with more than 20 household chickens. Rates of asymptomatic infection with Campylobacter were similar. Although corolling may be useful if corrals are distant from living quarters, it is not advisable as a control measure for Campylobacter in communities such as this.

INTRODUCTION

Campylobacter is a major cause of bacterial diarrhea among children in many developing countries.1–4 Chickens frequently excrete Campylobacter and are presumed to be the source of many human infections, but studies demonstrating household spread of Campylobacter between chickens and children are lacking.5–7 The large population of children living in periurban shanty-towns or pueblos jóvenes surrounding greater Lima, Peru are at high risk for Campylobacter infection, as demonstrated by the high rate of Campylobacter isolation from stool in Peruvian children with diarrhea. Most of these communities lack basic sanitary facilities, and fecal contamination from both human and animal sources is high. Families in these pueblos jóvenes often have chickens in the household, and Campylobacter has been isolated from 50% of free-ranging domestic chickens and in 88% of commercially sold chickens in studies done in pueblos jóvenes.1

We hypothesized that free-ranging chickens in the household are a major source of Campylobacter infections in these communities, and that corolling of these chickens would result in decreased rates of Campylobacter infections and Campylobacter-related diarrhea in children exposed to these chickens. Although corolling of chickens and ducks is already practiced in Las Pampas, the corrals are not of good-enough quality (most have holes), and corolling is not practiced consistently enough to have public health impact.8 This report describes the results of a trial designed to test this hypothesis as part of an ongoing study of the epidemiology of Campylobacter transmission in the Pampas de San Juan (the Pampas), a low-income pueblo joven community in periurban Lima, using a randomized intervention (corolling) versus control study design.8,9

This study is the final phase (phase III) of a project that began with epidemiologic investigation of endemic Campylobacter infections in households from the Pampas with many free-ranging chickens and correlation of human and avian Campylobacter strains by restriction fragment length polymorphism (RFLP) patterns within households in relation to diarrhea (phase I).9 Phase II was an assessment of attitudes and practices regarding corolling of chickens.8

METHODS

Sample size was calculated using the EpilInfo program (Centers for Disease Control and Prevention, Atlanta, GA) based on diarrhea surveillance data from this community collected in the previous year. Sample size was calculated for a 20% incidence of Campylobacter from stool of children with diarrhea (estimated for households with chickens), assuming a 50–60% reduction in Campylobacter incidence in the intervention group, with an alpha = 0.05 and power of 80%. Given that the incidence of diarrhea in the ≥3 year-old age group in this community is approximately three episodes/child/year based on data from a Lactobacillus diarrhea prevention study conducted in the same community within the previous three years,7 80 children (236/3 = 78.6, or approximately 40 per group) would be required for one year. Our study exceeded the necessary number of children and diarrhea episodes because we enrolled 137 children less than five years of age between both groups (150 child-years total versus 80 child-years needed based on sample size estimate). The number of diarrhea episodes identified in the cohort (n = 371) exceeded the minimum of 236 episodes needed for 80% power, which increased our power to detect significant differences.

Families selected as candidates were identified from a demographic database on the Pampas de San Juan, which is updated from ongoing surveillance conducted by PRISMA, a
Peruvian private voluntary organization with a long history of service projects in the community. Randomization was done by household instead of by individual, with an average of 2.5 children eligible for surveillance per household. Eligibility criteria for households included 1) residence in the Pampas de San Juan, 2) at least two adult chickens allowed to roam freely around the house, and 3) two or more children less than five years of age living in the household. Only one household per square block (manzana) was included to prevent cross-contamination from other study families. Potential family participants meeting eligibility criteria were assigned a number and selected using a random number generator, and informed consent was obtained from the head of the household. Assent statements were included for each participating family member. Informed consent was obtained from all human subjects or their parents or guardians and the human experimentation guidelines of the U.S. Department of Health and Human Services, Tulane Medical Center, and Asociacion Benefica PRISMA were followed in conducting this research. Families who declined to participate were replaced with the next family identified by the random number list. Families were maintained in the longitudinal study as long as they had at least one free-roaming chicken in the house, and families that lost or slaughtered all chickens without replacing them within a week were dropped and replaced with another family.

