UNITED STATES OF AMERICA
BEFORE THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
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In the Matter of:	FDA DOCKET: 00N-1571
	DATE: March 17, 2003
Enrofloxacin for Poultry: Withdrawal	
of Approval of Bayer Corporation's	
New Animal Drug Application	
(NADA) 140-828 (Baytril)	

Center for Veterinary Medicine's Submission of Proposed Findings of Fact

The Center for Veterinary Medicine respectfully submits the following proposed findings

of fact with record references:

Frank Aarestrup (G-1451)

1. Dr. Aarestrup is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

2. The first antimicrobial agents were introduced in the 1930s and a number of new compounds were discovered in the following decades. However, shortly after their introduction, bacteria began to show resistance. Since then, resistance mechanisms have been identified in bacteria for all known antimicrobial agents. This includes both natural and synthetic compounds. In addition, bacteria frequently acquire several mechanisms for resisting drugs, making them highly resistant to antimicrobial therapy. Aarestrup WDT: p. 1, lines 45-49 and p. 2, lines 1-2

3. Antimicrobial agents have saved millions of lives and are the most important weapon against infectious diseases. The greatest threat against the use of antimicrobial agents is the development of resistance in pathogenic bacteria. In general the occurrence of resistance follows the consumption of antimicrobial agents closely. Aarestrup WDT: p. 2, lines 4-7

4. *Campylobacter* followed by *Salmonella* are the most common causes of bacterial gastro-intestinal infections in man worldwide. Aarestrup WDT: p. 2, lines 9-10

5. Emergence of resistance in *Salmonella* and *Campylobacter* would have consequences for the possibilities to treat infections in man. Aarestrup WDT: p. 2, lines 10-11

6. Fluoroquinolones are the drug of choice for treatment of gastro-intestinal infections in humans in most countries. Thus, resistance to this group of antimicrobial agents would have the most severe consequences. Aarestrup WDT: p. 2, lines 12-14; p. 4, lines 5-6

7. Evolving resistant bacterial population does not respect traditional boundaries between countries. People travel and food of animal origin is traded worldwide. Thus, the development of resistance in any country is an impending problem for all countries. Aarestrup WDT: p. 2, lines 14-17

8. Antibiotics are used for the treatment of infectious diseases caused by bacteria. To be effective, an antibiotic should show activity against the infecting bacteria and have the ability to reach the infected organ or tissue in sufficiently high concentrations to stop the infection. Aarestrup WDT: p. 3, lines 3-5

9. Fluoroquinolones have activity against a wide range of different organisms and have very good distribution in the body. There is a good chance that this antibiotic will have a beneficial effect on almost all infections in all different organs/tissues. It is therefore a very easy antibiotic to use even in the absence of a proper diagnosis or accurate identification of the infectious agent. Aarestrup WDT: p. 3, lines 9-13

10. The first fluoroquinolones were introduced in human medicine in Europe in 1984 and in 1985 in USA. Since that time there has been an increasing use of these antimicrobial agents especially in hospitals. Aarestrup WDT: p. 4, lines 6-14; G-6

11. The introduction of fluoroquinolones in veterinary medicine was followed by an emergence and increase in resistance among bacteria in food animals, including zoonotic bacteria such as *Campylobacter* and *Salmonella.* Aarestrup WDT: p. 4, lines 6-14; G-6

12. In several countries the introduction of fluoroquinolones for veterinary use was followed by an emergence among resistance in bacteria in food animals, including *Campylobacter.* Aarestrup, WDT: p. 4, lines 11-14 and 25; G-191

13. The introduction of fluoroquinolones for veterinary use has been the driving force behind the emergence of fluoroquinolone-resistant *Campylobacter* giving infections in man. It can be observed that resistance emerged first in the countries that first approved fluoroquinolones for veterinary use. Aarestrup, WDT: p. 4, lines 31-32; G-191

14. Dr. Aarestrup's Figure 1, depicts trends in fluoroquinolone resistance among *Campylobacter* isolated from humans in 10 countries as reported in several studies. The figure shows that

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resistance for these countries emerged after approval of fluoroquinolones for veterinary use in that country. Aarestrup WDT: p. 5, Figure 1; G-191

15. The fluoroquinolones have also become widely used agents in veterinary medicine. In the Netherlands water medication with the fluoroquinolone enrofloxacin in poultry production was followed by an emergence of fluoroquinolone-resistant *Campylobacter* species among both poultry and humans. Aarestrup WDT: p. 4, lines 17-20

16. In Spain, an increase in the occurrence of fluoroquinolone-resistant *Campylobacter* infecting humans has been observed after the introduction of fluoroquinolones into veterinary medicine. More than half of the *Campylobacter* isolates from human infections were reported to be resistant two years after fluoroquinolones were licensed for animals compared to none before licensing. Aarestrup WDT: p. 4, lines 20-25

17. There is compelling evidence that the introduction of fluoroquinolones in veterinary medicine has lead to the emergence and increase in resistance among all different bacterial groups colonizing animals. This includes the zoonotic bacteria *Campylobacter* and *Salmonella*. Aarestrup WDT: p. 6, lines 3-6

18. A decreased usage of antimicrobial agents in food animals will lead to a decrease in resistance in the bacteria they carry to slaughter. Aarestrup WDT: p. 8, lines 4-5

19. Fluoroquinolones inhibit the activity of a bacterial enzyme called DNA gyrase. Aarestrup WDT: p. 8, line 8

20. In Campylobacter (unlike other enteric pathogens) full resistance to fluoroquinolones can be obtained by a single mutation in the gyrA gene, making it very easy for these bacteria to acquire resistance. Aarestrup WDT: p. 8, lines 17-20

21. Resistance to fluoroquinolones in *Campylobacter* is most often mediated by a single point mutation, indicating that resistance in this bacterium arises much more rapidly than in other bacteria such as *Salmonella* and *E. coli*. Aarestrup WDT: p. 8, lines 26-28

22. The use of fluoroquinolones will select for resistance in all bacteria living in animals, including bacteria capable of transferring to and causing infections in humans, such as *Campylobacter* and *Salmonella*. Aarestrup WDT: p. 9, lines 24-26

23. Quinolone resistance has emerged in *Campylobacter* and *Salmonella* causing infections in man as a consequence of the introduction of fluoroquinolones for food animals. Aarestrup WDT: p. 9, lines 28-30

24. A more limited usage of fluoroquinolones will lead to a decrease in resistance. Aarestrup WDT: p. 9, line 32

25. Fluoroquinolones are convenient drugs to use in veterinary medicine, but they are rarely important and never essential. Aarestrup WDT: p. 9, lines 34-35

Frederick Angulo (G-1452)

26. Dr. Angulo is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

27. Foodborne infections are an important public health challenge. Angulo WDT: p. 2, line 7 and 42-43; G-410.

28. The CDC estimates that foodborne infections cause 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year. Angulo WDT: p. 2, line 42-43; G-410.

29. *Campylobacter* causes a significant burden of illness in the population of the United States. Angulo WDT: p. 7, line 5-8 and 10-14; p. 9, line 16; G-410; G-1452, Attachment 1.

30. Despite a decline in incidence, *Campylobacter* continues to present a significant burden of infection in the U.S. population. Angulo WDT: p. 5, line 21-23; p. 7, line 5-8 and 10-14; p. 9, line 16; G-410; G-1452, Attachment 1.

31. Many cases of foodborne diseases are not reported. Angulo WDT: p. 6, line 17-18; G-410.

32. A large number of cases of campylobacteriosis are not reported to public health officials and therefore are not detected through routine public health surveillance. Angulo WDT: p. 6, line 22-45; G-410.

33. The number of laboratory-diagnosed *Campylobacter* cases reported to public health officials represents but a fraction of the many *Campylobacter* infections that occur in the United States. Angulo WDT: p. 6, line 22 through p. 7, line 2.

34. In 1999, the CDC estimated the degree of underreporting of *Campylobacter* to be approximately 38-fold. Angulo WDT: p. 7, line 4-5; G-410.

35. Using FoodNet surveillance data from 1996-1997, and correcting for underreporting, researchers estimated that *Campylobacter* causes 2.4 million infections, 13,000 hospitalizations, and 124 deaths a year in the United States, where the frequency of foodborne transmission of *Campylobacter* was estimated to be 80 percent. Angulo WDT: p. 7, line 5-8; G-410.

36. Using *Campylobacter* incidence in 1999 from FoodNet surveillance data and a simulation procedure developed by FDA in a *Campylobacter* risk assessment, CDC estimated that in 1999 *Campylobacter* infected an estimated 1.4 million persons. Angulo WDT: p. 7, line 10-14; G-1452, Attachment 1.

37. FoodNet is a collaborative project among the CDC, state health departments, the United States Department of Agriculture Food Safety and Inspection Service, and the United States Food and Drug Administration. Angulo WDT: p. 2, line 16-19.

38. In 2001, FoodNet conducted population-based active surveillance for clinical laboratory isolations of *Campylobacter, Cryptosporidium, Cyclospora*, Shiga-toxin producing *Escherichia coli* including *E. coli* O157:H7, *Listeria, Salmonella, Shigella, Vibrio*, and *Yersina* infections in Connecticut, Georgia, Maryland, Minnesota, and Oregon, and selected counties in California, Colorado, New York, and Tennessee. Angulo WDT: p. 2, line 34-38; G-1791.

39. In 2001, the total population in the area under FoodNet surveillance was greater than 37 million persons, which was greater than 13 percent of the population of the United States. Angulo WDT: p. 2, line 38-39; G-1791.

40. The populations in the FoodNet surveillance area and the United States had similar age and gender distributions. Angulo WDT: p. 4, line 7-19.

41. FoodNet surveillance data are generalizable to the United States population for the purpose of understanding the epidemiology of foodborne illness. Angulo WDT: p. 4, line 21-26; G-769.

42. The estimated incidence of laboratory-confirmed *Campylobacter* infections per 100,000 population in sites participating in FoodNet surveillance was 23.5 in 1996, 24.7 in 1997, 19.4 in 1998, 15.0 in 1999, and 15.4 in 2000. Angulo WDT: p. 4, line 44-46 and p.5, line 1-3; G-102; G-93; G-94; G-1791.

43. Preliminary data for 2001 ascertained 4,740 laboratory-confirmed *Campylobacter* infections, which correlate to an incidence of 13.8 laboratory-confirmed infections per 100,000 population in sites participating in FoodNet surveillance. Angulo WDT: p. 5, line 6-9; G-1791.

44. A log-linear Poisson regression model was used to estimate the effect of time on the incidence of *Campylobacter*, treating time (calendar year) as a categorical variable, with 1996 as the reference year to account for a near doubling between 1996 and 2001 in the number of sites and population under FoodNet surveillance and the variation in incidence among sites; in this model, the incidence of *Campylobacter* declined by 27 percent (95% CI: 19%, 35%) between 1996 and 2001. Angulo WDT: p. 5, line 15-21; G-1791.

45. A review of the epidemiology of *Campylobacter* infections using FoodNet surveillance data from 1996-1999 showed that ten percent of persons with laboratory-confirmed *Campylobacter* infections were hospitalized, with the highest hospitalization rate (27 percent) among persons 60 years of age or older. Angulo WDT: p. 5, line 25-33; G-1452, Attachment 1; G-555.

46. A review of the epidemiology of *Campylobacter* infections using FoodNet surveillance data from 1996-1999 showed that one person in every 3,000 persons with a laboratory-confirmed *Campylobacter* infection died. Angulo WDT: p. 5, line 25-33; G-1452, Attachment 1; G-555.

47. The vast majority of *Campylobacter* infections are not related to recognized outbreaks but occur as sporadic individual infections. Angulo WDT: p. 9, line 18-19.

48. *Campylobacter* does not tend to multiply in foods left out for many hours unlike some other bacteria; indeed, it does not tolerate exposure to atmospheric oxygen or to drying. Angulo WDT: p. 9, line 29-31.

49. Epidemiological investigations have been conducted in the United States and in other developed nations to determine risk factors for sporadic *Campylobacter* infections. Although these studies differed in location, technique, and sample size, they consistently indicate several dominant sources of infection, including contact with and consumption of chicken and turkey. Angulo WDT: p. 9, line 36-40; G-268; G-162; G-334; G-1718; G-10; G-182; G-1686.

50. Data from the 1998-1999 FoodNet *Campylobacter* case-control study on risk factors demonstrate that the dominant domestic source of *Campylobacter* infections in humans is poultry, particularly chicken but also turkey. Angulo WDT: p. 10, line 22 through p. 11, line 1; G-1452, Attachment 3; G-228.

51. The 1998-1999 FoodNet *Campylobacter* case-control study on risk factors was population-based and conducted in seven FoodNet sites --Connecticut, Georgia, Minnesota, Oregon, and selected counties in California, Maryland, and New York. Angulo WDT: p. 9, line 46 through p. 10, line 1; G-1452, Attachment 3; G-228.

52. The 1998-1999 FoodNet *Campylobacter* case-control study on risk factors determined that the largest population attributable fractions for *Campylobacter* infections were for eating chicken in a restaurant and eating non-poultry meat in a restaurant. Angulo WDT: p. 10, line 36-44.

53. The 1998-1999 FoodNet *Campylobacter* case-control study on risk factors used the following methods: (a) all selected cases with a culture-confirmed *Campylobacter* infection in the surveillance sites during the study period were attempted to be enrolled; (b) one age-matched well control was enrolled for each case; (c) 1316 *Campylobacter* cases and 1316 matched well community controls were enrolled; and (d) cases and controls were asked about foreign travel, food and water exposures, and food handling practices in the seven days prior to illness onset of the case. Angulo WDT: p. 10, line 1-5, 14-15; G-1452, Attachment 3.

54. In the 1998-1999 FoodNet *Campylobacter* case-control study on risk factors: (a) cases were 10.0 times more likely (95% CI: 6.0, 16.7) to have traveled internationally in the seven days prior to illness onset than controls (13% of cases traveled outside the United States in the seven days prior to illness onset compared with 1.5% of controls); and (b) the population attributable fraction for foreign travel was 12 percent, suggesting that 12 percent of sporadic cases of campylobacteriosis in the United States are due to travel outside the United States. Angulo WDT: p. 10, line 14-20; G-1452, Attachment 3.

55. In the final multivariate logistic regression model used to determine risk factors for acquiring a *Campylobacter* infection among persons who did not travel outside the United States, the 1998-1999 FoodNet *Campylobacter* case-control study found that: (a) cases were 2.2 times more likely (95% CI: 1.7, 2.9) to have eaten chicken in a restaurant in the seven days prior to illness onset than controls (44% of cases ate chicken in a restaurant compared with 26% of controls); (b) cases were 2.5 times more likely (95% CI: 1.3, 4.7) to have eaten turkey in a restaurant in the seven days prior to illness onset than controls (6% of cases ate turkey in a restaurant compared with 3% of controls); and (c) cases were 1.7 times more likely (95% CI: 1.3, 2.2) to have eaten non-poultry meat in a restaurant in the seven days prior to illness onset than controls (52% of cases ate non-poultry meat in a restaurant compared with 35% of controls). Angulo WDT: p. 10, line 22-32; G-1452, Attachment 3.

56. In the 1998-1999 FoodNet *Campylobacter* case-control study on risk factors, the population attributable fraction for eating: (a) chicken in a restaurant was 24 percent (95% CI: 17%, 30%); (b) non-poultry meat in a restaurant was 21 percent (95% CI: 13%, 30%); and (c) turkey in a restaurant was 4 percent (95% CI: 1%, 6%). Angulo WDT: p. 10, line 36-41; G-1452, Attachment 3.

57. The population attributable fraction determined in the 1998-1999 FoodNet *Campylobacter* case-control study on risk factors suggests that, among persons who did not travel outside the United States, 24 percent of sporadic cases of campylobacteriosis in the United States are due to eating chicken in a restaurant, 21 percent are due to eating non-poultry meat in a restaurant, and 4 percent are due to eating turkey in a restaurant in the seven days prior to illness onset. Angulo WDT: p. 10, line 36-44; G-1452, Attachment 3.

58. Several factors in addition to the high prevalence of *Campylobacter* on chickens and turkeys after processing contribute to the high number of human campylobacteriosis cases that occur each year in the United States. The high frequency that *Campylobacter*-contaminated chickens and turkeys are handled by food handlers and consumers contribute to the number of *Campylobacter* infections. Chickens and turkeys sold to restaurants are frequently contaminated with *Campylobacter* and are thereby handled by food handlers in restaurant kitchens during preparation. Chickens and turkeys sold in grocery stores are frequently contaminated with *Campylobacter* and therefore chicken or turkey contaminated with *Campylobacter* are commonly brought into consumer's kitchens in the United States. Once in a consumer's kitchen, *Campylobacter* on the chicken or turkey can easily contaminate other foods through routine kitchen activities. Angulo WDT: p. 12, line 37-48.

59. Consumers can reduce, but not eliminate, the frequency of the occurrence of *Campylobacter* cross-contamination in kitchens by careful washing and disinfecting of hands and surfaces after handling uncooked chicken and turkey. Angulo WDT: p. 12, line 48 through p. 13 line 2.

60. Because of 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

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eliminated. Angulo WDT: p. 12, line 9-28; p. 12, line 37 through p. 13, line 2; p. 13, line 24-30; G-1528.

61. A dominant source of *Campylobacter* infections in the U.S. population is poultry, particularly chicken, which is frequently contaminated with ciprofloxacin-resistant *Campylobacter*. Angulo WDT: p. 12, line 26-28; p. 17, line 22-24; G-1528.

62. The January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens was conducted in three FoodNet-participating state health departments (Georgia, Maryland, and Minnesota). Angulo WDT: p. 11, line 47-48; G-1528.

63. The January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens used the following sample collection methods: (a) each participating state health department purchased ten whole broiler chickens each month from supermarkets located within the state; (b) the public health department laboratories at each site tested the chicken samples for *Campylobacter*; (c) carcass rinse samples were centrifuged and pellets were incubated in enrichment broth and plated onto *Campylobacter* blood agar plates; and (d) if available, one isolate from each carcass rinse was forwarded to the CDC for species identification and antimicrobial susceptibility testing. Angulo WDT: p. 11, line 48 through p. 12, line 6.

64. The January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens used the following methods for species identification: (a) upon receipt at the CDC, isolates were tested for viability and purity; (b) isolates were confirmed as *Campylobacter* and then identified to species level by the hippurate test; (c) hippurate-positive isolates were classified as *C. jejuni*; (d) hippurate-negative isolates were additionally tested by a polymerase chain reaction to identify the presence or absence of the hippuricase gene; (e) isolates with the hippuricase gene were classified as *C. jejuni* and isolates without the gene were further tested to determine whether they are *C. coli*, *C. upsaliensas*, or another species of *Campylobacter*. Angulo WDT: p. 7, line 32-38; G-97; G-98; G-749.

65. The January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens used the following methods for antimicrobial susceptibility testing: (a) all *Campylobacter* isolates were tested with the E-test system for minimal inhibitory concentrations for ciprofloxacin and several other antimicrobial agents; and (b) ciprofloxacin resistance was defined as a ciprofloxacin minimum inhibitory concentration of greater than or equal to four micrograms per milliliter. Angulo WDT: p. 7, line 38-41; G-97; G-98; G-749.

66. In the January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens, 180 retail chicken products were purchased, representing multiple domestic brand names from over 20 grocery stores. Angulo WDT: p. 12, line 9-10; G-1528.

67. In the January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens, *Campylobacter* was isolated from 80 (44%) of the samples. Angulo WDT: p. 12, line 10; G-1528.

68. Among the 80 *Campylobacter* isolates in the January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens, 62 (78%) were C. jejuni, 16 (20%) were *Campylobacter coli*, and 2 were an unknown species. Angulo WDT: p. 12, line 10-12; G-1528.

69. In the January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens, a ciprofloxacin-resistant strain of *Campylobacter* was identified in 11 percent of 180 retail chicken products tested, demonstrating the frequent contamination of chicken with ciprofloxacin-resistant *Campylobacter*. Angulo WDT: p. 12, line 26-28; G-1528.

70. Among the 62 *C. jejuni* isolates in the January – June 1999 FoodNet *Campylobacter* microbiologic survey of grocery store chickens, 15 (24%) were resistant to ciprofloxacin. Angulo WDT: p. 12, line 19; G-1528.

71. Antibiotic resistance is a food safety problem. Angulo WDT: p. 3, line 9-12.

72. 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*. Angulo WDT: p. 3, line 10-12.

73. The primary purpose of the human NARMS surveillance program is to monitor antimicrobial resistance among foodborne enteric bacteria including *Campylobacter, Salmonella,* and *Escherichia coli* O157:H7. Angulo WDT: p. 3, line 17-19; G-749.

74. The human NARMS surveillance program 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. Angulo WDT: p. 3, line 26-27; G-749.

75. The human NARMS surveillance program testing of *Campylobacter* isolates began in 1997. Angulo WDT: p. 3, line 36; G-749.

76. As part of the human NARMS surveillance program, clinical laboratories isolate foodborne enteric bacteria usually from diagnostic specimens collected from ill persons and forward the isolates to state public health laboratories; each of the state health departments participating in FoodNet sends selected *Campylobacter* isolates each week to the CDC for susceptibility testing. Angulo WDT: p. 3, line 32-33 and line 38-40; G-749.

77. *Campylobacter* isolates in the human NARMS surveillance program exhibited two distinct populations with respect to their minimum inhibitory concentrations to ciprofloxacin: nearly all isolates either had a minimum inhibitory concentration of 0.5 or less micrograms per milliliter (susceptible isolates), or a minimum inhibitory concentration of 32 or more micrograms per milliliter (resistant isolates). Angulo WDT: p. 8, line 5-8; G-97; G-98; G-749.

78. In the human NARMS surveillance program, the percent of *Campylobacter* isolates resistant to ciprofloxacin was 13 percent (28 of 217) in 1997, 14 percent (48 of 345) in 1998, 18 percent (58 of 319) in 1999, 14 percent (46 of 324) in 2000, and 19 percent (75 of 387) in 2001. Angulo WDT: p. 8, line 9-11; G-1452, Attachment 2.

79. In the human NARMS surveillance program, the percent of *C. jejuni* isolates resistant to ciprofloxacin was 12 percent (26 of 209) in 1997, 14 percent (45 of 330) in 1998, 18 percent (52 of 295) in 1999, 14 percent (43 of 306) in 2000, and 18 percent (67 of 366) in 2001. Angulo WDT: p. 8, line 13-16; G-1452, Attachment 2.

80. To account for the potentially confounding effects of the changing population base and the site variability in ciprofloxacin resistance, the human NARMS surveillance program used a multivariate logistic regression to analyze the change between 1997 and 2001 in the proportion of *Campylobacter* isolates that were resistant to ciprofloxacin. Angulo WDT: p. 8, line 27-29.

81. In the multivariate logistic regression model used in the human NARMS surveillance program, the proportion of *Campylobacter* isolates resistant to ciprofloxacin in 2001, controlling for site variation and age, was 2.5 times higher (95% CI: 1.4, 4.4) than the proportion of *Campylobacter* isolates resistant to ciprofloxacin in 1997. Angulo WDT: p. 8, line 35-38; G-1452, Attachment 2.

82. When restricting the analysis to only the *C. jejuni* isolates in the multivariate logistic regression model used in the human NARMS surveillance program, the proportion of *C. jejuni* isolates resistant to ciprofloxacin in 2001 was 2.2 times higher (95% CI: 1.2, 4.0) than the proportion of *C. jejuni* isolates resistant to ciprofloxacin in 1997. Angulo WDT: p. 8, line 41-44; G-1452, Attachment 2.

83. No remarkable changes in the analysis were observed in either multivariate model when the cases from Connecticut were excluded from the multivariate logistic regression model used in the human NARMS surveillance program. Angulo WDT: p. 8, line 46-47.

84. Data from the human NARMS surveillance program demonstrate that a high proportion (approximately one-fifth) of human *Campylobacter* isolates in the United States are resistant to ciprofloxacin. Angulo WDT: p. 8, line 11; p. 9, line 1-2; G-1452, Attachment 2.

85. Ciprofloxacin-resistant *Campylobacter* presents a substantial burden of infection in the U.S. population. Angulo WDT: p. 8, line 9-11; p. 17, line 9-10; G-1452, Attachment 2.

86. When using a multivariate model to account for the marked regional variation and increasing population size in the human NARMS surveillance program, the proportion of human *Campylobacter* in the United States resistant to ciprofloxacin is two and a half times higher in 2001 than it was in 1997. Angulo WDT: p. 8, line 23-38; p. 9, line 2-5; G-1452, Attachment 2.

87. Ciprofloxacin-resistant *Campylobacter* infection in the U.S. population is increasing. Angulo WDT: p. 7, line 25 through p. 9, line 13; p. 17, line 17.

88. The trend between 1997 and 2001 of an increasing prevalence of ciprofloxacin resistance among human *Campylobacter* isolates is statistically significant. Angulo WDT: p. 9, line 3-6; p. 8, line 35-38.

89. 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. Angulo WDT: p.15, line 12 through p. 16, line 7; G-1452, Attachment 4.

90. Among persons treated with fluoroquinolones, persons with ciprofloxacin-resistant *Campylobacter* infections had a longer duration of diarrhea than persons with ciprofloxacin-susceptible *Campylobacter* infections. Angulo WDT: p.15, line 12 through p. 16, line 7; G-1452, Attachment 4.

91. It appears likely that fluoroquinolones are less efficacious against ciprofloxacin-resistant *Campylobacter*, thus prolonging the diarrheal illness. Angulo WDT: p. 15, line 12 through p. 16, line 7; G-1452, Attachment 4.

92. In the 1998-1999 FoodNet *Campylobacter* case-control study on medical consequences, 858 patients with culture-confirmed *Campylobacter* infections whose isolates had been susceptibility tested were asked about their medical treatment; persons who still had diarrhea at the time of interview, persons who were unable to give an estimated duration of diarrhea, and persons who reported not having diarrhea, were excluded from the analysis. Angulo WDT: p. 15, line 13-15, line 21-23; G-1452, Attachment 4.

93. Of the 740 persons included in the analysis of data from the 1998-1999 FoodNet *Campylobacter* case-control study on medical consequences, the mean duration of diarrhea was 8 days (range, 2 to 21 days) for the 82 (11%) persons with ciprofloxacin-resistant *Campylobacter* infections and 7 days (range, 1 to 60 days) for the 658 persons with ciprofloxacin-susceptible *Campylobacter* infections (p=0.1). Angulo WDT: p. 15, line 26-29; G-1452, Attachment 4.

94. In the 1998-1999 FoodNet *Campylobacter* case-control study on medical consequences, the mean duration of diarrhea among the 421 (57%) persons who did not take antidiarrheal medications (loperamide, diphenoxylate, or a prescribed antidiarrheal medication) for their illness was 9 days (range, 2 to 21 days) for the 39 patients with ciprofloxacin-resistant *Campylobacter* infections and 7 days (range, 2 to 60 days) for the 382 patients with ciprofloxacin-susceptible *Campylobacter* infections (p=0.05). Angulo WDT: p. 15, line 31-36; G-1452, Attachment 4.

95. In the 1998-1999 FoodNet *Campylobacter* case-control study on medical consequences, the mean duration of diarrhea among the 67 of 421 (16%) persons not taking an antidiarrheal

medication who also did not take an antimicrobial agent for their illness was 12 days (range, 8 to 20 days) for the 6 persons with ciprofloxacin-resistant infections and 6 days (range, 2 to 21 days) for the 61 persons with ciprofloxacin-susceptible infections (p<0.01). Angulo WDT: p. 15, line 36-40; G-1452, Attachment 4.

96. Of the 740 persons included in the analysis of data from the 1998-1999 FoodNet *Campylobacter* case-control study on medical consequences, 128 (17%), the mean duration of diarrhea among the 128 (17%) persons who took fluoroquinolones and no other antimicrobial agent or antidiarrheal medication for their illness was 8 days (range, 3 to 14 days) for the 17 patients with ciprofloxacin-resistant infections and 6 days (range, 2 to 31 days) for the 111 patients with ciprofloxacin-susceptible infections (p=0.08). Angulo WDT: p. 15, line 42-46; G-1452, Attachment 4.

97. The multivariate analysis of variance (ANOVA) model used to analyze factors that were potentially associated with duration of diarrhea for the persons with *Campylobacter* infections and susceptibility results in the 1998-1999 FoodNet *Campylobacter* case-control study on medical consequences controlled for antimicrobial agent use, loperamide, diphenoxylate, or prescribed antidiarrheal medication use, having an underlying condition, and age; in this model, the mean duration of diarrhea was 9 days in persons with ciprofloxacin-resistant *Campylobacter* infections compared to a mean duration of diarrhea of 8 days in persons with ciprofloxacin-susceptible *Campylobacter* infections (p=0.05). Angulo WDT: p. 16, line 1-7; G-1452, Attachment 4.

98. Ciprofloxacin-resistant *Campylobacter* may have some intrinsic factor or factors which make them more virulent than susceptible isolates. Angulo WDT: p. 16, line 27-28.

Timothy Barrett (G-1453)

99. Dr. Barrett is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

100. Nalidixic acid was the first quinolone drug used to treat bacterial infections, beginning in the mid-1960s. Barrett WDT: page 2, lines 1 and 2

101. The fluoroquinolones, including ciprofloxacin and enrofloxacin, were created synthetically by adding one or two fluorine molecules to the basic quinolone ring structure. Barrett WDT: page 2, lines 3 to 5

102. All of the quinolones physically interact with DNA gyrase, an enzyme essential for bacterial replication, and prevent it from functioning normally. Barrett WDT: page 2, lines 7 to 9

103. Bacteria that have become resistant to fluoroquinolones (most often through mutation in the genes coding for subunits of the DNA gyrase molecule) are also typically resistant to nalidixic acid. The use of any fluoroquinolone can select for bacteria that are resistant to nalidixic acid as well as to the specific fluoroquinolone used and to other fluoroquinolones.

It is not necessary that bacteria be exposed to nalidixic acid to become resistant to nalidixic acid. Barrett WDT: page 2, lines 9 to 15

104.   In any environment in which quinolones are present, bacteria that are resistant to those drugs will have a very large selective advantage over quinolone-susceptible bacteria. Barrett WDT: page 2, lines 16 to 18

105.   *C. jejuni, C. coli,* and *C. lari* are sometimes referred to as the "thermophilic (heat liking) campylobacters" because they grow well at 42° C, a temperature that inhibits the growth of most bacteria of medical importance (most medically important bacteria prefer 37° C, normal body temperature). Barrett WDT: page 2, lines 29 to 32

106.   Throughout the 1980s, susceptibility to nalidixic acid continued to be one of the primary criteria used to differentiate between the thermophilic campylobacters, with *C. jejuni* and *C. coli* considered to be susceptible. Barrett WDT: page 3, lines 1 to 3

107.   Up to 1988, Dr. Barrett considered *Campylobacter* jejuni resistance to nalidixic acid so rare that it could be used as a diagnostic criteria for distinguishing between *Campylobacter* strains. Barrett WDT: page 3, lines 10 to 12

108.   Dr. Barrett published a paper in 1988 describing finding only 2 of 42 *Campylobacter jejuni* resistant to nalidixic acid; and resistance to fluoroquinolones was even more unusual at that time. Barrett WDT: p. 3, lines 3-13; G-1609.

109.   As fluoroquinolone resistance emerged during the mid-1990s in *Campylobacter* isolates from human patients, nalidixic acid resistance emerged concordantly, making nalidixic acid-susceptibility a far less valuable test for speciation. During the 1990s, it was used by fewer and fewer researchers as a diagnostic criterion for identification of thermophilic *Campylobacters*. Barrett WDT: page 3, lines 20 to 24

110.   The emergence of quinolone resistance in *C. jejuni* and *C. coli* may have incorrectly reduced the apparent incidence of these organisms (especially quinolone-resistant strains) in surveillance studies. Barrett WDT: page 3, lines 29 to 31

111.   Because *C. jejuni* and *C. coli* were originally considered to be nalidixic acid-susceptible, a researcher relying on this criterion to identify *C. jejuni or C. coli* would have excluded all quinolone-resistant isolates from surveillance for these two species. Barrett WDT: page 3, lines 31 to 34

112.   In studies where a researcher relied on nalidixic acid susceptibility to identify *C. jejuni or C. coli*, the true incidence of fluoroquinolone-resistant *C. jejuni* and *C. coli* would have been drastically underreported. Barrett WDT: p. 3, lines 31-36.

113.   Despite the clinical use of nalidixic acid since the mid 1960s, nalidixic acid resistance was rare enough in *C. jejuni* that susceptibility to nalidixic acid was considered a critical

characteristic in differentiating *C. jejuni* from *C. lari* throughout the 1980s. Barrett WDT: page 4, lines 4 to 7

114.   The emergence of fluoroquinolone-resistant (and thus nalidixic acid-resistant) *C. jejuni* and *C. coli* in the mid-1990s has resulted in nalidixic acid-susceptibility being dropped as an identifying characteristic for these bacteria. Barrett WDT: page 4, lines 7 to 10

115.   The emergence of quinolone-resistant *C. jejuni* in humans in the 1990s does not appear to be the result of nalidixic acid use in clinical medicine. Barrett WDT: p. 4, lines 10-12.

116.   The purpose of bacterial subtyping is to take bacterial isolates that have already been characterized as belonging to a single species (*C. jejuni*, for example), and to further group them in some meaningful way. Barrett WDT: page 4, lines 19 to 21.

117.   By determining which bacterial isolates of the same species are most like each other, and thus most likely to have come from a common source, bacterial subtyping assists in finding links between patients and between patients and food or animal sources. Barrett WDT: page 4, lines 25 to 27.

118.   RAPD and PFGE are both techniques that enable scientists to compare strain similarity at the genetic level by examining large regions of the bacterial DNA. Barrett WDT: page 5, line 2 to 4.

119.   The purpose of molecular subtyping is to provide information that is useful to the type of investigation being conducted, not to identify the maximum number of types. Barrett WDT: page 5, lines 15 and 16.

120.   The data presented by Clow indicate that 77% of human *C. jejuni* isolates were types that were also seen in chickens. Barrett WDT: page 6, lines 40 and 41.

121.   There is no universally accepted "gold standard" method for molecular subtyping of *C. jejuni*. Barrett WDT: page 7, lines 29 and 30.

Mary Bartholomew (G-1454)

122.   Dr. Bartholomew is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

123.   Human food safety testing comprises a battery of toxicological studies, typically designed to look for chronic problems and cancers caused by long term exposures to trace amounts of chemicals in food or developmental problems in offspring of parents so exposed. The process of deciding on acceptable daily intakes (ADIs) from no observable effect levels (NOELs) based on these toxicology studies is a risk assessment activity. Bartholomew WDT: p. 2, lines 4-9

124. The use of fluoroquinolones in chickens and the development of resistant *Campylobacter* in chickens were of concern to CVM for several reasons. First, chickens are reservoirs for many food borne pathogens including *Campylobacter* and *Salmonella*. For example, broiler carcass contamination measured in the processing plant estimates that 20% of broiler chickens in the United States are contaminated with *Salmonella* and over 80% are contaminated with *Campylobacter*. Consumption of food contaminated with these bacteria can lead to illness in susceptible. Second, *Campylobacter* is the most common known cause of bacterial food borne illness in the United States. Sporadic cases of *Campylobacter* account for approximately 99% of all *Campylobacter* cases. Epidemiological investigations of sporadic infections have indicated that chicken is the most common source of human infection. Also, slaughter and processing of chickens may result in bacterial contamination on the carcass that can survive on retail product and result in human exposure during food preparation and consumption. Third, *Campylobacter* has been reported to develop resistance when fluoroquinolones are used. Finally, fluoroquinolones are used in human medicine empirically to treat gastrointestinal infections, such as campylobacteriosis and are important for use in many other therapeutic indications in human medicine. Increasing levels of resistance reduce the utility of fluoroquinolones in the empiric treatment of enteric illness. Bartholomew WDT: p. 3, lines 14-17, G-953

125. Although some information on fluoroquinolone-resistant *Campylobacter* was available at the time that fluoroquinolones were approved, CVM approved fluoroquinolones for use in poultry as prescription only medication and prohibited its extra-label use. CVM expected that these measures would minimize the development of resistance. Bartholomew WDT: p. 3, lines 20-23; G-953

126. As part of its plans to address concerns about antimicrobial resistance, CVM decided to develop a risk assessment model that would be applicable to the general problem of antimicrobial resistance in food borne pathogens where that resistance is attributed to the use of an antimicrobial drug in animals. Bartholomew WDT: p. 3, lines 34-38

127. CVM became concerned about the potential public health impact of veterinary uses of the fluoroquinolones when, despite the precautionary use restrictions, emerging resistance to fluoroquinolones in *Campylobacter* was noted in humans. This concern was reinforced by scientific literature reporting the emergence of fluoroquinolone resistance in human campylobacteriosis after approval of poultry fluoroquinolones in several foreign countries, notably in the Netherlands and Spain. Bartholomew WDT: p. 3, lines 40-46; Exhibits G-190, G-320, G-491, G-505 and G-671

128. A detailed description of the risk assessment model is given in the report document "The Human Health Impact of Fluoroquinolone Resistant Campylobacter Attributed to the Consumption of Chicken" which was made available to the public on the CVM website in its final version on January 5, 2001. Bartholomew WDT: p. 4, lines 10-13; G-953

129. At the time the risk assessment was initiated, there were no food animal approvals for fluoroquinolones except those in poultry. Bartholomew WDT: p. 4, lines 21-23

130. Predictive microbiology is used in microbial risk assessments to model the increases or decreases in microbial load under varied conditions of food processing or storage. Bartholomew WDT: p. 4, lines 28-29

131. At the time CVM conducted its risk assessment, the data describing the complex exposure, host-pathogen interactions necessary to recreate an accurate dose-response relationship were lacking. Bartholomew WDT: p. 4, lines 33-34

132. In its *Campylobacter* risk assessment, CVM used an estimate using data collected by the Centers for Disease Control (CDC) FoodNet program of the annual number of people with campylobacteriosis as a starting point in its risk assessment modeling process. Bartholomew WDT: p. 4, lines 44-47

133. The CDC had begun collecting prevalence data for campylobacteriosis in humans in FoodNet in 1996. FoodNet is an active surveillance system for food borne illness coordinated by the CDC with participating Health Departments reporting culture-confirmed cases. Bartholomew WDT: p. 5, lines 40-43

134. Estimates used in the CVM *Campylobacter* risk assessment for the proportion of times persons with diarrhea sought care and submitted stool samples were obtained from a telephone survey conducted by CDC. Bartholomew WDT: p. 6, lines 2-3

135. An estimate of the proportion of samples from persons with campylobacteriosis that actually yield *Campylobacter* was obtained from the literature. Bartholomew WDT: p. 6, lines 8-10

136. The CDC had begun collecting human *Campylobacter* isolates and testing for resistance in the National Antimicrobial Resistance Monitoring System (NARMS) in 1997. Bartholomew WDT: p. 6, lines 11-12

137. The amount of chicken consumed is available from the United States Department of Agriculture (USDA) Economic Research Service (ERS). As part of the NARMS program, USDA Agricultural Research Service (ARS) had in 1998 begun performing susceptibility testing on *Campylobacter* from chicken collected by the Food Safety Inspection Service (FSIS). Bartholomew WDT: p. 6, lines 17-21

138. The estimation used by CVM in its *Campylobacter* risk assessment of the number of campylobacteriosis cases seeking care who are prescribed a fluoroquinolone and who have fluoroquinolone-resistant campylobacteriosis attributed to the use of fluoroquinolones in chickens consists of four sub-component estimates. The estimates are for the total number of cases of campylobacteriosis; the number of campylobacteriosis cases attributed to chicken; the proportion of cases with resistant *Campylobacter* where the resistance is attributed to the use of fluoroquinolones in chickens; and the number of resistant cases attributed to chicken that seek medical care and are treated with fluoroquinolones. Bartholomew WDT: p. 6, line 47- p. 7, line 2

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139. The total number of cases of campylobacteriosis contracted in the United States annually is estimated by the CDC from the total number of reported isolations in a year. The CVM used the process developed by the CDC and modeled the uncertainties in parameters used in the process of deriving the estimate. The CDC estimates the total number of cases by taking the observed number of culture-confirmed cases within FoodNet sites and multiplying it by factors that account for undercounting and under-reporting. Bartholomew WDT: p. 7, lines 5-10

140. The CVM model derives the uncertainty distribution for the estimated mean number of cases of campylobacteriosis cases attributed to chicken. Two case-control studies from the literature were used for input values for determining the proportion of all campylobacteriosis cases attributable to chicken (Harris et al. 1986; Deming et al. 1987). In the Harris study, an attributable fraction of 45.2 percent was given; in the Deming study, the attributable fraction was 70 percent. Bartholomew WDT: p. 8, lines 12-16

141. Data from the *Campylobacter* case-control study assisted in the removal of proportions of resistance attributed to other sources. Bartholomew WDT: p. 9, lines 5-7

142. The proportion of resistance among domestically acquired cases was multiplied by the number of chicken-associated cases to estimate the mean number of cases resistant and attributed to resistance from chicken. Bartholomew WDT: p. 9, lines 29-31

143. To estimate the mean number of resistant cases who had infections attributed to consumption of chicken who seek care and are treated with a fluoroquinolone and its associated uncertainty distribution, the mean number of cases with resistant campylobacteriosis attributed to chicken is multiplied by the respective care-seeking proportions discussed in the Bartholomew written direct testimony. Bartholomew WDT: p. 10, lines 13-16

144. The proportion of cases who receive antibiotics and the proportion who receive a fluoroquinolone were derived from the 1998-1999 CDC *Campylobacter* case control study. Bartholomew WDT: p. 10, lines 20-21.

145. CVM's *Campylobacter* Risk Assessment included estimation of the quantity of chicken with fluoroquinolone-resistant *Campylobacter* consumed and modeling the uncertainties in the estimate. Bartholomew WDT: p. 11, lines 13-14

146. Inputs to estimate the quantity of consumed chicken were taken from USDA Economic Research Service (ERS) with product sent for rendering, product diverted for pet food, exports, water added during processing and imports subtracted. The proportion of chicken with *Campylobacter* and the portion of *Campylobacter* that were fluoroquinolone-resistant were determined from samples that FSIS and ARS analyzed as part of the NARMS project. Bartholomew WDT: p. 11, lines 14-19

147. The CVM *Campylobacter* risk assessment enables the estimation of the probability that a pound of chicken meat with fluoroquinolone-resistant *Campylobacter* will result in a case of

fluoroquinolone-resistant campylobacteriosis in a specific year. Bartholomew WDT: p. 12, lines 22-24

148. For the years for which the CVM *Campylobacter* risk assessment was done, the model indicates that there would be a case of human fluoroquinolone-resistant campylobacteriosis from chicken for every 7900 pounds of chicken contaminated with fluoroquinolone-resistant *Campylobacter*. Bartholomew WDT: p. 13, Figure 6 and lines 3-11

149. In 1995 about 88 percent of chicken carcasses yielded *Campylobacter* in the slaughterhouse, as reported by the USDA FSIS. This compares to 1-4 percent found on beef at slaughter in 1993. Bartholomew WDT: p. 14, lines 8-10

150. The microbial load of *Campylobacter* is higher on chicken than on other food animal products. Bartholomew WDT: p. 14, lines 10-11

151. The CVM risk assessment calculated that approximately 50 pounds of domestically produced chicken per person was consumed in 1998/1999. This value was derived by subtracting off water weight and amounts sent for rendering and export from the pounds of chicken produced as given by USDA ERS. The combined information about *Campylobacter* contamination levels on chicken and the substantial exposure through consumption provide further credence to the epidemiologic study findings. Bartholomew WDT: p. 14, lines 11-16

152. The mean value for the distribution of the estimated proportion of cases that is attributed to chicken in the CVM *Campylobacter* risk assessment is 57%. The proportion of cases attributable to chicken was multiplied times the number of cases of campylobacteriosis to estimate the number of cases of campylobacteriosis attributed to chicken. Bartholomew WDT: p. 14, lines 19-22

153. The mean value for the distribution of the estimated proportion of cases that is attributed to chicken in the CVM *Campylobacter* risk assessment is 57%. Bartholomew WDT: p. 14, lines 19-20

154. Dr. Bartholomew concluded that the reported proportions of resistance among isolates from poultry are likely to be underestimates, and that the estimates of resistance among humans in the CVM *Campylobacter* risk assessment obtained by subtraction of other sources of resistance are likely to be nearer to the real values. Bartholomew WDT: p. 15, lines 41-44

155. The proportion of resistant cases attributed to domestically-produced chicken among all cases attributed to domestically-produced chicken was estimated to be about 14.2% in 1998 in the CVM *Campylobacter* risk assessment. Bartholomew WDT: p. 16, lines 29-31

156. The CVM risk assessment used an epidemiological approach to estimate the mean number of individuals impacted by fluoroquinolone-resistant campylobacteriosis attributable to chicken consumption. Individuals considered to be impacted were those with campylobacteriosis, who had fluoroquinolone-resistant infections, had sought care, and were

prescribed a fluoroquinolone antibiotic to treat the infections. Bartholomew WDT: p. 17, line 43 -p. 18, line 2

157.   In 1998, the mean number (the number of cases that would occur on average if 1998 were to be repeated many times) was estimated to be 8,678 between the 5th percentile, 4,758 and the 95th percentile, 14,369. This corresponds to a risk of one impacted individual in every 34,945 individuals in the United States. Bartholomew WDT: p. 18, lines 2-6

158.   In 1999, the mean number was estimated to be 9,261 between the 5th percentile, 5,227 and the 95th percentile, 15,326. This corresponds to a risk of one impacted individual in every 32,912 individuals in the United States. Bartholomew WDT: p. 18, lines 6-8

John Besser (G-1455)

159.   Dr. Besser is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

160.   The use of antibiotics in food production has a number of potential impacts on human health, including facilitating the emergence of drug resistance and increasing the proportion of drug-resistant bacteria transmitted to humans. Besser WDT: p. 2, line 45 through p. 4, line 29.

161.   The chance that a resistant clone will emerge from antibiotic use is related to the amount of antibiotic used, and the manner in which it is used. Besser WDT: p. 3, line 8-24; G-366.

162.   Evidence for the role that fluoroquinolone use in poultry plays in the emergence of fluoroquinolone resistance in humans includes: (a) temporal and epidemiologic association between the approval of fluoroquinolones for use in animals and the emergence of fluoroquinolone-resistant disease in humans in multiple countries; (b) experimental feeding experiments in poultry showing the rapid emergence of fluoroquinolone resistance in *Campylobacter* following fluoroquinolone treatment ; and (c) biologic plausibility, i.e. that the observations in (a) and (b) above fit with what is known about the molecular mechanisms of fluoroquinolone resistance and the relative rapidity with which the mutations leading to fluoroquinolone resistance would be expected to occur. Besser WDT: p. 3, line 10-24; G-586; G-403; G-315; G-1350.

163.   The proportion of drug-resistant bacteria transmitted from food animals to humans is likely to increase as a result of antibiotic use in the animal source. Besser WDT: p. 3, line 26 through p. 4, line 22; G-1350.

164.   The use of antibiotics in a food animal population where resistant clones have already emerged increases the proportion of resistant bacteria in the food animals, which increases the probability that a human who becomes ill directly or indirectly (via cross-contamination of other material) from that food animal source will acquire a resistant infection. Besser WDT: p. 3, line 26 through p. 4, line 22; G-1350.

165.   Increasing antibiotic use in the food source increases the number of resistant infections in humans. Besser WDT: p. 3, line 26 through p. 4, line 22.

166.   Control of antibiotic resistance spread relies both on limiting the emergence of resistance and limiting its spread. Besser WDT: p. 2, line 45 through p. 4, line 29.

167.   Withdrawal of fluoroquinolones for animal use should reduce the proportion of fluoroquinolone-resistant bacteria in the animals, and hence reduce fluoroquinolone-resistant infections in humans. Besser WDT: p. 2, line 45 through p. 4, line 29.

168.   Antimicrobial susceptibility testing is used to determine if a given bacterium is likely to be inhibited or killed by antibiotics used to treat the infections that they cause. Besser WDT: p. 4, line 34-36.

169.   The most common way to measure antibiotic resistance is by reporting a "minimal inhibitory concentration," or "MIC." Besser WDT: p. 4, line 36-37.

170.   MIC is a laboratory test designed to predict the minimum amount of antibiotic needed to inhibit or kill the bacterium in question. Besser WDT: p. 4, line 37-39.

171.   The higher the MIC, the more antibiotic is needed to kill the organism or inhibit its growth. Besser WDT: p. 4, line 39-40.

172.   In general, very high MIC values correspond to "resistant" bacteria (unaffected by treatment with the antibiotic), and very low MIC values correspond to "susceptible" bacteria (easily treated with the antibiotic). Besser WDT: p. 4, line 40-42.

173.   Standardized antimicrobial susceptibility testing methods are available for a number of infection-causing bacteria and antibiotics commonly used to treat them. Besser WDT: p. 4, line 43-44.

174.   The term "breakpoint" refers to an MIC value (or the diameter of a zone of growth inhibition with some methods) used to indicate susceptible, intermediately susceptible, or resistant bacteria. Besser WDT: p. 4, line 46 through p. 5, line 2.

175.   Antimicrobial susceptibility testing standards, MIC values and established breakpoints do not exist for many disease-causing bacteria and associated antibiotics. Besser WDT: p. 5, line 8-9.

176.   When the Smith study of fluoroquinolone resistance in *Campylobacter jejuni* was conducted, there existed neither standardized methods nor established breakpoints. Besser WDT: p. 5, line 15-16.

177.   The Smith study of fluoroquinolone resistance in *Campylobacter jejuni* chose a commonly used antimicrobial susceptibility testing method called the E-test, and used breakpoints for the most similar bacterial family for which standards have been established, *Enterobacteraceae*. Besser WDT: p. 5, line 16-19.

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178. In the Smith study of fluoroquinolone resistance in *Campylobacter jejuni*, most isolates (96%) had MICs of >32 μg/ml, or 8 times the breakpoint level of 4.0 μg/ml specified in the NCCLS standards for *Enterobacteraceae*. Besser WDT: p. 5, line 29-31.

179. Given the broad bimodal nature of MIC values (i.e., very high or very low levels) in the Smith study of fluoroquinolone resistance in *Campylobacter jejuni*, the basic interpretations, i.e., "resistant" or "susceptible," are valid. Besser WDT: p. 5, line 35-37.

180. DNA fingerprinting tells us whether a group of disease-causing bacteria with identical DNA fingerprints is more likely to have a common origin than a group with different DNA fingerprints. Besser WDT: p. 6, line 23-25.

181. DNA fingerprinting serves to strengthen statistical associations that may already be present by removing from consideration cases less likely to be associated. Besser WDT: p. 6, line 28-30.

182. DNA fingerprinting of bacteria allows patterns of disease in the population to be seen that might otherwise be too difficult to differentiate from background disease activity. Besser WDT: p. 6, line 41-43.

183. DNA fingerprinting works by facilitating recognition of "clusters" of disease. Besser WDT: p. 7, line 1-3.

184. DNA fingerprinting involves the use of "restriction" enzymes that cut the bacterial DNA into different sized fragments, which, when electrically separated from each other, form a pattern; the locations of bacterial DNA sequences that the enzymes recognize determine the sizes of the fragments. Besser WDT: p. 7, line 13-43.

185. By using more enzymes in DNA fingerprinting, one can essentially examine more locations, identify more patterns, and find more differences between bacteria, thus increasing the "resolution" of the test. Besser WDT: p. 7, line 45 through p. 8, line 1.

186. Since bacteria are constantly multiplying and changing, it is always possible to find differences between samples in DNA fingerprinting. Besser WDT: p. 8, line 1-2.

187. The "right" level of resolution in DNA fingerprinting is achieved when cases cluster in a manner that proves to be meaningful after epidemiologic analysis. Besser WDT: p. 8, line 12-13.

188. In DNA fingerprinting, the most useful classification level is one where meaningful relationships can be drawn from the associated epidemiologic analyses. Besser WDT: p. 9, line 5-7.

189. No test method for DNA fingerprinting measures all of the differences between the DNA of the samples; rather, each method examines differences in selected "markers," which are used to reflect broader differences. Besser WDT: p. 9, line 12-15.

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190.   The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method examines differences in the gene that codes for the bacterial flagellum, which is an appendage of the bacterium that gives it motility. Besser WDT: p. 9, line 23-27.

191.   Since the flagellum is exposed to the immune system of animal hosts, it is thought that selective pressure would cause this gene to change at a rate which would make it a good indicator of short-term epidemiologic linkage. Besser WDT: p. 9, line 27-30.

192.   The flagellar gene (*"fla*A") is not in any way associated with the genes that cause resistance to ciprofloxacin or any other antibiotic, and one would not expect the method to reflect differences in susceptibility. Besser WDT: p. 9, line 30-33.

193.   The polymerase chain reaction restriction fragment length polymorphism method was chosen for the Smith study of fluoroquinolone resistance in *Campylobacter jejuni* since it was being considered as a national subtyping standard at that time, and is relatively fast and simple to perform. Besser WDT: p. 9, line 33-35.

194.   The pulsed-field gel electrophoresis (PFGE) method measures gene sequences, which could occur on any part of the bacterial DNA. Besser WDT: p. 9, line 37-44.

195.   Pattern differences in the pulsed-field gel electrophoresis (PFGE) method *may* reflect differences in antibiotic susceptibility, or any other factor. Besser WDT: p. 9, line 41-46.

196.   The Smith study of fluoroquinolone resistance in *Campylobacter jejuni* found a high degree of concordance between fluoroquinolone-resistant subtypes found in domestically acquired human infections and domestically produced chicken (92.3%), which implicates chicken as a likely source for fluoroquinolone-resistant human infections. Besser WDT: p. 10, line 7-10; G-589.

197.   The association between fluoroquinolone-resistant subtypes in domestically acquired human infections and domestically produced chicken found in the Smith study of fluoroquinolone resistance in *Campylobacter jejuni* was further strengthened by the observation that other groups of infected humans, such as those with fluoroquinolone-sensitive infections or those who likely acquired their infections through foreign travel, did not share the same high proportion of common subtypes with fluoroquinolone-resistant domestic chicken isolates (44.4% and 35% shared subtypes, respectively). Besser WDT: p. 10, line 10-15; G-589.

John Carey (G-1456)

198.   Dr. Carey is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

199.   Young chickens that are produced for meat are called broilers. Carey WDT: page 2, line 4

200.   Male turkeys are called Toms and female turkeys are called Hens. Carey WDT: page 2, line 6

201. Cleanout frequency (for poultry raising facilities) varies depending on the operation and owner husbandry practice but broiler facilities are typically cleaned out every 12-18 months. Broiler facilities may have as many as 5-7 successive flocks per year and turkey facilities 4 successive flocks per year, depending on final body weight and market considerations. Carey WDT: p. 3, lines 3-9

202. In all poultry housing facilities except all-slat broiler breeder facilities, birds are in contact with fecal material. Since it is normal behavior for birds to scratch and peck at the ground, they ingest fecal material (coprophagy) and related contamination from other birds in the facility. Carey WDT: p. 3, lines 18-21

203. Feeding, environmental and all other husbandry practices are largely applied to the flock as a whole. Individual birds are observed and care for if necessary; but generally, individual bird management or treatment is not feasible and is seldom necessary. Carey WDT: page 4, lines 27-30

204. Since birds are housed in large common groups, with common disease threats, health related care is typically administered on a flock basis via the water or feed. In such cases, all birds receive medicated feed or water. This is viewed as the most practical manner to treat poultry health episodes since the entire flock has exposure to the challenge due to their common housing, feeding, drinking, and litter exposure. Carey WDT: page 4, lines 31-37
205.

Hubert Endtz (G-1457)

206. Dr. Endtz is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

207. Although many subspecies of *Campylobacter* have been described in the last 20 years, *Campylobacter jejuni* is by far the most frequently isolated as it is responsible for >90% of clinical infections. Endtz WDT: page 2, lines 17-20

208. Three distinct forms of infections caused by *C. jejuni* are known: (a) acute diarrhea; (b) extra-intestinal infections; and (c) non-supurative post-infectious complications. Endtz WDT: page 2, lines 22-23

209. The most common manifestation of *C. jejuni* is an acute diarrhea. The incubation period ranges from 1 to 7 days. There is a so-called prodromal phase preceding the diarrhea of 12-48 hours with fever, headache and abdominal pain. Endtz WDT: page 2, lines 31-33

210. Diarrhea associated with *Campylobacter jejuni* varies from very mild to massive, watery or grossly, bloody stools. Fifteen or more stools may pass on the worst day of illness. Endtz WDT: page 2, lines 35-36

211.   The mortality associated with *Campylobacter* diarrhea in the United States has been estimated at 8/10,000 and 24/10,000, respectively, cultured-confirmed cases in two different studies. Endtz WDT: page 2, lines 44-46; G-1644 and G-1783.

212.   The most important non-suppurative infective complications of *Campylobacter* infections include reactive arthritis and the Guillain-Barre syndrome (GBS). Endtz WDT: page 3, lines 4-5

213.   The DALY (Disability Adjusted Life Year) is a measure to assess the global health burden of a disease in a standardized manner. This integrated measure combines years of life lost by premature mortality with years lived in disability. Endtz WDT: page 3, lines 28-31

214.   The total health burden of *Campylobacter* infection is similar to diseases as meningitis, sepsis, upper respiratory infections and stomach and duodenal ulcers. This underscores the impact of *Campylobacter* in society and the importance as a primary Public Health problem. Endtz WDT: page 3, lines 35-38

215.   *Campylobacter* are zoonotic bacteria (i.e. they are transmitted from animals to humans and cause disease in humans). Endtz WDT: page 3, lines 43-44

216.   *Campylobacter* and *Salmonella* are the two most common causes of foodborne illness in the developed world. Endtz WDT: page 3, lines 44-45

217.   The great majority of *Campylobacter* infections are sporadic infections. Endtz WDT: page 3, lines 45-46

218.   Human-to-human transfer of *Campylobacter* is very rare and probably of no epidemiological importance. Endtz WDT: p. 4, lines 1-3

219.   In developed countries poultry is often considered to be the most important reservoir of *C. jejuni*. Endtz WDT: page 4, lines 6-7

220.   Live poultry are often colonized by large numbers of *Campylobacters* without showing any signs of clinical illness. Colonization levels in the small intestine and ceca usually ranges from 10^5 - 10^9 CFU/g feces. Endtz WDT: page 4, lines 7-9

221.   Several epidemiological studies suggest that the handling and consumption of poultry meat (either home or commercially prepared) is a dominant sources of sporadic infection. Endtz WDT: page 4, lines 12-15

222.   In many studies handling and/or consumption of poultry have been found to be independent risk factors and may account for up to approximately 70% of the sporadic cases. Endtz WDT: page 4, lines 15-17

223.   Foreign travel is also a risk factor for acquiring *Campylobacter* infection. These cases likely result from consumption of contaminated food or water in the countries visited. Endtz WDT: page 4, lines 17-21

224.   It is important to note that in a few studies, the consumption of chicken has been found to protect against *Campylobacter* infection. This paradox can be explained on the basis of acquired immunity after repeated challenges with contaminated meat. Endtz WDT: p. 4, lines 23-25

225.   In 1999, Belgium had a dioxin crisis caused by dioxin-contaminated feed being fed to livestock. The contamination led to the withdrawal of all Belgian chicken and eggs from the market for a period of four weeks. Belgium has had a *Campylobacter* surveillance network of sentinel and reference laboratories since 1983, which provided an unique opportunity to investigate the effect of withdrawal of particular food products from the market on the prevalence of campylobacteriosis. Based on a model that was generated with reports from preceding years, they observed a significant decline of 40% of the number of *Campylobacter* infections upon this intervention. One has to stress that only Belgian poultry was withdrawn from the market and that foreign poultry remained on the market. In 1999, 41% of the poultry available for consumption has been imported. Thus, non-Belgian-poultry-related *Campylobacter* infections were still present in the reported numbers. The total percentage of poultry-related *Campylobacter* infections in Belgium in 1999 is likely to have exceeded the reported figure of 40%. Endtz WDT: page 4, lines 29-41; G-672

226.   In rural areas in the developing world, close direct contact with animals, in particular poultry, is the most important risk factor for cases of campylobacteriosis in humans. Endtz WDT: page 4, lines 43-44

227.   A study from Denmark studied the serotype distribution, using the Penner scheme, of *Campylobacter* isolates from Danish patients and from major food production animals. The most commonly observed human serotypes showed large overlap with the distribution of *Campylobacter* serotypes in cattle and broilers, thereby suggesting that these food animals could be a major source of human campylobacteriosis. Endtz WDT: p. 5, lines 17-21; G-459

228.   Serotyping studies in the Netherlands in the late eighties have observed that the five most prevalent human serotypes were also frequently found in isolates from poultry. Endtz WDT: p. 5, lines 23-26

229.   A study in Taiwan investigated the relatedness of quinolone-resistant *Campylobacters* from poultry products and from humans with PFGE and *flaA* RFLP (Restriction Fragment Length Polymorphism of the *flaA* gene). They found that 40% of the human types were shared with the populations isolated from poultry products. They concluded that domestic poultry is an important source of quinolone-resistant campylobacters. Endtz WDT: page 5, lines 26–32; G-1775

230.   In a comparable study in Canada using PFGE, 20% of the human *Campylobacter* isolates were genetically related to genotypes found in poultry indicating a potential important source of human infections. Endtz WDT: page 5, lines 33-35; G-1684

231.   Treatment of bacterial diarrhea is often empirical and antimicrobial therapy has to be initiated before the results of fecal cultures become available. Endtz WDT: page 6, lines 46-47

232.   To cover causes of bacterial diarrhea other than *Campylobacter,* like *Salmonella* and *Shigella*, where macrolides are not effective, the fluoroquinolones are the preferred agents since they are active against all major causes of bacterial diarrhea. Endtz WDT: page 6, lines 47 – p. 7, line 3

233.   With the introduction of quinolone resistance in *Campylobacter* species empirical treatment of patients with quinolones may result in treatment failures. Endtz WDT: page 7, lines 3-5

234.   With increasing levels of fluoroquinolone resistance, empirical treatment with these drugs will become hazardous. Most patients with *Campylobacter* diarrhea are not hospitalized and one has therefore to rely on oral drugs. No other oral drug with comparable activities and toxicity profile is currently available as an alternative treatment. Endtz WDT: p. 7, lines 5-9

235.   Twenty-five years of study of the epidemiology of *Campylobacter* infections in the US and Europe has not come up with data that refute the hypothesis that epidemiology of *Campylobacter* in the two continents is in essence very comparable. Therefore, data from outside the US include valuable information that may be extrapolated to the US situation. Endtz WDT: p. 7, lines 14-17

236.   Fluoroquinolone resistance in *Campylobacter* from poultry must be the result of the use of these drugs in animal husbandry. Endtz WDT: p. 8, lines 6-7

237.   It is unlikely that the use of norfloxacin (in human medicine) alone may have led to the development of fluoroquinolone resistance in strains isolated from humans. It seems more probable that the use of enrofloxacin in poultry contributed significantly to the resistance problems in humans. Endtz WDT: p. 8, lines 7-10

238.   In Spain, before licensing of enrofloxacin for veterinary use in 1990, the prevalence of fluoroquinolone-resistant *Campylobacter* in human ranged from 0 to 3%. After licensing, fluoroquinolone resistance percentages in human *Campylobacter* increased dramatically to 39-88%. The sharpest increase occurred from 1990 to 1991, the first year following introduction of enrofloxacin. Endtz WDT: p. 8, lines 16-20

239.   In the U.S., sarafloxacin was licensed in 1995 and enrofloxacin in 1996 for use in poultry. Endtz WDT: p. 8, lines 32-33

240. Smith observed an increase of fluoroquinolone-resistant *Campylobacter* infecting humans from 1.3% in 1992 to 10.2% in 1998. Although part of the rise of fluoroquinolone resistance may be explained by foreign travel and quinolone use prior to the collection of stool specimens, the prevalence of domestically acquired quinolone-resistant infections, not related to prior human use, also increased during the study period, largely due to acquisition from poultry. Endtz WDT: p. 8, lines 33-38; G-589

241. In another study conducted between 1982 and 1992, no *C. jejuni* or *C.*coli isolated from humans were resistant to the fluoroquinolones, thereby strengthening the hypothesis that prior to the licensing of fluoroquinolones in poultry, the prevalence of fluoroquinolone-resistant *Campylobacter* was very low. Endtz WDT: p. 8, lines 38-41

242. Fluoroquinolones are not registered for use in the food-producing animals but they are registered for use in human medicine in Australia. Endtz WDT: p. 8, lines 45-47

243. Very few Australian patients with diarrhea with fluoroquinolone-resistant *Campylobacter* have been reported in the English-language literature. The cases that have been reported acquired the infection abroad. Endtz WDT: p. 8, line 47 – p. 9, line 2; B-225; B-421

244. In the absence of animal use, fluoroquinolone resistance in *Campylobacter* in Australia remains extremely low. Endtz WDT: p. 9, lines 3-4

245. *Campylobacter* is an important pathogen in terms of prevalence, morbidity, mortality and total health burden. Endtz WDT: page 9, lines 12-13

246. Treatment options for acute bacterial diarrhea, including *Campylobacter*, are greatly compromised by the emergence of fluoroquinolone resistance. Endtz WDT: page 9, lines 15-16

247. Poultry is an important source of campylobacters causing infections in humans. Endtz WDT: page 9, lines 18-19

248. There is substantial evidence that the use of fluoroquinolone in poultry leads to fluoroquinolone resistance in *Campylobacter* from poultry. Endtz WDT: page 9, lines 21-22

249. In the absence of significant person-to-person transmission, one may deduce that a significant proportion of fluoroquinolone-resistant *Campylobacter* is reaching people via poultry. Endtz WDT: page 9, lines 24-26

Marja-Liisa Hanninen (G-1458)

250. Dr. Hanninen is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

251. Baytril has been used for treatment of infections caused by *E. coli* or *Mycoplasma pneumoniae* in poultry since the early 1990s in various countries. Hanninen WDT: p. 1, ¶ 2

252. In most countries the use of fluoroquinolones in human medicine started in the 1980s. Hanninen WDT: p. 1, ¶ 2

253. In veterinary medicine, fluoroquinolone use has been regulated and restricted for the treatment of poultry and other animal illnesses, but in some countries it has also been used extensively for prophylaxis. Hanninen WDT: p. 1-2, ¶ 2

254. Spain was one of the first countries where Baytril was used in veterinary medicine, beginning in 1987. Hanninen WDT: p. 2, ¶ 2

255. Most European Union countries started a more systematic monitoring of antibiotic resistance among animal and human *C. jejuni/C. coli* in the middle of the 1990s as required by the European Union. Hanninen WDT: p. 2, ¶ 3

256. The US started a national monitoring program in 1996, called the National Antimicrobial Resistance Monitoring System (NARMS). Hanninen WDT: p. 2, ¶ 3

257. In Finland, enrofloxacin has never been used in poultry. Hanninen WDT: page 2, ¶ 3

258. In Spain, Thailand and Portugal, enrofloxacin has been used in poultry. Hanninen WDT: page 2, ¶ 3

259. Spain, Thailand and Portugal are common destinations for Finnish tourists. Hanninen WDT: page 2, ¶ 3

260. The fluoroquinolone resistance among Finnish poultry *Campylobacter* isolates is low and among Spanish and Thai poultry isolates it is high. Hanninen WDT: page 2, ¶ 3

261. In 1999, ciprofloxacin resistance was very low among the *C. jejuni* isolates from patients who had not traveled abroad before their illness. In contrast, most of the strains from the patients who had been in Spain or Thailand before their illness were ciprofloxacin-resistant. These Finnish results indicate a very low resistance among human domestic *C. jejuni* strains in spite of the fact that these antimicrobial agents have been used for treatment of diarrhea in humans since 1987. The finding that a high percentage of ciprofloxacin-resistant strains from patients who traveled to Spain before their illness in concordant with the Spanish results on high ciprofloxacin resistance among Spanish *C. jejuni* isolates. Hanninen WDT: p. 3, ¶ 4

262. Our results from Finland show that where fluoroquinolones are not used in poultry, there is a high level of susceptibility to fluoroquinolones among chicken and human *C. jejuni* strains even after more than ten years of fluoroquinolone use to treat human diarrhea. Hanninen WDT: p. 4, ¶ 4

263. In Sweden fluoroquinolones resistance among domestic poultry *Campylobacter* isolates is low because Baytril has not been approved for treatment of poultry. Similarly, fluoroquinolone resistance among human *Campylobacter* isolates of domestic origin seems

to be low, and an increasing resistance has been recognized among isolates from patients who have acquired the infection while traveling in Spain or Thailand. These results are concordant with the Finnish experience. Hanninen WDT: p. 4, ¶ 5

264.	In 1995 Denmark started an integrated program to monitor antibiotic resistance (Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP)) in animals, food and humans. Hanninen WDT: p. 4, ¶ 6

265.	The data suggest that fluoroquinolone resistance in *Campylobacter* has appeared after the use of Baytril in Denmark's chicken industry started after 1993. Hanninen WDT: p. 5, ¶ 6

266.	The number of human patients infected with ciprofloxacin- or nalidixic acid-resistant *Campylobacter* strain acquired in Denmark has increased very much from 1997 to 2000: from 6-7% to 22-25%, respectively. In 2000, 22% of domestic infections were ciprofloxacin resistant when 43% of patients with travel history had ciprofloxacin-resistant strain. In studies at the county level no correlation was found on the quantity of fluoroquinolones used for treatment of human domestic infections and the percentage of NAL resistant human isolates. These results suggest that human clinical use does not produce increased resistance to fluoroquinolones. Hanninen WDT: p. 5, ¶ 6

267.	The Netherlands is one of the first countries where an increased resistance to fluoroquinolones was observed among human *Campylobacter* isolated in the early 1990s (Endtz et al 1991). Baytril was approved for use in the Netherlands, in 1987, and used there extensively in poultry since 1987. Hanninen WDT: p. 5, ¶ 7

268.	In the Netherlands, no fluoroquinolone-resistant human strains of *Campylobacter* were identified in the first half of the 1980s and resistance soon appeared after the use of Baytril started in poultry. During the 1990s, an increasing resistance to fluoroquinolones in *Campylobacter* has been reported in both poultry and human isolates. Hanninen WDT: page 5, ¶ 7

269.	In Spain, fluoroquinolone resistance among chicken strains was nonexistent among strains isolated before 1987. In 1997 – 1998, ten years after the fluoroquinolone use started in poultry in Spain, a high percentage (99%) of *C. jejuni* strains isolated from poultry (fecal and meat samples) were fluoroquinolone-resistant, strongly suggesting association between veterinary medical use of fluoroquinolones and high level of resistance among *C. jejuni* from poultry. Hanninen WDT: p. 6, ¶ 9

270.	Fluoroquinolone resistance of human *Campylobacter* strains in Spain was nonexistent before 1987. In 1997 – 1998, 72% of human strains were fluoroquinolone-resistant. Hanninen WDT: p. 6, ¶ 9

271.	Studies indicate a strong temporal and spatial association between fluoroquinolone-resistant human *Campylobacter* strains and a high level of resistance among chicken *Campylobacter* strains after use of FQs in poultry. Hanninen WDT: p. 6, ¶ 9

272.   *Campylobacter jejuni* and *coli* are zoonotic bacteria. Zoonotic bacteria are those bacteria that can be acquired from animals. Consumption of chicken or handling chicken has been shown in most of the epidemiologic studies from USA and Europe to be a recognized risk factor for acquisition of the infection. Hanninen WDT: p. 7, ¶ 10

273.   Serotyping and molecular typing are important tools in tracing the sources and routes of transmission of human *Campylobacter* infections. Hanninen WDT: p. 7, ¶ 11

274.   Hanninen compared Finnish chicken and human strains of Campylobacter using several genotyping techniques (PFGE, AFLP ribotyping) in combination with serotyping and found several human isolates were identical to those found in chicken. Hanninen WDT: page 7, ¶ 11

275.   An additional evidence for transmission of fluoroquinolone-resistant strains from chickens to humans comes from the studies of Smith et al. (1999) where the authors found that the PCR-RFLP genotypes were partially overlapping among fluoroquinolone-resistant strains from chickens and fluoroquinolone-resistant strains from humans. Hanninen WDT: p. 7, ¶ 11

276.   Jacobs-Reitsma's study indicates a rapid induction of Baytril resistance in chickens colonized with *Campylobacter* and then treated with fluoroquinolones. Hanninen WDT: p. 7, ¶ 11

277.   McDermott's study showed that Baytril exposure at doses used in practical conditions induce high level ciprofloxacin MICs, and that these resistant strains persist weeks after stopping treatment. Hanninen WDT: p. 7, ¶ 12

278.   Rapid and persistent induction of resistance to fluoroquinolones after the use of Baytril in chickens with approved doses has been shown to take place in two separate studies which both have concordant results. Hanninen WDT: p. 8, ¶ 13

279.   Human-to-human transmission of *C. jejuni/C. coli* has not been reported as a significant factor. Hanninen WDT: p. 8, ¶ 13

280.   Poultry meat is frequently contaminated by *Campylobacter*. Hanninen WDT: p. 8, ¶ 13

281.   Many epidemiological studies show an association between poultry and an increased risk for human *Campylobacter* disease. Hanninen WDT: p. 8, ¶ 13

282.   Enrofloxacin has been used for treatment of poultry in a large number of countries all over the world starting from the middle of the 1980s. In all countries which have reported antimicrobial sensitivity data on poultry *Campylobacter* isolates, increasing resistance to enrofloxacin has been reported soon after use has started. In follow-up studies for a longer period of time, such as in Spain or The Netherlands, (where poultry use began in 1987), an increased resistance has been identified in both chicken and human strains. Hanninen WDT: p. 8, ¶ 13

283.   In countries such as the UK, USA, and Denmark where the use in poultry started in the middle of 1990s, the early stages of emerging resistance among chicken and human *Campylobacter* strains have been observed. Hanninen WDT: p. 8, ¶ 13

284.   In countries where fluoroquinolones have never been used for treatment of poultry, enrofloxacin resistance among chicken strains is very low or nonexistent (Finland, Sweden). Similarly fluoroquinolone resistance among human *Campylobacter* strains of domestic origin has been low before the fluoroquinolone era and has remained low even where ciprofloxacin has been in use in human medicine (Finland, Sweden). Hanninen WDT: p. 9, ¶ 13

285.   In countries (Finland, Sweden) where fluoroquinolone resistance among human domestically acquired *C. jejuni* strains is low, high and increasing frequency of resistant strains have been isolated from patients who have acquired the infection in traveling to countries where fluoroquinolone has been in extensive use in poultry (Spain, Portugal, Thailand). Similarly in countries where fluoroquinolone resistance has been increasing among domestic *Campylobacter* isolates (e.g. UK), a more intensive increase in resistant strains has been found among travelers to Spain and Thailand. Hanninen WDT: p. 8, ¶ 13

286.   Human-human transmission in *Campylobacter* infections is extremely uncommon. Hanninen WDT: p. 9, ¶ 17

287.   Ciprofloxacin is frequently used for treatment of human diarrhea including diarrhea caused by *Campylobacter*. Hanninen WDT: p. 9, ¶ 18

Wilma Jacobs-Reitsma (G-1459)

288.   Dr. Jacobs-Reitsma is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

289.   The most important species of *Campylobacter* in relation to human medicine is *Campylobacter jejuni* and to a lesser extent *Campylobacter coli*. Jacobs-Reitsma WDT: p. 2, lines 4-5

290.   The optimum temperature for *C. jejuni* and *C. coli* to grow is 37°C-42°C. Jacobs-Reitsma WDT: p. 2, lines 10-11

291.   The normal body temperature of poultry is 42°C. Jacobs-Reitsma WDT: p. 2, lines 11-12

292.   The normal body temperature of humans is 37°C. Jacobs-Reitsma WDT: p. 2, lines 11-12

293.   Transmission of *Campylobacter* organisms from animals to humans is via food products produced from those colonized animals. Jacobs-Reitsma WDT: p. 2 , lines 17-18

294. Colonized refers to the growth of bacteria in or on an animal. Jacobs-Reitsma WDT: p. 2, lines 18-19

295. During slaughter, intestinal contents of poultry may spread on the carcasses causing contamination of end-products. Jacobs-Reitsma WDT: p. 2, lines 19-21

296. *Campylobacter* is not considered normal intestinal flora of humans. Jacobs-Reitsma WDT: p. 2, line 28

297. *Campylobacter* is considered normal intestinal flora in broilers, laying hens, breeders and turkeys. Jacobs-Reitsma WDT: p. 2, lines 28-29

298. Normal flora refers to the types of bacteria that are present in or on a healthy animal without causing disease. Jacobs-Reitsma WDT: p. 2, lines 29-30

299. Broiler chicks typically become colonized after two weeks of age. Jacobs-Reitsma WDT: p. 2, lines 32-33; p. 7, lines 27-28

300. No clinical symptoms are seen in broiler chicks even when the broiler chicks carry large numbers of *Campylobacter* in their intestines. Jacobs-Reitsma WDT: p. 2, lines 33-35

301. In humans, the replication of *Campylobacter* in the intestines results in acute inflammatory enteritis. Jacobs-Reitsma WDT: p. 2, lines 41-42

302. Humans continue to excrete *Campylobacter* in their feces for several weeks after they have clinically recovered. Jacobs-Reitsma WDT: p. 2, lines 44-45

303. Long term carriage of *Campylobacter* has been observed in patients with immune deficiency. Jacobs-Reitsma WDT: p. 2, lines 45-46

304. *Campylobacter* colonization of broilers is mainly found in the caecum, as well as other parts of the intestinal tract. Jacobs-Reitsma WDT: p. 2, lines 48-49

305. There are approximately $10^7 - 10^9$ (10 million – 1 billion) *Campylobacter* colony forming units (CFUs) per gram of caecal content in a colonized broiler. Jacobs-Reitsma WDT: p. 2, line 49 – p 3, line 2; p. 7, lines 28-30

306. The average concentration of *Campylobacters* in turkeys is between 1.2×10^4 to 1.5×10^7 CFUs of *C. jejuni* per gram of caecal content. Jacobs-Reitsma WDT: p. 3, lines 5-9; p. 7, lines 30-32

307. Colonized broilers excrete the *Campylobacter* bacteria in their droppings and continue to do so during several weeks (at least up to slaughter at 6-7 weeks of age). Jacobs-Reitsma WDT: p. 3, lines 9-11

308. Once colonized, turkeys continue to excrete *Campylobacter* in their fecal droppings until slaughter. