

UNITED STATES OF AMERICA  
BEFORE THE FOOD AND DRUG ADMINISTRATION  
DEPARTMENT OF HEALTH AND HUMAN SERVICES

\_\_\_\_\_  
)  
)  
)  
In the Matter of: )  
)  
Enrofloxacin for Poultry: Withdrawal )  
of Approval of Bayer Corporation's )  
New Animal Drug Application )  
(NADA) 140-828 (Baytril) )  
)  
)  
)  
\_\_\_\_\_ )

FDA DOCKET: 00N-1571  
DATE: December 10, 2002

0011 02 10 10

Center for Veterinary Medicine's Unopposed Motion to Substitute Original Signed Written Direct Testimony of Dr. Frederick J. Angulo for the Signed Facsimile Version of Testimony of Dr. Frederick J. Angulo filed December 9, 2002

The Center for Veterinary Medicine ("the Center" or "CVM") respectfully moves to substitute the original signed written direct testimony of Dr. Frederick J. Angulo for the facsimile version (signed) of Dr. Angulo's written testimony. Although Dr. Angulo sent, via federal express, his signed written direct testimony to CVM on Friday, December 6, 2002, that testimony did not arrive at the Center in time to include it in yesterday's Motion to Enter Written Direct Testimony and Specified Exhibits and Documents. Robert Nicholas, counsel for Bayer, has indicated that this Motion is unopposed.

Respectfully submitted, this 10th day of December by:

  
Nadine Steinberg  
Counsel for the Center for Veterinary Medicine  
5600 Fishers Lane (GCF-1)  
Rockville, MD 20857  
(301) 827-5050



Enrofloxacin Hearing  
Docket No: 00N-1571

**CERTIFICATE OF SERVICE**

I hereby certify that an original and one copy of the foregoing Center for Veterinary Medicine's Unopposed Motion to Substitute the Original Written Direct Testimony of Dr. Frederick Angulo for the Facsimile Written Direct Testimony of Dr. Frederick Angulo was hand delivered this 10th day of December, 2002, to:

Dockets Management Branch (HFA-305)  
Food and Drug Administration  
5630 Fishers Lane (Room 1061)  
Rockville, MD 20852

I also certify that a copy of the pleading has been hand delivered and e-mailed, this 10th day of December, 2002, to:

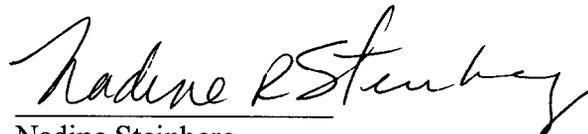
The Office of the Administrative Law Judge  
Food and Drug Administration  
Room 9-57, HF-3  
5600 Fishers Lane  
Rockville, MD 20857

I also certify that a copy of this pleading was e-mailed and mailed by First Class U.S. mail, this 10th day of December, 2002, and a copy of the paper submission, along with a pdf file on a CD will be delivered this 10th day of December, to:

Robert B. Nicholas  
McDermott, Will & Emery  
600 13th Street, NW  
Washington, DC 20005

Kent D. McClure  
Animal Health Institute  
1325 G Street, NW, Suite 700  
Washington, DC 20005

**Dated:** 12/10/02



Nadine Steinberg  
Counsel for the Center for  
Veterinary Medicine  
5600 Fishers Lane (GCF-1)  
Rockville, MD 20857  
(301) 827-5050

1  
2  
3 UNITED STATES OF AMERICA  
4 BEFORE THE FOOD AND DRUG ADMINISTRATION  
5 DEPARTMENT OF HEALTH AND HUMAN SERVICES  
6

7  
8  
9 In the Matter of: )

FDA DOCKET: 00N-1571

DATE: December 9, 2002

10 Enrofloxacin for Poultry: Withdrawal )  
11 of Approval of Bayer Corporation's )  
12 New Animal Drug Application )  
13 (NADA) 140-828 (Baytril) )  
14 )  
15 )  
16 )  
17 )  
18 )

19  
20  
21 Written Direct Testimony of Frederick J. Angulo  
22  
23

24 1. I am Frederick J. Angulo, Chief of the FoodNet/NARMS Unit of the Foodborne and Diarrheal  
25 Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious  
26 Diseases, Centers for Disease Control and Prevention (CDC).  
27

28 2. I earned a Doctor of Philosophy in Epidemiology from the School of Public Health at the  
29 University of California at Los Angeles in 1993, a Masters of Preventive Veterinary Medicine from  
30 the School of Veterinary Medicine at the University of California at Davis in 1984, a Doctor of  
31 Veterinary Medicine from the School of Veterinary Medicine at the University of California at  
32 Davis in 1983, a Masters of Science from the University of San Francisco in 1979, and a Bachelors  
33 of Science from the University of San Francisco in 1978.  
34

35 3. I am the lead scientist at the CDC on the epidemiology of antimicrobial resistance in bacteria  
36 that are predominately acquired from eating contaminated food. I have conducted extensive  
37 research on antimicrobial resistance in foodborne pathogens, including fluoroquinolone-resistant  
38 *Campylobacter*. I have authored or co-authored over 100 peer-reviewed articles or book chapters in  
39 the medical scientific literature. I have served as a consultant on several pertinent national and  
40 international committees including the United States Food and Drug Administration's Veterinary  
41 Medicine Advisory Committee, the United States Surgeon General's Veterinary Professional  
42 Advisory Committee, and several consultations with the World Health Organization. I have  
43 received several national awards for my professional work including the James Steele Award by the  
44 CDC for exceptional contributions in veterinary public health and recognition as a Distinguished  
45 Diplomat by the American College of Veterinary Preventive Medicine. My curriculum vitae,  
46 which includes a list of publications, is attached to this testimony and can be found in this Docket as  
47 Exhibit G-1402, pp. 1-26.

1 4. I grew up on a cattle ranch in California. My professional background includes serving as a  
2 veterinarian in the United States Army (1984-1990), and as an Epidemic Intelligence Service  
3 Officer (1993-1995) and a veterinary epidemiologist (1995-present) in the Foodborne and Diarrheal  
4 Diseases Branch at the CDC.  
5

#### 6 **Foodborne Disease Surveillance and FoodNet**

7 5. Foodborne infections are an important public health challenge. The CDC is actively involved in  
8 preventing foodborne disease. The principal role of the CDC's National Center for Infectious  
9 Diseases in the interagency Food Safety Initiative has been to enhance surveillance for and  
10 investigation of infections that are often foodborne. These efforts will provide crucial data to  
11 identify control points, focus future prevention strategies and decision-making by food safety  
12 regulatory agencies, measure changes in the burden of disease, and track trends in specific  
13 infections over time as prevention measures are implemented.  
14

15 FoodNet is the Foodborne Diseases Active Surveillance Network (1). FoodNet is the principal  
16 foodborne disease component of the CDC's Emerging Infections Program. FoodNet is a  
17 collaborative project among the CDC, state health departments, the United States Department of  
18 Agriculture Food Safety and Inspection Service (FSIS), and the United States Food and Drug  
19 Administration (FDA). FoodNet augments, but does not replace, longstanding activities at the CDC,  
20 participating state health departments, the FSIS, and the FDA, to identify, control, and prevent  
21 foodborne disease hazards.  
22

23 The objectives of FoodNet are to determine the frequency and severity of foodborne diseases;  
24 determine the association of common foodborne diseases with eating specific foods; and describe  
25 the epidemiology of new and emerging bacterial, parasitic, and viral foodborne pathogens. To  
26 address these objectives, FoodNet uses active surveillance and conducts related epidemiologic  
27 studies. Enhanced surveillance and investigation are integral parts of developing and evaluating new  
28 prevention and control strategies that can improve the safety of our food and the public's health. By  
29 monitoring the burden of foodborne diseases over time, FoodNet can document the effectiveness of  
30 new food safety control measures, such as the FSIS Pathogen Reduction and Hazard Analysis and  
31 Critical Control Point Systems, in decreasing the number of cases of foodborne disease in the  
32 United States each year.  
33

34 FoodNet conducts population-based active surveillance for clinical laboratory isolations of  
35 *Campylobacter*, *Cryptosporidium*, *Cyclospora*, Shiga-toxin producing *Escherichia coli* including *E.*  
36 *coli* O157:H7, *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, and *Yersina* infections in Connecticut,  
37 Georgia, Maryland, Minnesota, and Oregon, and selected counties in California, Colorado, New  
38 York, and Tennessee. The total population in the area under surveillance is greater than 37 million  
39 persons, which is greater than 13 percent of the population of the United States (2). FoodNet  
40 personnel identify cases of infection by regularly contacting each of the more than 450 clinical  
41 laboratories that serve residents of the surveillance areas. Using data from FoodNet and other  
42 sources, the CDC estimates that foodborne infections cause 76 million illnesses, 325,000  
43 hospitalizations, and 5,000 deaths each year (3).  
44

References for paragraph 5:

1. FoodNet website at <http://www.cdc.gov/foodnet>

2. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of foodborne illnesses--selected sites, United States, 2001. *Morbidity and Mortality Weekly Report* 2002;51(15):325-329. (Exhibit G-1791)
3. Mead PS, Slutsker L, Dietz V, McCaig L, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. Food-related illness and death in the United States. *Emerging Infectious Diseases* 1999;5(5):607-625. (Exhibit G-410)

### Antimicrobial Resistance Monitoring and NARMS

6. Antibiotic resistance is a food safety problem for several reasons. One important reason is that, as antibiotic resistance increases, resistance threatens the utility of antibiotics that are commonly used to treat serious human infections caused by bacteria commonly found in food, such as *Campylobacter* and *Salmonella*. The CDC's active involvement in preventing foodborne disease includes preventing antimicrobial-resistant infections. To achieve this goal, the CDC's National Center for Infectious Diseases conducts surveillance for and investigation of antimicrobial-resistant foodborne infections.

NARMS is the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (1). The primary purpose of NARMS is to monitor antimicrobial resistance among foodborne enteric bacteria including *Campylobacter*, *Salmonella*, and *Escherichia coli* O157:H7. NARMS detects emerging resistance and guides studies that evaluate where and how people become infected with resistant foodborne bacteria. NARMS data are used by the CDC and state health departments to investigate outbreaks caused by particular bacteria, conduct other studies to better understand the circumstances under which resistant bacteria arise and spread, and guide efforts to mitigate antimicrobial resistance.

NARMS is a collaborative project among the CDC, participating state health departments, the United States Food and Drug Administration, and the United States Department of Agriculture. Many NARMS activities are conducted within the framework of the CDC's Emerging Infections Program, including within FoodNet. Activities conducted at the CDC focus on foodborne enteric bacteria isolated from humans.

Clinical laboratories isolate foodborne enteric bacteria usually from diagnostic specimens collected from ill persons and forward the isolates to state public health laboratories. Specimens yielding foodborne enteric bacteria are commonly stool specimens, but also include blood, cerebrospinal fluid and other specimens. NARMS testing of human *Salmonella* and *Escherichia coli* O157:H7 isolates began in 1996; NARMS testing of human *Campylobacter* isolates began in 1997. In NARMS, participating state health departments forward selected *Salmonella* and *E. coli* O157:H7 isolates to the CDC for susceptibility testing. In addition, each of the state health departments participating in FoodNet also sends selected *Campylobacter* isolates each week to the CDC for susceptibility testing.

References for paragraph 6:

1. NARMS website at <http://www.cdc.gov/narms>.

### Representativeness of FoodNet and NARMS

7. Several NARMS activities, including susceptibility testing of human *Campylobacter*, are conducted exclusively in FoodNet sites. FoodNet is a sentinel network that is producing more stable and accurate estimates of the burden and sources of specific foodborne diseases in the United

1 States. While a key objective of FoodNet is to estimate the national burden and sources of  
2 foodborne diseases, FoodNet activities are conducted within selected state health departments. The  
3 selection of these participating state health departments was not chosen specifically to be  
4 representative of the United States population; selection was based upon written responses to  
5 Request for Proposals published in the Federal Register.  
6

7 FoodNet has evaluated the comparability of the population residing in the FoodNet surveillance  
8 area to the population residing in the United States (1). Using 1996 United States Census Bureau  
9 data and Community Health Status Indicator Project data, we performed a demographic comparison  
10 between the population in the FoodNet surveillance area and the United States on the basis of age,  
11 gender, race, urban residence, population density (persons per square mile), and percent at or below  
12 poverty (defined as having a household income less than the poverty thresholds established by the  
13 United States Census Bureau).  
14

15 The populations in the FoodNet surveillance area and the United States had similar age and gender  
16 distributions. Compared to the United States population, the population in the FoodNet surveillance  
17 area was slightly more likely to be Asian and less likely to be Black or Hispanic. The population in  
18 the FoodNet surveillance area was also more likely to include urban residents and residents in  
19 counties with lower population density, and less likely to include persons living at or below poverty.  
20

21 Taken together, these data indicate slight demographic differences between the populations residing  
22 in the FoodNet surveillance area and the United States. Despite these differences, the distribution of  
23 the FoodNet population across several other demographic factors, particularly age and gender, and  
24 health indicators, is similar to that of the United States population. These data support the  
25 generalizability of FoodNet data to the United States population for the purpose of understanding  
26 the epidemiology of foodborne illness.  
27

28 References for paragraph 7:

- 29 1. Hardnett F, Hoekstra R, Kennedy M, Angulo F, and the EIP FoodNet Working Group.  
30 Comparability of FoodNet and United States Populations. International Conference on  
31 Emerging Infectious Diseases. Atlanta, GA, March, 2002. Available at the FoodNet website  
32 at <http://www.cdc.gov/foodnet>. Also: Hardnett F, Hoekstra R, Johnson S, Kennedy M, and  
33 Angulo F. Comparability of the 1996 FoodNet and United States Populations. (Exhibit G-  
34 769)  
35

### 36 **FoodNet *Campylobacter* data**

37 8. FoodNet conducts active laboratory-based surveillance for culture-confirmed cases of  
38 *Campylobacter* and other foodborne pathogens. To identify cases, FoodNet personnel contact each  
39 clinical laboratory in their surveillance area either weekly or monthly depending on the size of the  
40 laboratory. Cases represent the first isolation of *Campylobacter* from a person by a clinical  
41 laboratory; most specimens are obtained for diagnostic purposes from ill persons.  
42

43 FoodNet surveillance began in 1996 in Minnesota and Oregon and selected counties in California,  
44 Connecticut, and Georgia. The estimated incidence of laboratory-confirmed *Campylobacter*  
45 infections was 23.5 infections per 100,000 population in 1996 and 24.7 infections per 100,000  
46 population in 1997 (1,2). In 1998, the surveillance area in Connecticut became statewide and active  
47 surveillance began in selected counties in Maryland and New York. In 1999, the remaining  
48 counties in Georgia were added, along with additional counties in New York. In 2000, surveillance

1 expanded to selected counties in Tennessee and additional counties in California. The estimated  
2 incidence of laboratory-confirmed *Campylobacter* infections per 100,000 population in 1998, 1999,  
3 and 2000 was 19.4, 15.0, and 15.4, respectively (3-5).

4  
5 In 2001, the surveillance area included Connecticut, Georgia, Minnesota, and Oregon, and selected  
6 counties in California, Colorado, Maryland, New York, and Tennessee. Preliminary data for 2001  
7 indicate that there were 4,740 laboratory-confirmed *Campylobacter* infections ascertained in  
8 FoodNet sites, which correlate to an incidence of 13.8 laboratory-confirmed infections per 100,000  
9 population (6). The incidence of laboratory-confirmed infections was highest in infants (children  
10 less than one year of age) with 33.5 infections per 100,000 population. There was substantial  
11 geographic variation in incidence. The incidence of laboratory-confirmed *Campylobacter* infections  
12 ranged from 7.0 infections per 100,000 population in Maryland to 31.7 infections per 100,000  
13 population in California.

14  
15 The number of sites and population under surveillance nearly doubled between 1996 and 2001.  
16 Because of substantial variation in incidence among sites, adding new sites influences overall crude  
17 incidence. To account for the increased population and variation in the incidence among sites, a log-  
18 linear Poisson regression model was used to estimate the effect of time on the incidence of  
19 *Campylobacter*, treating time (i.e., calendar year) as a categorical variable, with 1996 as the  
20 reference year (6). Between 1996 and 2001, the incidence of *Campylobacter* declined by 27 percent  
21 (95 percent confidence interval, 19 percent decline to 35 percent decline). Although there has been  
22 a decline in incidence, *Campylobacter* continues to present a significant burden of infection in the  
23 U.S. population.

24  
25 FoodNet has also recently completed a review of the epidemiology of *Campylobacter* infections  
26 using FoodNet data from 1996-1999 (7). During this period, the incidence of laboratory-confirmed  
27 *Campylobacter* infections was highest among Asians (33.2 infections per 100,000 population) and  
28 Hispanics (31.8 infections per 100,000 population). Infections were more common among males.  
29 Importantly, one percent of laboratory-confirmed infections were invasive resulting in  
30 *Campylobacter* isolation from blood. Furthermore, ten percent of persons with laboratory-  
31 confirmed infections were hospitalized. The highest hospitalization rate (27 percent) was among  
32 persons 60 years of age or older. One person in every 3,000 persons with a laboratory-confirmed  
33 *Campylobacter* infection died.

34  
35 References for paragraph 8:

- 36 1. Centers for Disease Control and Prevention. 1996 Final FoodNet Surveillance Report.  
37 (Exhibit G-102)
- 38 2. Centers for Disease Control and Prevention. 1997 Final FoodNet Surveillance Report.  
39 (Exhibit G-93)
- 40 3. Centers for Disease Control and Prevention. FoodNet Surveillance Report for 1998 (Final  
41 Report). Available at the FoodNet website at <http://www.cdc.gov/foodnet>. Also: Centers for  
42 Disease Control and Prevention. FoodNet 1998 Surveillance Results. Preliminary Report.  
43 (Exhibit G-94)
- 44 4. Centers for Disease Control and Prevention. FoodNet Surveillance Report for 1999 (Final  
45 Report). Available at the FoodNet website at <http://www.cdc.gov/foodnet>.
- 46 5. Centers for Disease Control and Prevention. FoodNet Surveillance Report for 2000 (Final  
47 Report). Available at the FoodNet website at <http://www.cdc.gov/foodnet>.

6. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of foodborne illnesses--selected sites, United States, 2001. *Morbidity and Mortality Weekly Report* 2002;51(15):325-329. (Exhibit G-1791)
7. Samuel M, Vugia DJ, Shallow S, Marcus R, Segler S, McGivern T, Kassenborg H, Reilly K, Kennedy M, Angulo F, Tauxe R, and the EIP FoodNet Working Group. Epidemiology of sporadic *Campylobacter* infection in the United States and declining trend in incidence, FoodNet 1996-1999. *Clinical Infectious Diseases* (in press). (Attachment 1) Also: Samuel M, Reilly K, Shallow S, Marcus R, Segler S, McGivern T, Kassenborg H, Hollinger K, Vose D, Bartholomew M, Kennedy M, Vugia D, and the EIP FoodNet Working Group. Burden of *Campylobacter* Infection in the United States and Declining Trend in Incidence, FoodNet 1996-1999. (Exhibit G-555)

### **Estimates of campylobacteriosis in the United States**

9. FoodNet studies have been used to estimate the total number infections, hospitalizations and deaths caused by *Campylobacter* in the United States. These estimates are determined using the incidence of laboratory-confirmed infections ascertained in FoodNet, and adjusting the FoodNet incidence to account for underreporting (1,2). Because many cases of foodborne diseases are not reported, it is necessary to account for underreporting to calculate the total number of *Campylobacter* infections.

Although many cases of campylobacteriosis, including many cases of severe illnesses and deaths caused by *Campylobacter*, are reported to public health officials, a large number of cases of campylobacteriosis are not detected through routine public health surveillance (i.e., not reported to public health officials) (1). For a person with a *Campylobacter* infection to be reported through routine surveillance, several steps along a chain of events are necessary; these steps may be described as a "surveillance pyramid." The surveillance pyramid requires that the ill person seeks medical care, the health-care provider obtains a specimen for diagnosis, the laboratory performs the necessary diagnostic tests, and the illness is ascertained by public health officials (2).

Although there are many cases of campylobacteriosis in the general population (at the bottom of the surveillance pyramid), many ill people do not seek medical care. There are numerous reasons why a person with campylobacteriosis might not seek medical care. These reasons include mild perceived illness and poor access to medical care.

Although many people with campylobacteriosis seek medical care, many of these patients do not have a specimen obtained and submitted to a clinical laboratory for diagnosis. There are numerous reasons, including difficulty in obtaining a specimen, why a person with campylobacteriosis who has sought medical care might not have a specimen obtained and submitted to a clinical laboratory.

Although many people with campylobacteriosis seek medical care and provide a specimen to a clinical laboratory, clinical laboratories do not always perform the necessary tests to diagnose *Campylobacter* or campylobacteriosis, and do not always communicate the results of the diagnostic tests to public health officials. Furthermore, some patients are treated with antibiotics prior to collection of the specimen, making it difficult to diagnose *Campylobacter* infections in these patients.

Taken together, the number of laboratory-diagnosed *Campylobacter* cases reported to public health officials (at the top of the surveillance pyramid) represents but a fraction of the many

1 *Campylobacter* infections that occur in the United States (at the bottom of the surveillance  
pyramid).

3  
4 In 1999, the CDC estimated the degree of underreporting of *Campylobacter* to be approximately 38-  
5 fold (1). Using the FoodNet data from 1996-1997, and correcting for this underreporting, it was  
6 estimated that *Campylobacter* causes 2.4 million infections, 13,000 hospitalizations, and 124 deaths  
7 a year in the United States (1). The frequency of foodborne transmission of *Campylobacter* was  
8 estimated to be 80 percent.

9  
10 The CDC also used FoodNet data in a more recent model to estimate the burden of *Campylobacter*  
11 (2). This more recent calculation used FoodNet 1999 *Campylobacter* incidence and a simulation  
12 procedure developed by Vose et al. at the United States Food and Drug Administration in a  
13 *Campylobacter* risk assessment. Using these data and that model, it is estimated that *Campylobacter*  
14 infected an estimated 1.4 million persons in 1999.

15  
16 References for paragraph 9:

- 17 1. Mead P, Slutsker L, Dietz V, McCaig L, Bresee JS, Shapiro C, Griffin PM, Tauxe R. Food-  
18 related illness and death in the United States. *Emerging Infectious Diseases* 1999;5(5):607-  
19 625. (Exhibit G-410)
- 20 2. Samuel M, Vugia DJ, Shallow S, Marcus R, Segler S, McGivern T, Kassenborg H, Reilly K,  
21 Kennedy M, Angulo F, Tauxe R, and the EIP FoodNet Working Group. Epidemiology of  
22 sporadic *Campylobacter* infection in the United States and declining trend in incidence,  
23 FoodNet 1996-1999. *Clinical Infectious Diseases* (in press).

#### 4 **NARMS *Campylobacter* data and resistance to fluoroquinolones in humans**

5  
6 10. The National Antimicrobial Resistance Monitoring System (NARMS) began antimicrobial  
7 susceptibility testing of human *Campylobacter* isolates in 1997 when laboratories in California,  
8 Connecticut, Georgia, Minnesota, and Oregon selected and began forwarding *Campylobacter*  
9 isolates each week to the CDC. Laboratories were added in Maryland and New York in 1998, in  
10 Tennessee in 1999 and in Colorado in 2000.

11  
12 Upon receipt at the CDC, isolates are tested for viability and purity. Isolates are confirmed as  
13 *Campylobacter* and then identified to species level (*Campylobacter jejuni*, *C. coli*, etc.) by the  
14 hippurate test. Hippurate-positive isolates are classified as *C. jejuni*. Hippurate-negative isolates are  
15 additionally tested by a polymerase chain reaction to identify the presence or absence of the  
16 hippuricase gene; isolates with the hippuricase gene are classified as *C. jejuni* and isolates without  
17 the gene are further tested to determine whether they are *C. coli*, *C. upsaliensis*, or another species  
18 of *Campylobacter*. All *Campylobacter* isolates are tested with the E-test (AB Biodisk, Solna,  
19 Sweden) system for minimal inhibitory concentrations for ciprofloxacin and several other  
20 antimicrobial agents. Ciprofloxacin resistance is defined as a ciprofloxacin minimum inhibitory  
21 concentration of greater than or equal to four micrograms per milliliter.

22  
23 Between 1997 and 2001, 1932 *Campylobacter* isolates were received at the CDC through NARMS;  
24 317 were either not viable, contaminated with other bacteria, or determined not to be  
25 *Campylobacter*. Analysis was, in general, restricted to one *Campylobacter* isolate per site per week.  
26 The 2001 CDC *Campylobacter* data are preliminary; therefore, the analysis discussed in this  
27 section, when involving data from 2001, is preliminary. Furthermore, the manuscripts describing  
28 these findings are currently undergoing the review process involved with CDC clearance.

1 Of the 1592 *Campylobacter* isolates included in the analysis, 1506 (95 percent) were *C. jejuni* and  
2 64 (4 percent) were *C. coli* (1). For the 1543 isolates for which sex and age of the patient were  
3 known, 693 (45 percent) were female and the median age was 33 years (CDC unpublished data).  
4

5 *Campylobacter* isolates exhibited two distinct populations with respect to their minimum inhibitory  
6 concentrations to ciprofloxacin. Nearly all isolates either had a minimum inhibitory concentration of  
7 0.5 or less micrograms per milliliter (susceptible isolates), or a minimum inhibitory concentration of  
8 32 or more micrograms per milliliter (resistant isolates). Overall, 16 percent (255 of 1592)  
9 *Campylobacter* isolates were resistant to ciprofloxacin (1). The percent of *Campylobacter* isolates  
10 resistant to ciprofloxacin was 13 percent (28 of 217) in 1997, 14 percent (48 of 345) in 1998, 18  
11 percent (58 of 319) in 1999, 14 percent (46 of 324) in 2000, and 19 percent (75 of 387) in 2001 (1).  
12

13 Overall, 15 percent (233 of 1506) *C. jejuni* isolates were resistant to ciprofloxacin (1). The percent  
14 of *C. jejuni* isolates resistant to ciprofloxacin was 12 percent (26 of 209 isolates) in 1997, 14 percent  
15 (45 of 330) in 1998, 18 percent (52 of 295) in 1999, 14 percent (43 of 306) in 2000, and 18 percent  
16 (67 of 366) in 2001 (1). The percent of *C. jejuni* isolates resistant to ciprofloxacin varied between  
17 years and between sites, from 7 percent in Minnesota in 1997 to 30 percent in Connecticut and  
18 Georgia in 2001 (CDC unpublished data). Overall, 31 percent (20 of 64) *C. coli* isolates were  
19 resistant to ciprofloxacin (1). The percent of *C. coli* isolates resistant to ciprofloxacin was 33 (2 of  
20 6) in 1997, 20 percent in 1998 (2 of 10), 30 percent in 1999 (6 of 20), 25 percent in 2000 (3 of 12),  
21 and 44 percent in 2001 (7 of 16) (1).  
22

23 Two sources of variation need to be accounted for when determining whether or not there is an  
24 increasing (or decreasing) trend in the prevalence of ciprofloxacin resistance among *Campylobacter*  
25 isolates received in NARMS from 1997 to 2001. First, the population under surveillance more than  
26 doubled from 1997 to 2001 with the addition of four FoodNet sites. Second, there is substantial site-  
27 to-site variation in the rates of ciprofloxacin resistance among *Campylobacter* isolates. To account  
28 for the potentially confounding effects of the changing population base and the site variability in  
29 ciprofloxacin resistance, we used a multivariate logistic regression to conduct a trend analysis.  
30 Furthermore, because the prevalence of ciprofloxacin resistance was high in Connecticut,  
31 particularly in 1999, we repeated the multivariate logistic regression model dropping data from  
32 Connecticut from the model, to ensure that the observed trend seen in the data was not driven by  
33 data from a single state (even though site variation was included in the multivariate model).  
34

35 In the multivariate logistic regression model, the proportion of *Campylobacter* isolates resistant to  
36 ciprofloxacin in 2001, controlling for site variation and age, was 2.5 times higher (95 percent  
37 confidence interval, 1.4 to 4.4 times higher) than the proportion of *Campylobacter* isolates resistant  
38 to ciprofloxacin in 1997 (1). The logistic regression model also demonstrated a relatively consistent  
39 increasing trend, with the proportion of *Campylobacter* isolates resistant to ciprofloxacin increasing  
40 every year compared to the previous year except in 2000. Similar results were obtained in the  
41 multivariate logistic regression model when restricting the analysis to only the *C. jejuni* isolates. In  
42 that model, the proportion of *C. jejuni* isolates resistant to ciprofloxacin in 2001 was 2.2 times  
43 higher (95 percent confidence interval, 1.2 to 4.0 times higher) than the proportion of *C. jejuni*  
44 isolates resistant to ciprofloxacin in 1997 (1). Again, a relatively consistent increasing trend was  
45 observed with the proportion of *C. jejuni* isolates resistant to ciprofloxacin increasing every year  
46 compared to the previous year except in 2000. No remarkable changes were observed in either  
47 multivariate model when the cases from Connecticut were excluded from the analysis.

1 These data demonstrate that a high proportion (approximately one-fifth) of human *Campylobacter*  
2 isolates in the United States are resistant to ciprofloxacin. Furthermore, when using a multivariate  
3 model to account for the marked regional variation and increasing population size in NARMS, the  
4 proportion of human *Campylobacter* in the United States resistant to ciprofloxacin is two and a half  
5 times higher in 2001 than it was in 1997; the trend of an increasing prevalence of ciprofloxacin  
6 resistance among human *Campylobacter* isolates is statistically significant, is relatively consistent  
7 from year-to-year, and is not due solely to an increasing prevalence observed in a single site.

8  
9 References for paragraph 10:

- 10 1. NARMS for Enteric Bacteria. Summary tables and preliminary trend analyses for 1997-  
11 2001. Draft (in CDC Clearance). (Attachment 2). Also: NARMS 1997 Annual Report  
12 (Exhibit G-97); NARMS 1998 Annual Report (Exhibit G-98); NARMS 1999 Annual  
13 Report (Exhibit G-99, pp 1-4); NARMS 2000 Annual Report (Exhibit G-749).

### 14 15 **Risk factors for acquiring campylobacteriosis**

16 11. *Campylobacter* causes a significant burden of illness in the United States. Using FoodNet data,  
17 for example, there were an estimated 1.4 million *Campylobacter* infections in the United States in  
18 1999 (1). The vast majority of *Campylobacter* infections are not related to recognized outbreaks but  
19 occur as sporadic individual infections. The sources of sporadic *Campylobacter* infections can be  
20 quite different from those of outbreaks.

21  
22 Sources in outbreaks have commonly included contaminated water and food (2). Among foodborne  
23 outbreaks, milk, usually raw, unpasteurized milk, has historically been the most common single  
24 food source. The most typical scenario for these outbreaks is a school field trip to a dairy farm  
25 where a drink of raw milk is part of the experience. Such outbreaks have been rare in recent years,  
26 perhaps due to public health warnings following such events. The broad variety of other foods  
27 implicated in campylobacteriosis outbreaks is consistent with cross-contamination events in  
28 kitchens from raw meat, poultry in particular, to a variety of other foods. Transmission from ill food  
29 handlers occurs occasionally but is not common. Unlike some other bacteria, *Campylobacter* does  
30 not tend to multiply in foods left out for many hours; indeed, it does not tolerate exposure to  
31 atmospheric oxygen or to drying. This would explain the rarity of large outbreaks related to solid  
32 foods. Typical scenarios for foodborne outbreaks involve simple cross-contamination between raw  
33 poultry and other foods in the kitchen.

34  
35 As stated above, the vast majority of *Campylobacter* infections occur as sporadic infections.  
36 Epidemiological investigations to determine risk factors for sporadic *Campylobacter* infections  
37 have been conducted several times in the United States and in other developed nations (3-10).  
38 Although these studies differed in location, technique, and sample size, they consistently indicate  
39 several dominant sources of infection, including contact with and consumption of chicken and  
40 turkey. Other identified risk factors for infections include contact with the feces of pets and other  
41 animals, consumption of raw milk, and contaminated drinking water. Despite the identification of  
42 these risk factors for infections, the incidence of campylobacteriosis remains substantial. To address  
43 this ongoing public health problem we conducted a nationwide case-control study of patients with  
44 sporadic *Campylobacter* infections in FoodNet (11).

45  
46 The largest case-control study of sporadic *Campylobacter* infections was conducted in the United  
47 States in the FoodNet sites in 1998 and 1999. The 12-month population-based case-control study  
48 was conducted in the seven FoodNet sites; Connecticut, Georgia, Minnesota, Oregon, and selected

1 counties in California, Maryland, and New York. We attempted to enroll all selected cases with a  
2 culture-confirmed *Campylobacter* infection in the surveillance sites during the 12-month study  
3 period. One age-matched well control was enrolled for each case. Cases and controls were asked  
4 about foreign travel, food and water exposures, and food handling practices in the seven days prior  
5 to illness onset of the case.  
6

7 Data from the 1998-1999 FoodNet case-control study have been used in three analyses: (a)  
8 comparison of *Campylobacter* cases and well community controls to determine the risk factors for  
9 becoming infected with *Campylobacter*; (b) comparison of ciprofloxacin-resistant *Campylobacter*  
10 cases and well community controls to determine the risk factors for becoming infected with  
11 ciprofloxacin-resistant *Campylobacter*; and (c) comparison of the medical consequences of  
12 ciprofloxacin-resistant and ciprofloxacin-susceptible *Campylobacter* cases.  
13

14 To determine risk factors for becoming infected with *Campylobacter*, 1316 *Campylobacter* cases  
15 and 1316 matched well community controls were enrolled in the study (11). Cases were 10.0 times  
16 more likely to have traveled internationally in the seven days prior to illness onset than controls (95  
17 percent confidence interval, 6.0 to 16.7); 13 percent of cases traveled outside the United States in  
18 the seven days prior to illness onset compared to 1.5 percent of controls. The population attributable  
19 fraction for foreign travel was 12 percent, suggesting that 12 percent of sporadic cases of  
20 campylobacteriosis in the United States are due to travel outside the United States.  
21

22 A multivariate logistic regression model was used to determine risk factors for acquiring a  
23 *Campylobacter* infection among persons who did not travel outside the United States. In the final  
24 multivariate model, cases were 2.2 times more likely to have eaten chicken in a restaurant in the  
25 seven days prior to illness onset than controls (95 percent confidence interval, 1.7 to 2.9); 44  
26 percent of cases ate chicken in a restaurant compared with 26 percent of controls. Cases were 2.5  
27 times more likely to have eaten turkey in a restaurant in the seven days prior to illness onset than  
28 controls (95 percent confidence interval, 1.3 to 4.7); 6 percent of cases ate turkey in a restaurant  
29 compared with 3 percent of controls. Cases were also 1.7 times more likely to have eaten non-  
30 poultry meat in a restaurant in the seven days prior to illness onset than controls (95 percent  
31 confidence interval, 1.3 to 2.2); 52 percent of cases ate non-poultry meat at a restaurant compared  
32 with 35 percent of controls. Other independent risk factors included drinking untreated surface  
33 water, drinking raw milk, eating undercooked poultry, eating raw seafood, having a pet puppy,  
34 having contact with farm animals, and having contact with animal feces.  
35

36 The largest population attributable fractions were for eating chicken in a restaurant and eating non-  
37 poultry meat in a restaurant. The population attributable fraction for eating chicken in a restaurant  
38 was 24 percent (95 percent confidence interval, 17 to 30 percent), and for eating non-poultry meat  
39 in a restaurant was 21 percent (95 percent confidence interval, 13 to 30 percent). The population  
40 attributable fraction for eating turkey in a restaurant was 4 percent (95 percent confidence interval,  
41 1 to 6 percent). The population attributable fraction suggests that, among non-travelers, 24 percent  
42 of sporadic cases of campylobacteriosis in the United States are due to eating chicken in a  
43 restaurant, 21 percent are due to eating non-poultry meat in a restaurant, and 4 percent are due to  
44 eating turkey in a restaurant in the seven days prior to illness onset.  
45

46 In summary, data from the large, recent, national case-control study of sporadic *Campylobacter*  
47 infections conducted in the FoodNet sites in 1998 and 1999, demonstrate that sporadic  
48 *Campylobacter* infections have multiple sources. Among these sources, the dominant domestic

1 source is poultry, particularly chicken, but also turkey. Other important domestic sources include  
2 non-poultry meats.

3  
4 References for paragraph 11:

- 5 1. Samuel M, Vugia DJ, Shallow S, Marcus R, Segler S, McGivern T, Kassenborg H, Reilly K,  
6 Kennedy M, Angulo F, Tauxe R, and the EIP FoodNet Working Group. Epidemiology of  
7 sporadic *Campylobacter* infection in the United States and declining trend in incidence,  
8 FoodNet 1996-1999. *Clinical Infectious Diseases* (in press).
- 9 2. Friedman CR, Neimann J, Wegener HC, Tauxe RV. Epidemiology of *Campylobacter jejuni*  
10 infections in the United States and other industrialized nations. In: *Campylobacter*. Second  
11 edition. Eds: Nachamkin I, Blaser MJ. American Society of Microbiology 2000.  
12 Washington, D.C. (Exhibit G-1644)
- 13 3. Adak GK, Cowden JM, Nicholas S, Evans HS. The Public Health Laboratory national case-  
14 control study of primary indigenous sporadic cases of campylobacter infection.  
15 *Epidemiology and Infection* 1995;115(1):15-22. (Exhibit G-10)
- 16 4. Eberhart-Phillips J, Walker N, Garrett N, et al. Campylobacteriosis in New Zealand: results  
17 of a case-control study. *Journal of Epidemiology and Community Health* 1997;51(6):686-  
18 691. (Exhibit G-182)
- 19 5. Kapperud G, Skjerve E, Bean NH, Ostroff SM, Lassen J. Risk factors for sporadic  
20 *Campylobacter* infections: results of a case-control study in southeastern Norway. *Journal of*  
21 *Clinical Microbiology* 1992;30(12):3117-3121. (Exhibit G-334)
- 22 6. Neal KR, Slack RC. Diabetes mellitus, anti-secretory drugs and other risk factors for  
23 campylobacter gastroenteritis in adults: a case-control study. *Epidemiology and Infection*  
24 1997;119(3):307-311. (Exhibit G-1686)
- 25 7. Neimann J, Engberg J, Moelbak K, Wegener HC. Foodborne risk factors associated with  
26 sporadic campylobacteriosis in Denmark. *Dansk Veterinaertidsskrift* 1998;81(19):702-705.
- 27 8. Schorr D, Schmid H, Rieder HL, Baumgartner A, Vorkauf H, Burnens A. Risk factors for  
28 *Campylobacter* enteritis in Switzerland. *Zentralblatt fur Hygiene und Umweltmedizin*  
29 1994;196(4):327-337. (Exhibit G-1718)
- 30 9. Harris NV, Weiss NS, Nolan CM. The role of poultry and meat in the etiology of  
31 *Campylobacter jejuni/coli* enteritis. *American Journal of Public Health* 1986;76(4):407-411.  
32 (Exhibit G-268)
- 33 10. Deming MS, Tauxe RV, Blake PA, et al. *Campylobacter* enteritis at a university:  
34 transmission from eating chicken and from cats. *American Journal of Epidemiology*  
35 1987;126(3):526-534. (Exhibit G-162)
- 36 11. Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J, Shiferaw B, Reddy S, Ahuja  
37 SD, Helfrick DL, Hardnett F, Carter M, Anderson B, Tauxe RV, and the FoodNet Working  
38 Group. Risk factors for sporadic *Campylobacter* infections in the United States: a case-  
39 control study in FoodNet sites. Draft (in CDC Clearance). (Attachment 3) Also: Friedman  
40 C, Reddy S, Samuel M, Marcus R, Bender J, Desai S, Shiferaw B, Helfrick D, Carter M,  
41 Anderson B, Hoekstra M, and the EIP Working Group. Risk factors for sporadic  
42 *Campylobacter* infections in the United States: a case-control study on FoodNet sites. 2nd  
43 International Conference on Emerging Infectious Diseases. Atlanta, GA, July 2000.  
44 (Exhibit G-228)

45  
46 **Prevalence of fluoroquinolone-resistant *Campylobacter* isolated from retail poultry**

47 12. Three state health departments (Georgia, Maryland, and Minnesota) participated in a FoodNet  
48 microbiologic survey of chickens in grocery stores (1). Between January and June 1999, each

1 participating state health department purchased ten whole broiler chickens each month from  
2 supermarkets located within the state. The public health department laboratories at each site tested  
3 the chicken samples for *Campylobacter*. Carcass rinse samples were centrifuged and pellets were  
4 incubated in enrichment broth and plated onto *Campylobacter* blood agar plates. If available, one  
5 isolate from each carcass rinse was forwarded to the CDC for species identification and  
6 antimicrobial susceptibility testing. Upon receipt at the CDC, isolates were tested using methods  
7 described above in paragraph 10.  
8

9 Of the 180 retail chicken products purchased, representing multiple domestic brand names from  
10 over 20 grocery stores, *Campylobacter* was isolated from 80 (44 percent) of the samples (1).  
11 Among the 80 *Campylobacter* isolates, 62 (78 percent) were *C. jejuni*, 16 (20 percent) were  
12 *Campylobacter coli*, and 2 were an unknown species (CDC unpublished data). The prevalence of  
13 *Campylobacter* isolated from retail chicken products varied by site; the prevalence of  
14 *Campylobacter* was 33 percent in Georgia, 37 percent in Maryland, and 63 percent in Minnesota  
15 (1). This difference among the three sites in the isolation of *Campylobacter* was due to the  
16 difference in isolation of *C. coli*; 14 (88 percent) of the 16 *C. coli* isolates came from retail chicken  
17 purchased in Minnesota (CDC unpublished data).  
18

19 Among the 62 *C. jejuni* isolates, 15 (24 percent) were resistant to ciprofloxacin (CDC unpublished  
20 data). Among 16 *C. coli* isolates, 3 (19 percent) were resistant to ciprofloxacin (CDC unpublished  
21 data). *Campylobacter* isolates from chickens exhibited two distinct populations with respect to their  
22 minimum inhibitory concentrations to ciprofloxacin. Similar to *Campylobacter* isolates obtained  
23 from humans, isolates from chickens predominantly had either a minimum inhibitory concentration  
24 of 0.5 or less micrograms per milliliter (susceptible isolates), or a minimum inhibitory concentration  
25 of 32 or more micrograms per milliliter (resistant isolates), with very few intermediate phenotypes.  
26 A ciprofloxacin-resistant strain of *Campylobacter* was identified in 11 percent of 180 retail chicken  
27 products tested (1), demonstrating the frequent contamination of chicken with ciprofloxacin-  
28 resistant *Campylobacter*.  
29

30 References for paragraph 12:

- 31 1. Rossiter S, Joyce K, Ray M, Benson J, Mackinson C, Gregg C, Sullivan M, Vought K,  
32 Leano F, Besser J, Marano N, Angulo F. High prevalence of antimicrobial-resistant,  
33 including fluoroquinolone-resistant, *Campylobacter* on chicken in US grocery stores.  
34 American Society for Microbiology 2000 General Meeting. (Exhibit G-1528)  
35

### 36 **Contribution of food handling practices to campylobacteriosis**

37 13. Several factors, in addition to the high prevalence of *Campylobacter* on chickens and turkeys  
38 after processing, contribute to the high number of human campylobacteriosis cases that occur each  
39 year in the United States. The high frequency that chickens and turkeys are handled by food  
40 handlers and consumers, for example, contribute to the number of infections. Over 8 billion  
41 chickens and turkeys are slaughtered and processed each year in the United States. Many of these  
42 chickens and turkeys are sold to restaurants and thereby handled by food handlers in restaurant  
43 kitchens during preparation. Many of these chickens and turkeys are sold in grocery stores and  
44 thereby handled by consumers in kitchens during preparation and cooking. Since chickens and  
45 turkeys sold in grocery stores are frequently contaminated with *Campylobacter*, chicken or turkey  
46 contaminated with *Campylobacter* are commonly brought into consumer's kitchens in the United  
47 States. Once in a consumer's kitchen, the *Campylobacter* on the chicken or turkey can easily  
48 contaminate other foods through routine kitchen activities. Consumers can reduce, but not

eliminate, the frequency of the occurrence of cross-contamination in kitchens by careful washing and disinfecting of hands and surfaces after handling uncooked chicken and turkey.

Although a high proportion of consumers report washing their hands after handling raw chicken or turkey, it is not a universal practice. The CDC conducted a telephone survey of the almost 7,500 adults in five FoodNet sites (California, Connecticut, Georgia, Minnesota, and Oregon) in 1996 and 1997 (1). Respondents were selected and interviewed by telephone using methods similar to those used in the Behavioral Risk Factor Surveillance System (2). Ninety-three percent of respondents said they almost always washed their hands after handling raw poultry. Young adults compared to older adults were less likely to wash their hands after handling raw poultry (88 percent versus 95 percent), and men washed their hands less often than women (89 percent versus 97 percent). Although there were differences between demographic groups in the frequency of this important prevention practice, there were insufficient differences between groups to warrant targeted education programs; instead, all groups need to be frequently reminded of the importance of appropriate washing and disinfection of hands and surfaces to reduce the likelihood of cross-contamination by *Campylobacter*-contaminated chicken and turkey.

The higher frequency of riskier food handling practices by young men probably contributes to the higher incidence of *Campylobacter* infections observed among men compared with women in FoodNet (3). This finding was also observed in a case-control study of sporadic infections; a higher risk of campylobacteriosis was observed among young men compared with young women in college apparently in part due to poor cooking and handling practices (4).

Since risky (i.e., not washing hands after handling raw poultry) food handling practices increase the risk of acquiring a *Campylobacter* infection, reducing the frequency of risky food handling practices will reduce the incidence of *Campylobacter* infections. However, given the high prevalence of *Campylobacter* contamination of chickens and turkeys in grocery stores, and the high frequency that chickens and turkeys are purchased from stores and handled by consumers, it is likely that the incidence of *Campylobacter* infections in people would remain high even if all risky food handling practices in the United States were eliminated.

#### References for paragraph 13:

1. Shiferaw B, Yang S, Cieslak P, Vugia D, Marcus R, Koehler J, Deneen V, Angulo F, and the FoodNet Working Group. Prevalence of high-risk food consumption and food-handling practices among adults: a multistate survey, 1996 to 1997. *Journal of Food Protection* 2000;63(11):1538-1543.
2. Remington RL, Smith MY, Williamson DF, Anda RF, Gentry EM, Hogelin GC. Design, characteristics, and usefulness of state-based behavioral risk factor surveillance, 1981-1987. *Public Health Reports* 1988;103:366-375.
3. Centers for Disease Control and Prevention. FoodNet Surveillance Report for 2000 (Final Report). Available at the FoodNet website at <http://www.cdc.gov/foodnet>.
4. Deming MS, Tauxe RV, Blake PA, et al. Campylobacter enteritis at a university: transmission from eating chicken and from cats. *American Journal of Epidemiology* 1987;126(3):526-534. (Exhibit G-162)

1 **Levels of fluoroquinolone resistance in *Campylobacter* isolates from humans prior to the**  
2 **approval of fluoroquinolones for use in poultry**

3 14. Before the National Antimicrobial Resistant Monitoring System (NARMS) was established,  
4 the CDC monitored antimicrobial resistance among foodborne enteric bacteria from humans using  
5 periodic surveys of isolates from a panel of sentinel counties in the United States. In 1989 and 1990,  
6 the CDC conducted a twelve-month *Campylobacter* sentinel county study. Detailed methods of this  
7 study are described elsewhere (1). In brief, isolates of the first five sporadic *Campylobacter*  
8 infections identified each month of the study period in 19 counties were identified by biochemical  
9 tests by the submitting laboratory and forwarded to the CDC for confirmation and further testing.  
10

11 At the CDC, the isolates were confirmed by dark-field examination and hippurate hydrolysis.  
12 Isolates were also tested for susceptibility to azithromycin, chloramphenicol, ciprofloxacin,  
13 clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline using broth microdilution  
14 methods. Ciprofloxacin resistance was defined as a minimum inhibitory concentration greater than  
15 or equal to four micrograms per milliliter. A preliminary summary of these results was published in  
16 1992 (2). Of note, in this preliminary report, it was reported that one of the 332 isolates tested was  
17 ciprofloxacin-resistant. Subsequent testing revealed, however, that this isolate was misclassified and  
18 is actually a *Campylobacter lari* isolate (CDC unpublished data). *Campylobacter lari* isolates are  
19 inherently resistant to ciprofloxacin; therefore, this isolate was deleted from the analysis of  
20 ciprofloxacin resistance.  
21

22 In the 1989-1990 sentinel county study, interviews were conducted with 313 patients from whom  
23 specimens had yielded *Campylobacter* isolates and whose isolates were received at the CDC for  
24 susceptibility testing (CDC unpublished data). Eight percent (24 of 313) patients traveled outside  
25 the United States in the seven days prior to their illness onset. None of the 313 isolates was  
26 ciprofloxacin-resistant (CDC unpublished data). Since most of these infections were acquired  
27 domestically, these data suggest that the prevalence of ciprofloxacin resistance among  
28 *Campylobacter* isolates was, at most, very low in the United States in 1989 and 1990. Furthermore,  
29 although eight percent of patients in this study traveled internationally in the seven days prior to  
30 illness onset, none of these patients had a ciprofloxacin-resistant infection. Taken together, these  
31 data indicate that the prevalence of ciprofloxacin resistance among *Campylobacter* in the United  
32 States and abroad was, at most, very low in 1989 and 1990. These findings are particularly  
33 noteworthy because this study was conducted four years after ciprofloxacin was first approved for  
34 use in humans in the United States, indicating that human use of ciprofloxacin provides little  
35 selective pressure for the emergence of ciprofloxacin resistance among *Campylobacter* isolates in  
36 the United States.  
37

38 **References for paragraph 14:**

- 39 1. Patton CM, Nicholson MA, Ostroff SM, Ries AA, Wachsmuth IK, Tauxe RV. Common  
40 somatic O and heat-labile serotypes among *Campylobacter* strains from sporadic infections  
41 in the United States. *Journal of Clinical Microbiology* 1993;31(6):1525-1530. (Exhibit B-  
42 589)
- 43 2. Tenover FC, Baker CN, Fennell CL, Ryan CA. Antimicrobial resistance in *Campylobacter*  
44 species. In: Nachamkin I, Blaser MJ, Tompkins LS, editors. *Campylobacter jejuni* current  
45 status and future trends. Washington D.C.:American Society of Microbiology; 1992. p.66-  
46 73. (Exhibit G-624)

1 **Effect of fluoroquinolone resistance on the duration of diarrhea in *Campylobacter* infections**

2 15. The impact of antibiotics, including fluoroquinolones, on the duration of diarrhea caused by  
3 *Campylobacter* was demonstrated in the 1989-1990 *Campylobacter* sentinel county study  
4 conducted by the CDC (1). In that study, a questionnaire was administered to patients with culture-  
5 confirmed *Campylobacter* infections in 19 sentinel counties. In the 460 interviewed patients, early  
6 antimicrobial treatment reduced illness duration. Initiation of antimicrobial therapy (predominately  
7 ciprofloxacin (CDC unpublished data)) within three days of symptom onset reduced the duration of  
8 diarrhea from a mean of nine days to six days ( $p<0.02$ ); initiation of antimicrobial therapy within  
9 five days of symptom onset reduced duration of diarrhea from a mean of nine days to seven days  
10 ( $p<0.02$ ).

11  
12 Additional information on the medical consequences of fluoroquinolone resistance is available from  
13 the 1998-1999 FoodNet *Campylobacter* case-control study (2). During the study, 858 patients with  
14 culture-confirmed *Campylobacter* infections whose isolates had been susceptibility tested, were  
15 asked about their medical treatment. Eighty-three percent of persons with culture-confirmed  
16 *Campylobacter* infections took an antimicrobial agent for their illness; of these, 53 percent took  
17 fluoroquinolones. The 1998-1999 FoodNet *Campylobacter* analyses discussed in this section are  
18 preliminary; the manuscript describing these findings is currently undergoing the review process  
19 involved with CDC clearance.

20  
21 Persons who still had diarrhea at the time of interview, persons who were unable to give an  
22 estimated duration of diarrhea, and persons who reported not having diarrhea, were excluded,  
23 leaving 740 persons for the analysis. The mean duration of diarrhea for the 740 patients was 7 days  
(range, 1 to 60 days).

24  
25 Of the 740 persons, 82 (11 percent) had a ciprofloxacin-resistant *Campylobacter* infection. The  
26 mean duration of diarrhea was 8 days (range, 2 to 21 days) for the 82 patients with ciprofloxacin-  
27 resistant *Campylobacter* infections and 7 days (range, 1 to 60 days) for the 658 patients with  
28 ciprofloxacin-susceptible *Campylobacter* infections ( $p=0.1$ ).

29  
30  
31 Of the 740 persons, 421 (57 percent) did not take antidiarrheal medication (loperamide,  
32 diphenoxylate, or a prescribed antidiarrheal medication) for their illness. The mean duration of  
33 diarrhea among the 421 persons who did not take these antidiarrheal medications was 9 days (range,  
34 2 to 21 days) for the 39 patients with ciprofloxacin-resistant *Campylobacter* infections and 7 days  
35 (range, 2 to 60 days) for the 382 patients with ciprofloxacin-susceptible *Campylobacter* infections  
36 ( $p=0.05$ ). Of the 421 persons who did not take an antidiarrheal medication, 67 (16 percent) reported  
37 not taking an antimicrobial agent for their illness. The mean duration of diarrhea among persons  
38 who did not take an antidiarrheal medication or an antimicrobial agent was 12 days (range, 8 to 20  
39 days) for the 6 persons with ciprofloxacin-resistant infections and 6 days (range, 2 to 21 days) for  
40 the 61 persons with ciprofloxacin-susceptible infections ( $p<0.01$ ).

41  
42 Of the 740 persons, 128 (17 percent) took fluoroquinolones and no other antimicrobial agent or  
43 antidiarrheal medication for their illness. The mean duration of diarrhea among persons who took  
44 fluoroquinolones and no other antimicrobial agent or antidiarrheal medication was 8 days (range, 3  
45 to 14 days) for the 17 patients with ciprofloxacin-resistant infections and 6 days (range, 2 to 31  
46 days) for the 111 patients with ciprofloxacin-susceptible infections ( $p=0.08$ ).

1 A multivariate analysis of variance (ANOVA) model was used to analyze factors that were  
2 potentially associated with duration of diarrhea for the persons with *Campylobacter* infections and  
3 susceptibility results. Factors included were antimicrobial agent use; loperamide, diphenoxylate, or  
4 prescribed antidiarrheal medication use; having an underlying condition; and age. Controlling for  
5 these factors, the mean duration of diarrhea was 9 days in persons with ciprofloxacin-resistant  
6 *Campylobacter* infections compared to a mean duration of diarrhea of 8 days in persons with  
7 ciprofloxacin-susceptible *Campylobacter* infections ( $p=0.05$ ).  
8

9 Taken together, the data from the sentinel county study (1989-1990) and the data from the FoodNet  
10 *Campylobacter* case-control study (1998-1999) indicate that: (a) persons infected with  
11 *Campylobacter* are commonly treated with fluoroquinolones; (b) antibiotics, including  
12 fluoroquinolones, shorten the duration of diarrhea caused by *Campylobacter*, particularly when  
13 given early in the infection; and (c) among persons treated with fluoroquinolones, persons with  
14 ciprofloxacin-resistant *Campylobacter* infections had a longer duration of diarrhea than persons  
15 with ciprofloxacin-susceptible *Campylobacter* infections. It appears likely that fluoroquinolones are  
16 less efficacious against ciprofloxacin-resistant *Campylobacter*, thus prolonging the diarrheal illness.  
17 Similar findings have been described in two other studies. A study by Smith et al. in Minnesota  
18 found that, among patients treated with a fluoroquinolone after the collection of a stool specimen,  
19 those with fluoroquinolone-resistant *Campylobacter* infections had a three-day longer median  
20 duration of diarrhea than those with fluoroquinolone-susceptible infections (3). A study by Neimann  
21 et al. in Denmark found that persons with ciprofloxacin-resistant *Campylobacter* infections who  
22 were treated with fluoroquinolones had a five-day longer median duration of diarrhea (personal  
23 communication with Kare Molbak).

24 More studies are needed to determine why ciprofloxacin-resistant *Campylobacter* infections might  
25 result in a longer duration of diarrhea than ciprofloxacin-susceptible infections. One possible  
26 explanation is that ciprofloxacin-resistant *Campylobacter* have some intrinsic factor or factors  
27 which make them more virulent than susceptible isolates. More severe illnesses resulting from  
28 infections with fluoroquinolone-resistant bacteria have also been seen with *Salmonella*, another  
29 foodborne pathogen. A recent study by Varma et al. found that infection with antimicrobial-resistant  
30 *Salmonella*, including fluoroquinolone-resistant *Salmonella*, was associated with an increased rate  
31 of hospitalization (4). Also, in a recent Danish study, more severe illnesses were reported in patients  
32 with fluoroquinolone-resistant infections; persons with fluoroquinolone-resistant *Salmonella*  
33 infections were more likely to die in the two years following their infection than persons infected  
34 with susceptible *Salmonella* infections (5). Again, the reasons for these differences are not known;  
35 it is possible that fluoroquinolone-resistant strains are more virulent than fluoroquinolone-  
36 susceptible strains.  
37

38  
39 References for paragraph 15:

- 40 1. Sobel J, Tauxe R, Ries A, Patton C, Maloney K. The burden of *Campylobacter jejuni*  
41 infections: a target for early treatment. Centers for Disease Control and Prevention. 45<sup>th</sup>  
42 Annual Epidemic Intelligence Service Conference. 1996. (Exhibit G-592)
- 43 2. Nelson JM, Marano N, Joyce K, Vugia DJ, Rabatsky-Ehr T, Segler S, Kassenborg H,  
44 Zansky S, Hoekstra M, Smith K, Angulo FJ. Prolonged diarrhea due to ciprofloxacin-  
45 resistant *Campylobacter* infections. Draft (in CDC Clearance). (Attachment 4)
- 46 3. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni*  
47 infections in Minnesota, 1992-1998. *New England Journal of Medicine* 1999;340:1525-  
48 1532. (Exhibit G-589)

4. Varma JK, Molbak K, Rossiter S, et al. Antimicrobial resistance in *Salmonella* is associated with increased hospitalization – NARMS and FoodNet, 1996-2000. Available at the NARMS web site at <http://www.cdc.gov/narms>.
5. Helms M, Vastrup P, Gerner-Smidt P, et al. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerging Infectious Diseases* 2002;8(5):490-495. (Exhibit G-1758)

### Conclusions

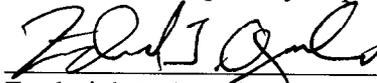
16. Ciprofloxacin-resistant *Campylobacter* presents a substantial burden of infection in the U.S. population. An estimated 1.4 million persons in the U.S. in 1999 were infected with *Campylobacter*, and resistance to ciprofloxacin in human *Campylobacter* isolates in 2001 was estimated at nearly 20 percent. In some clinical laboratories in the United States, the prevalence of fluoroquinolone resistance is remarkably higher than that reported by NARMS; for example, the University of Pennsylvania recently reported that 40 percent of *Campylobacter jejuni* isolates from ill humans submitted from several Philadelphia-area hospitals were fluoroquinolone-resistant in 2001 (1).

Ciprofloxacin-resistant *Campylobacter* infection in the U.S. population is increasing; resistance to ciprofloxacin in human *Campylobacter* isolates was two and a half times higher in 2001 than it was in 1997. Compared to persons with a ciprofloxacin-susceptible *Campylobacter* infection, persons with a ciprofloxacin-resistant *Campylobacter* infection are likely to have diarrhea for a longer duration, including in persons who have been treated with fluoroquinolones, which are commonly used to treat *Campylobacter* infections. A dominant source of *Campylobacter* infections in the U.S. population is poultry, particularly chicken, which is frequently contaminated with ciprofloxacin-resistant *Campylobacter*. Prior to approval in the U.S. of fluoroquinolones for use in poultry, the prevalence of ciprofloxacin resistance in the U.S. among human *Campylobacter* isolates appears to have been very low.

### References for paragraph 16:

1. Nachamkin I, Ung H, Li M. Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA, 1982-2001. *Emerg Infect Dis* 2002 Dec. Available at <http://www.cdc.gov/ncidod/EID/vol8no12/02-0115.htm>. (Attachment 5). Also: Nachamkin I, Ung H, Li M. Increasing fluoroquinolone resistance in *Campylobacter jejuni* in Philadelphia, 1982-2001. Accepted for publication, *Emerg Infect Dis*. (Exhibit G-1517)

I declare under penalty of perjury that the foregoing is true and correct.

  
\_\_\_\_\_  
Frederick J. Angulo

12-6-02  
\_\_\_\_\_  
Date