EXHIBIT G

TO DECLARATION OF
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SURVEILLANCE OF THE FLOW OF SALMONELLA AND CAMPYLOBACTER IN A COMMUNITY

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I. ABSTRACT

In an effort to learn more about possible linkages between human infection with Salmonella and Campylobactor jejuni and contamination of food by those bacteria, a two-pronged surveillance system was established. Food of animal origin from retail outlets in King County was systematically cultured for Salmonella and C. jejuni for a period of 20 months. Simultaneously, cases of Salmonella and C. jejuni enteritis diagnosed in enrollees at Group Health Cooperative of Puget Sound, a 320,000 member health maintenance organization, were investigated. A case/control study was included in the evaluation of the C. jejuni infections.

Salmonella and C. jejuni isolates from food and human cases were subjected to antibiotic susceptibility testing, plasmid analysis, and serotyping in an effort to identify phenotypic characteristics which might facilitate comparisons between the two groups of isolates.

The predominant finding in the food surveillance system was contamination of retail poultry by C. jejuni; 192 (22.3%) of 862 specimens cultured C. jejuni, only 30 (3.5%) cultured Salmonella. Other types of retail meat had, at most, negligible contamination by either bacterium.

In the population under study, C. jejuni was found to be a more common enteric pathogen than Salmonella. During the period of case surveillance, 218 C. jejuni cases (matched with 526 controls) and 72 Salmonella cases were diagnosed and investi-

gated. The case/control study disclosed that a number of exposures during the 7 days prior to the onset of illness were associated with cases more frequently than with controls, thus implicating those exposures as risks for C. jejuni infection. The most prominent of those exposures were travel to undeveloped countries, drinking raw milk, consumption of chicken, exposure to untreated water, contact with humans ill with enteritis, and contact with pets, particularly dogs, with diarrhea.

Calculation of the "etiologic fraction," the proportion of all cases attributable to specific exposures, revealed that consumption of chicken was responsible for 48.2% of cases of C. jejuni enteritis in the Group Health population during the period of study.

The information provided by the case/control study showing that chicken consumption was the predominant risk factor associated with C. jejuni enteritis was supported by microbiological study of C. jejuni isolates. Thus, isolates from human cases and those from retail poultry had similar antibiotic susceptibility patterns including prevalences of 29.7% and 32.8%, respectively, of tetracycline resistance, which was found to be plasmid-mediated. In addition, the distribution of the six most frequent Lior serotypes of isolates of C. jejuni from retail poultry was identical to that among isolates from humans.

In summary, work performed under Contract #223-81-7041 indicates that enteritis due to Campylobacter jejuni is more common than that due to Salmonella. Furthermore, C. jejuni does appear to flow from chickens to man via consumption of poultry products.

VI. DISCUSSION

Details of the total scope of work performed under Contract #223-81-7041 can appropriately be discussed in relation to the four stipulated objectives of the contract. One objective was to determine the magnitude and the character of the problem of human illness caused by Salmonella and C. jejuni in a defined population. The present study was well designed to accomplish that objective, because its case-surveillance system was established at Group Health Cooperative of Puget Sound (GHC), a 320,000 member health maintenance organization serving residents of western Washington. Surveillance of the two diseases of interest at GHC permitted the derivation of incidence rates because of the availability of patient enrollment statistics to serve as denominators to match case information or numerator data.

The overall incidence of C. jejuni infection at GHC was 71.5 cases diagnosed per 100,000 population per year, some two and one-half times the incidence of Salmonella infection (27.7/100,000/year). Other investigators have reported C. jejuni infections to be more frequent than salmonellosis (11, 46), and this newly recognized infection may in fact, be more common than the two traditionally predominant bacterial infections, salmonellosis and shigellosis combined (46).

Only two reportable diseases, gonorrhea, with an incidence of 415 per 100,000 and chicken pox, with a rate of 72.2 per 100,000, were reported in the U.S. in 1982 at an incidence greater than that of C. jejuni (47). To provide further com-

parison, in 1982 the incidence of tuberculosis in the U.S. was 11.0 per 100,000, that of syphilis, 32.7 per 100,000 (47).

A difference was also noted in the age-specific incidence of C. jejuni infection as compared to that for salmonellosis. Along with a peak in incidence in infants, very similar to that of Salmonella infection, there was with C. jejuni a second distinct peak in the young adult years, ages 20-39, which was entirely absent in salmonellosis.

This distinctive diphasic curve that comprises the age-specific incidence for C. jejuni infections has also been noted in Great Britain (48). The absence of a male preponderence of cases within the peak in the young adult years suggests that male homosexuals, known to acquire C. jejuni anorectal infections (49), do not account for a large proportion of cases in the GHC population. Nevertheless, this observed difference between C. jejuni and Salmonella age-specific rates suggests fundamental differences in the epidemiology of the two infections, which are currently viewed by many to have common pathways of transmission to and among humans.

Clinically, C. jejuni and Salmonella enteritis produced illnesses that were virtually indistinguishable. Nearly all patients with both infections had diarrhea and cramps, with a substantial minority suffering from bloody diarrhea. Fortunately, it was necessary to hospitalize only a small fraction of patients with both infections, and none died.

In comparing the morbidity of C. jejuni and Salmonella enteritis two facts suggested that the former was more severe.

First, a significantly larger proportion of patients with C. jejuni infection experienced weight loss with their illness, and secondly, the mean duration of symptoms was significantly longer.

One estimate of the magnitude of the problem caused by C. jejuni is a derivation of days lost to normal activities due to the infection. The mean duration of C. jejuni illness was 13.52 days in the present study. If it is assumed that approximately half of that time, or 7 days, was lost to normal activity such as employment, then one can calculate that 40 person-weeks of normal activity would be lost per 100,000 population per year due to C. jejuni infection. In a city of one million population, therefore, 400 person-weeks or 7.69 person-years of normal activity such as employment, would be lost due to this infection.

Furthermore, if it is true that C. jejuni infection is more prevalent than salmonellosis and produces as great or greater morbidity, then it is possible to make crude estimates on the importance of C. jejuni infection based upon statistics derived for salmonellosis. For example, it has been estimated by the Centers of Disease Control that as many as two million cases of salmonellosis occur annually in the U.S., at a cost of more than a billion dollars to the U.S. economy (1). An infection such as C. jejuni enteritis, which occurs twice as frequently as salmonellosis and is at least as severe, might be expected to produce an economic impact twice that of salmonellosis, or two billion dollars a year upon the U.S. As crude as these estimates are, it can be appreciated that the potential quantitative impact of C. jejuni upon a population similar to that where the current case sur- veillance system was conducted is considerable whether

viewed in terms of disease morbidity or economic burden.

It should be kept in mind that all the foregoing estimates were made without consideration of related C. jejuni infections within a family. Our culture surveillance in family contacts of cases and our case/control study documented that the organism is prevalent in the environment of cases, and that contacts of cases are at increased risk for the illness, but the morbidity associated with these findings is yet to be determined.

Two of the three remaining objectives of Contract #223-81-7041 may be considered together as a basis for further discussion of the present study because the two objectives are closely related. The first was to define the character of the movement of bacteria from animals to man, using as markers Salmonella and C. jejuni in foods from animals; the second, to utilize specific microbiological markers to link human illness caused by Salmonella and C. jejuni to specific animal sources. With respect to the use of C. jejuni and Salmonella as "markers" for bacterial contamination of animal-derived food, the present study is the first, to our knowledge, to report comparative contamination rates for these two pathogens in the same pool of food products. C. jejuni was isolated over four times more frequently than Salmonella but was found almost exclusively in poultry products. Thus, in retail poultry at least, C. jejuni appears to provide a better marker for bacterial contamination than does Salmonella.

Because C. jejuni contamination of poultry products was the only significant positive finding in the food surveillance compo-

nent of this study, further discussion of possible linkages between food contamination and human illness will be restricted to considerations relating to that phenomenon.

Considerable effort was extended in the present project to characterize all C. jejuni isolates from food and human cases with respect to three specific microbiological markers which might, to paraphrase the contract objective under question, link a human illness, C. jejuni enteritis, to a specific animal source, poultry. The three markers were antibiotic susceptibility testing, plasmid analysis, and serotyping (as noted in Results Section H, serotyping of C. jejuni isolates was performed by the Centers for Disease Control, Atlanta, GA).

The most interesting observations to emerge from the study of these phenotypic markers of C. jejuni was a relationship between tetracycline resistance and plasmid carriage. First, a surprisingly high rate of tetracycline resistance was noted in poultry products (32.8%) and in human cases (29.7%). Secondly, there was a strong correlation between tetracycline resistance and plasmid carriage; 93% of tetracycline resistant strains harbored plasmids. Thirdly, 30 of 31 plasmid-containing isolates were able to transfer the tetracycline resistance phenotype, confirming the fact that the resistance gene is located on extrachromosomal elements. Finally, there were no differences in the MIC's of tetracycline for the isolates tested with and without pre-exposure to the antibiotic, which suggests that tetracycline resistance is not inducible in Campylobacter species, and therefore not located on the bacterial chromosome.

Previous work with R-factors obtained from cynomologous

monkeys (35) and Taylor and associates' early studies with human isolates (50) suggested that the R-factors mediating tetracycline resistance in C. jejuni would be of a single type and size, since both of the above reports describe a conjugal 57 kilobase plasmid. However, during the present study, it became apparent that C. jejuni plasmids both larger and smaller than 57 kilobase were capable of transferring resistance to tetracycline within Campylobacter species. Although the BgIII and BcII endonuclease patterns of these plasmids were highly diverse, further analysis revealed that several fragments were highly conserved. These data confirm and extend the data recently published by Taylor and associates (50), who also noted a high degree of nucleotide homology among Campylobacter R-factors. Further studies are needed to explain the reasons for the diversity in size of these closely related C. jejuni plasmids.

Further work must also be done to determine the relationship between carriage of a tetracycline R-factor by C. jejuni and tetracycline supplementation of poultry feed; the present study did not address this issue directly. However, we did observe variation, unlikely to be due to chance, between tetracycline resistance of C. jejuni cultured from Washington-reared poultry and that of poultry originating in other states. It is tempting to speculate that geographic variability in plasmid mediated tetracycline resistance of C. jejuni could be related to regional differences in poultry feeding patterns. Further specific study should be undertaken to unravel any relationships that may exist between poultry feeding practices and C. jejuni plasmid profiles

and susceptibility patterns in a given region. It is noteworthy that the strain specific analysis preformed as a part of the case/control study showed that humans were no more likely to acquire tetracycline-resistant than tetracycline-susceptible C. jejuni infections from poultry. This finding suggests that poultry does not serve as the only reservoir of tetracycline-resistant C. jejuni infection.

Also yet to be determined is the clinical significance of tetracycline resistance in C. jejuni. Most patients with C. jejuni infection do not receive antibiotic therapy (51), and tetracycline is not the drug of choice should an antibiotic be needed (10). Furthermore, previous studies (50) and work done under the present contract all demonstrate that the C. jejuni R-factors could not be introduced into E. coli by conjugation or transformation. This suggests that these plasmids are incapable of replicating at least in E. coli and probably in the Enterobacteriaceae in general. Whether these plasmids could be transferred and maintained in other members of the bowel flora, such as in Bacteroides species, remains to be tested. It is possible, therefore, that there is little practical clinical significance to the finding of a related family of C. jejuni R-factors, even should they ultimately be traced to poultry feeding practices.

To conclude a review of microbiological markers identified in the present study, one other interesting phenomenon was noted: C. jejuni isolates from human cases and retail poultry possessed distributions of the six most frequent Lior serotypes that were virtually identical. This correlation has been noted previously with Salmonella serotypes isolated from human and non-

human sources (2), and has been used as evidence that human Salmonella infections arise from an animal reservoir, presumably through contamination of animal-derived food (3).

However, a word of caution must be raised against the casual use of microbiological markers to link bacteria from different sources, or more specifically, to prove that human C. jejuni infections arise from animal-derived food, as the two contract objectives under question stipulate.

The precedence for the use of phenotypic bacterial markers to identify sources of human disease originated with the study of foodborne outbreaks. In that setting, laborataory tests often show similarities between pathogenic bacteria isolated from food eaten by ill individuals and those isolated from the patients themselves. In fact, the investigation of the single C. jejuni foodborne outbreak in the present project (Appendix B) fit this pattern perfectly, with the outbreak-associated bacterial strains, isolated from all implicated patients and from several goats at the implicated dairy, showing virtual homology among the following tests: susceptibility to five antibiotics, plasmid pattern, and Lior serotype.

However informative bacterial phenotypic markers may be in the evaluation of foodborne outbreaks, it is quite a different matter to presume that they will be useful in identifying sporadic, isolated infections as being foodborne. Similar phenotypic markers in C. jejuni isolates from retail poultry and patients could, taken alone, as easily support a hypothesis that chickens were infected by humans, or perhaps more reasonably, that chick-

e... and humans derived their C. jejuni isolates from another large enviornmental reservoir.

For purposes of linking C. jejuni isolates from different groups, it would have been helpful had significant C. jejuni contamination of another type of meat been detected. In that case, isolates from humans could have been compared to those of the two meat species by analysis of the microbiological markers and perhaps subsequently labeled as more similar to isolates found in one meat species than to those of the other. This was not the case, although C. jejuni isolates from retail poultry did show closer marker similarities to human strains than did those from processor products.

This dilemma concerning appropriate interpretations of data on microbiological markers of C. jejuni was largely resolved by information gained from the case/control study of C. jejuni infections in humans. Case/control analysis is one of the tools routinely applied by epidemiologists to address questions of cause and effect in human disease. The dual approach employing microbiological and epidemiological (case/control) methodologies employed in this study has recently been proposed by the CDC to determine the cause(s) of sporadic cases of salmonellosis (correspondence from John C. Feeley, Acting Director, Division of Bacterial Diseases, CDC, to State and Territorial Epidemiologists dated June 6, 1984. Subject: Salmonella antimicrobial resistance patterns in the United States).

Because the epidemiology of human C. jejuni infection is so poorly understood, it was considered essential to explore numerous potential causal factors by means of an extensive inquiry

into the backgrounds of affected and unaffected persons. During this process, a wide variety of potential food exposures and habits were sought in order to maximize the likelihood of addressing a prime objective of Contract #223-81-7041, which was to link human illness caused by C. jejuni to specific animal sources through food derived from those animals.

There were a number of exposures that were more frequent among cases than among controls during the week prior to the onset of symptoms. Consequently those exposures were identified as potential causes of C. jejuni enteritis. It is noteworthy that many of the previously implicated types of transmission of C. jejuni infection to humans summarized from the literature in a recent epidemiological review (45) were also found to be risk factors for sporadic C. jejuni enteritis in the present study. Among those were travel to an undeveloped country, consumption of chicken, drinking raw milk, consumption of untreated surface water, contact with humans ill with enteritis, and contact with pets, particularly dogs, with diarrhea.

The most surprising food exposure found to have an elevated relative risk in the present study was mushroom consumption. Mushrooms have not previously been implicated as a vehicle for C. jejuni infection. However, that vegetable is grown commercially in high concentrations of animal manure, including that of chickens. Thus, C. jejuni proliferation is plausible in this setting. Furthermore, because mushroom eating is a common exposure, there is considerable potential profit for disease prevention in establishing a causal relationship. Further work should be done to

substantiate this finding.

Considering the objectives of Contract #223-81-7041, it is appropriate to focus critical attention upon chicken consumption, found in our study to have an elevated relative risk. Is this association, in fact, a causal one?

Several well-accepted observational criteria for causation of associations found in case/control studies (52) may be applied to that between eating chicken and C. jejuni enteritis. First, the temporal sequence must be consistent: a causal event precedes its effect. In the present study, the questions on food exposures applied specifically to the 7-day period prior to onset of illness. This time interval was chosen because existing information suggested that the incubation period for C. jejuni infection ranges between two and seven days. Assuming no recall bias was present, the temporal sequence criterion appears to have been satisfied for the association under question.

A second condition for causation is that the association under question should be consistently observed under different conditions. As previously noted, there is now an accumulating body of evidence that eating chicken is a risk factor for C. jejuni infection. Brouwer et al. (53) described an outbreak in a military academy which affected 89 of 123 first year cadets during a survival exercise. Each cadet had killed, skinned, and prepared his own chicken over a wood fire. In several recent case/control studies (24,25,54,55), the cases reported greater frequencies of chicken consumption (25,54,55), handling of chicken during meal preparation (24,55), and greater frequency of shorter cooking time (55), than did controls.

Thirdly, the strength of the association provides evidence for causation. In the present study, the relative risk for chicken consumption was not large, as cases were only two and one-half times as likely as controls to have named that exposure in the week prior to onset of illness. It is probable that the strength of the association would have been much greater had it been possible to ascertain the consumption of C. jejuni- contaminated chicken, found in our food surveillance study to represent approximately 22% of all retail specimens. According to this evidence, almost 80% of chicken eaters were not at risk for C. jejuni enteritis because the chicken that was eaten was not contaminated. This fact led to considerable dilution of the association between chicken consumption and C. jejuni enteritis, and produced the relatively modest relative risk.

A final important condition supporting causation of an observed association is consonance of the association with existing knowledge. It is in this context that the microbiological information acquired in our study assumes importance. The association of chicken eating with C. jejuni infection is strongly supported by the observation in this and previous studies (11-14) that retail poultry products are frequently contaminated with C. jejuni because this is a plausible mechanism by which eating chicken could "cause" C. jejuni infection.

Furthermore, the similarities in antibiograms, plasmid content, and serotypes of C. jejuni isolates from retail poultry and humans found in the present study would be expected should a substantial fraction of human C. jejuni infections derive from

t pool of organisms that are found in poultry. In addition, the similar seasonal patterns of C. jejuni contamination of retail poultry and human C. jejuni cases, with a summer-fall peak, is evidence for a causal association. It should also be noted that the absence of C. jejuni isolation from the other major meat species, beef and pork, supports the lack of association of those foods with C. jejuni illness in the case/control study.

The fourth and last objective of Contract #223-81-7041 was to determine the proportion of human salmonellosis and C. jejuni enterities which has its origin from animal foods obtained from poultry, swine, and beef. For C. jejuni this proportion was estimated, in the determination of the "etiologic fraction," to be 48.2% for poultry and none for either beef or swine.

The etiologic fraction of a disease is viewed as the expected reduction in the disease following removal of the exposure. Thus, we estimate that almost 50% of C. jejuni infections at Group Health Cooperative during the period of study would not have occurred had no poultry been consumed. We have no reason to believe that the same effect would not accrue to the general population if C. jejuni contamination of retail poultry were controlled.

Thus, work performed by Seattle-King County Health Department and its subcontractors under Contract #223-81-7041 provides an important insight into the epidemiology of infections due to Camplylobacter jejuni by showing that almost half of cases in a defined population over an 18-month period were attributable to eating poultry. This information, derived from the case/control

study, was supported by results of food surveillance showing that over 20 percent of poultry products sampled at King County retail outlets over the same period were contaminated with C. jejuni. Furthermore, C. jejuni isolates from those human cases and retail poultry were similar with respect to antibiotic susceptibilities, plasmid carriage, and serotype.

The food surveillance component of the present study suggested several possible strategies for at least reducing C. jejuni contamination of poultry. For example, isolation rates for C. jejuni in retail markets were lowest on Mondays, when display shelves were cleanest and products had been freshly received. On the other hand, handling of poultry at the retail level appeared to increase the likelihood of culturing C. jejuni. The most dramatic effect on C. jejuni contamination of poultry was movement of products from processor, where the isolation rates approached 60%, to retail sites, where it fell to just over 20%. Observations such as these provide hypotheses for studies aimed at reducing C. jejuni contamination in retail poultry.