Chicken coops or corrals were donated and installed by the study team, which replaced any existing homemade corrals for the duration of surveillance. Dimensions of the coop for each study household were determined by 1) number of chickens observed in the household on enrollment visits, and 2) the size of the available area on the subject’s property and outside of the enclosed living area, using spaced deemed to be acceptable by the householder. Any homemade corrals in the intervention households were either temporarily dismantled or stored. Control group families could continue to use homemade corrals in homes where they were already in place. Maintenance of the project corrals were the responsibility of the householder, and corrals were inspected daily by field workers during home visits. However, if major damage occurred to a project corral that compromised its ability to confine chickens and which could not be repaired by the householder, a project staff member arranged for repair of the corral. As previously reported, chicken feed is already purged by homeowners raising chickens in the Pampas, and householders in both intervention and control groups were responsible for feeding their own chickens.

Surveillance for Campylobacter infections between August 2000 and December 2001 was conducted using methods previously described by our group. Samples from all children less than six years of age and chickens were cultured for Campylobacter jejuni/coli at the start of surveillance, and field workers trained in collection of chicken feces collected specimens from all chickens. Specimens were cultured according to laboratory techniques outlined in this report. Samples from household members less than six years of age and two sentinel chickens from each household were cultured for Campylobacter monthly. The decision to culture samples from two chickens from each family was based on practical limitations of the laboratory capacity. Initial culture data were not used in determining which chickens to sample on a monthly basis. Additional cultures were obtained from children less than six years of age with diarrhea according to the criteria of Black and others. Briefly, an episode of diarrhea was defined as at least one day with at least four liquid stools for children within the age group included in the protocol, and the duration of diarrhea was defined as the number of days both preceding and following the day that meets this main criteria in which at least three liquid stools were passed. At least two days without diarrhea by this definition must be present for a day, which meets the main criteria to be classified as a new episode. If a diarrhea stool specimen was culture positive for Campylobacter jejuni/coli, stool cultures of family contacts and all chickens in the household were obtained during the week after initial Campylobacter isolation.

Use of chicken coops was monitored by field workers on every other day home visits, and only families that used the chicken coop as a corral at least 90% of the time remained in the intervention group. Participants who used the corral less than 90% of the time (i.e., in use during 9 of 10 unscheduled home visits over the period of one month) were replaced with another eligible family.

The day-to-day operation of the project was coordinated by a nurse supervisor. During every other day home visits field workers administered a brief study questionnaire regarding recent diarrhea in any household participant using a precoded form. Daily frequency of stools, stool consistency, and the presence of blood or mucus in the stools were recorded.

Microbiologic techniques. Microbiologic assays for recovery of Campylobacter jejuni/coli were performed by trained laboratory technicians at the Naval Medical Research Institute Detachment in Lima. Fresh stool specimens were collected in stool containers, and most specimens were transported to the laboratory within one hour after sampling. All specimens were examined for Campylobacter by direct plating onto Campylobacter blood agar plates prepared at Naval Medical Research Institute Detachment with Brucella agar base, sheep blood, and antibiotic supplements (with vancomycin, trimethoprim, polymyxin B, amphotericin B and cephalothin). Incubated plates were incubated with CampyPaks (Becton Dickinson, Cockeysville, MD) at 42°C for 48 hours. Campylobacter isolates were identified using conventional techniques, including gram stain, oxidase testing, and speciation using hippurate and indoxyl acetate hydrolysis and susceptibility to cephalothin and nalidixic acid. Stools from children with diarrhea were also cultured for Shigella, Salmonella, Aeromonas, Pleismononas, and Vibrio cholerae using standard microbiologic techniques, but no attempt was made to identify other bacterial, viral, or parasitic agents because the etiology of pediatric diarrhea in this population has been well described in previous reports.

Data analysis. Dates of initiating and ending surveillance for each child less than six years of age were recorded for each child, and data from individual surveillance were pooled to compare disease rates by intervention group (with or without corrals), in subgroups of the intervention groups defined by age (subjects less than 36 months of age), and number of chickens (households with ≥ 21 chickens, comprising the top one-third of households based on exposure to larger number of chickens). Other subgroups were not evaluated separately because of sample size constraints. Surveillance data were only analyzed until a subject reached his or her sixth birthday, after which surveillance for that subject ended. Incidence of Campylobacter-associated diarrhea in the two intervention-
based groups was corrected for the proportion of diarrhea episodes without a corresponding stool specimen (defined as a specimen collected within 48 hours before or after the onset of the episode, and processed by stool culture). Because the observation period exceeded 12 months, the incidence rates observed may not reflect incidence based on a calendar year. Data analysis was performed at Tulane School of Public Health, Universidad Peruana Cayetano Heredia, and the Naval Medical Research Institute Detachment using SPSS version 7.5 (SPSS Inc., Chicago, IL), STATA 8.0 (Stata Corporation, College Station, TX), and EpiInfo version 6 software programs. Chi-square tests and, where necessary, Fisher’s exact tests were used to measure strengths of association between categorical variables. A two-tailed t-test was used to compare continuous variables. The association of the intervention group with the risk of Campylobacter infection during a diarrhea episode, was evaluated in multivariable logistic and Poisson regressions. In both cases, the regressions were performed with and without the consideration of temporal occurrence of events and adjustment for the correlation structure of subjects was performed with the generalized estimating equations approach. Consecutive nested models were compared with the likelihood ratio test, starting from the model containing the totality of covariates followed by removal of the least significant covariates.

RESULTS

A total of 55 families (27 with corrals and 28 without corrals) were followed during 17 months of surveillance. No family invited to participate refused to take part in the study. Except for the two households added to replace households that were non-compliant, enrollment occurred over a two-week period at the beginning of the 17-month study period. The entire study group included 137 participants (71 in the corral group [45 less than three years of age and 26 3–5 years of age] and 66 in the no corral group [40 less than three years of age and 26 3–5 years of age]). Demographic features compared between the two study groups included age and sex distribution, educational level of the head of household, home ownership and construction, crowding, and monthly income. No differences between the study groups were found based on any of these parameters. During this study, 4,266 human stool specimens and 3,950 avian stool specimens were cultured for Campylobacter. Human stool specimens included 338 initial/enrollment specimens, 3,498 monthly control specimens, 277 diarrhea specimens (183 specimens from the corral group and 94 specimens from the no corral group), 37 diarrhea follow-up specimens, and 116 family contact specimens. Campylobacter was the most commonly isolated bacterial pathogen in diarrhea specimens, being present in 53 (19.1%) of 277 cases. Other pathogens encountered included Shigella sp. (15 of 277, 5.4%), Aeromonas sp. (3 of 277, 1.1%) and non-O1 V. cholerae (1 of 277, 0.4%). Of 570 Campylobacter isolates from humans, 415 (72.8%) were C. jejuni, 147 (25.7%) were C. coli, 3 (0.5%) were C. lari, and 5 were of undetermined species. Of 2,409 avian isolates, 1,850 (76.8%) were C. jejuni, 542 (22.5%) were C. coli, 5 (0.2%) were C. lari, and 12 were of undetermined species.

Campylobacter was isolated from 2,418 (61.2%) of 3,950 avian stool specimens. Chickens in the corral group were colonized with Campylobacter less frequently than those in the control group (1,165 [58.1%] of 2,005 Campylobacter-positive specimens in the corral group and 1,283 [63.9%] of 1,945 specimens in the control group, P < 0.001), but both groups were heavily colonized with Campylobacter species. Because of logistical constraints, stool specimens were not available for analysis from 51 (21.8%) of 234 diarrhea episodes in the corral group and from 43 (31.4%) of 137 diarrhea episodes in the no corral group. Rates of Campylobacter-associated diarrhea were adjusted proportionately for these episodes without specimens in the analysis, as described in Methods. Overall, the rate of diarrhea from all causes was significantly higher in the corral group (2.79 episodes per person per year [epy]) than in the no corral group (2.07 epy; P = 0.017) (Table 1). In comparison with the overall study group, diarrhea rates were higher in subgroups based on presence of more than 21 chickens in the home and based on a child’s age of less than 36 months. In most of these subgroups, diarrhea incidence was higher in the corral group than in the no corral group, although this difference did not reach statistical significance because of the smaller sample size in these subgroups.

We also compared the incidence of Campylobacter infections documented in humans in each intervention group.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Incidence of all diarrhea episodes in humans with chickens in the household, grouped according to corralling practices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea cases</td>
<td>Corral</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>All age groups</td>
<td></td>
</tr>
<tr>
<td>From all households</td>
<td>234 episodes/83.9 person-years (2.79 epy)</td>
</tr>
<tr>
<td>Residents of homes with &gt; 21 chickens</td>
<td>86 episodes/27.1 person-years (3.16 epy)</td>
</tr>
<tr>
<td>Children &lt; 36 months of age</td>
<td></td>
</tr>
<tr>
<td>From all households</td>
<td>84 episodes/50.7 person-years (3.62 epy)</td>
</tr>
<tr>
<td>Residents of homes with &gt; 21 chickens</td>
<td>65 episodes/15.6 person-years (4.17 epy)</td>
</tr>
</tbody>
</table>

NS = not significant
* Number of diarrhea episodes divided by total number of person-years of observation in the study group (corral or no corral).
† Incidence of diarrhea episodes in the study group (based on data from the previous column), expressed in episodes per person per year (epy).
Symptomatic (diarrhea-related) and asymptomatic infections were evaluated separately (Table 2). Incidence of *Campylobacter*-associated diarrhea in the two intervention-based groups was corrected for the proportion of diarrhea episodes without a corresponding stool specimen. Rates of *Campylobacter*-associated diarrhea among children in households with corrals were approximately twice the rates found among children in houses without corrals, in both the entire study group (0.57 epy with corral versus 0.27 epy without corral; $P = 0.006$) and in children less than 36 months of age (0.77 epy with corral versus 0.46 epy without corral; $P = 0.08$). In children living in homes with ≥ 21 chickens, differences were even more striking (approximately seven-fold differences) in both the overall study group (0.77 epy with corral versus 0.077 without corral; $P = 0.002$) and in children less than 36 months of age (1.15 epy with corral versus 0.15 epy without corral; $P = 0.021$). Family contacts of culture-positive cases also tended to be culture positive more frequently in the corral group (12 [43%] of 28 specimens) than in the non-corral group (2 [22%] of 9 specimens).

Corralling was associated with a non-significant trend toward fewer asymptomatic *Campylobacter* infections (2.68 episodes/person/year in the corral group versus 3.12 episodes/person/year in the no corral group; two-tailed $P = 0.12$). However, this trend toward fewer asymptomatic infections in the households with corrals is probably a reflection of the higher rates of symptomatic diarrhea-associated infections in the children from households with corrals. Consequently, the children in households with corrals did not experience fewer overall *Campylobacter* infections (when symptomatic and asymptomatic infections were combined). This outcome has been communicated to households with corrals in place.

The effect of other demographic covariates on the outcome measurements of interest was determined in multivariate logistic and Poisson regressions. These covariates included family size, breastfeeding practices, number of rooms in the house, property space and child ages. Multivariate analysis did not alter the significance of the described differences in incidence of diarrhea and incidence of *Campylobacter*-associated diarrhea shown in Tables 1 and 2. Although the incidence of *Campylobacter* diarrhea differed between intervention groups, the proportions of *Campylobacter* infection during a diarrhea episode were not significantly different between the intervention groups in the multivariate model (0.21, 95% confidence interval [CI] = 0.15–0.28 in the corral group versus 0.15, 95% CI = 0.08–0.24 in the no corral group).

**DISCUSSION**

In summary, corralling of chickens in the household was not associated with reduced risk of *Campylobacter*-related diarrhea in our study. Rates of all diarrhea episodes and *Campylobacter*-associated diarrhea were significantly higher among children living in homes with corrals versus children living in homes without corrals (Tables 1 and 2). The trend toward fewer asymptomatic infections in the households with corrals is probably a reflection of the higher rates of symptomatic diarrhea-associated infections in the households with a corral. Consequently, the children in households with corrals did not experience fewer overall *Campylobacter* infections (and their rate of *Campylobacter*-related disease was significantly higher). The corralling of chickens in this environment was not protective against *Campylobacter*-related diarrhea, and appeared to enhance rates of this diarrheal illness.

In phase I of this project, where we conducted RFLP analysis of 838 *Campylobacter* strains from humans and free-ranging chickens from households without effective corralling, we found that recovery of a strain from a human with the same RFLP type as an isolate from a household chicken was highly associated with the absence of diarrhea (odds ratio = 0.07, $P < 0.005$); i.e., symptomatic infections were much less likely to be caused by an isolate recovered from a household chicken than asymptomatic infections. This suggested that in the usual situation in this community, in which chickens are not confined, most diarrhea-associated *Campylobacter* infections are due to infections acquired outside the home. Our corralling intervention may have changed this balance of diarrhea-associated infections from sources inside versus outside the home, but we were unable to definitively prove this with our currently available strain typing data. Nevertheless, the key message from a public health perspective is that corralling of chickens in this environment was not protective against *Campylobacter* diarrhea, and it may have increased the risk of campylobacteriosis in young children.

### Table 2

Incidence of asymptomatic and symptomatic *Campylobacter* infections in humans with chickens in the household, grouped according to corralling practices

<table>
<thead>
<tr>
<th>Corral</th>
<th>No corral</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Campylobacter</strong> diarrhea cases</td>
<td></td>
</tr>
<tr>
<td>All age groups</td>
<td></td>
</tr>
<tr>
<td>From all households</td>
<td>48/83.9</td>
</tr>
<tr>
<td>Residents of homes with ≥ 21 chickens</td>
<td>21/27.1</td>
</tr>
<tr>
<td>Children &lt; 36 months of age</td>
<td></td>
</tr>
<tr>
<td>From all households</td>
<td>39/50.7</td>
</tr>
<tr>
<td>Residents of homes with ≥ 21 chickens</td>
<td>18/15.6</td>
</tr>
<tr>
<td>Asymptomatic <em>Campylobacter</em> infections</td>
<td></td>
</tr>
<tr>
<td>From all households</td>
<td>225/83.9</td>
</tr>
<tr>
<td>Residents of homes with ≥ 21 chickens</td>
<td>67/27.1</td>
</tr>
</tbody>
</table>

* Number of positive cultures for *Campylobacter* (corrected for missing samples) divided by total number of person-years of observation in the study group (corral or no corral).
† Incidence of positive cultures for *Campylobacter* in the study group (based on data from the previous column), expressed in episodes per person per year (epy).
If the effect of coralling was exacerbating the risk of symptomatic campylobacteriosis and of diarrhea in general, why did this happen? We can only speculate, but several observations may be relevant. First, families in this community have relatively little property space, which is usually enclosed with walls, so corrals had to be located near the families’ living quarters. Although the corrals were built as far away from the living area as possible, frequently they had to be located on the roof or a rear patio that are not as ideal as sites located well away from the home. Corrals reduced the amount of chicken feces in the home, but concentrated them in a single area that required cleaning by someone in the household. Scarcity of water in this desert environment and poor hand washing practices may have also contributed to concentration of Campylobacter in the corrals. Although we did not observe children playing inside the corrals and families with corrals were counseled against this (based on observations from phase II of the study), children could have entered the corrals when field workers were not present, increasing their risk of Campylobacter infections. Since there were very few damaged corrals, and all damaged corrals were repaired immediately by project staff, no association between this variable and Campylobacter infections or Campylobacter diarrhea was observed.

Our study design offered an unusual opportunity to examine the impact of a behavioral and public health intervention, i.e., coralling of chickens, on the incidence of both symptomatic and asymptomatic Campylobacter infections in a community where this organism is a leading cause of diarrheal disease morbidity. We demonstrated that coralling chickens in the household in this environment was clearly not protective against symptomatic campylobacteriosis, and appeared to increase the risk. However, this study is subject to several limitations. Sample size considerations do not allow us to determine the impact of coralling on rates of Campylobacter diarrhea in narrower age groups, such as children less than one year of age who often have high rates of campylobacteriosis. Our data cannot be directly extrapolated to other less common organisms carried by chickens that could cause diarrhea in children, such as Salmonella sp. Financial limitations did not allow us to adequately explore the relationship between Campylobacter strains carried by chickens in the corral and those causing disease in children living in the household. Phase I of this study suggested that in the absence of corrals, asymptomatic infections were much less likely than asymptomatic infections to be caused by an isolate recovered from a household chicken.9 Our Phase III data on incidence of Campylobacter infections suggests that the presence of corrals changed this dynamic by increasing the risk of symptomatic disease from strains carried by household chickens, but the extensive RFLP typing needed to address this questions was not economically feasible.

Therefore, although effective coralling may be accomplished at low cost using materials available in this pueblo joven community, this coralling did not have an impact on the main outcome variable of interest, i.e., Campylobacter-associated diarrhea. The rate of Campylobacter-related diarrhea was increased among young children in the group with corrals, increasing the possibility that coralling increased the risk of this disease due to behavioral factors. Future control measures may be more effective if efforts are directed toward sanitary preparation of potentially contaminated foods and limiting contact with Campylobacter isolates outside of the home. Although coralling may be useful if corrals are distant from living quarters, it is not advisable as a control measure for Campylobacter in periurban shanty town communities such as our study site where chickens are commonly raised.

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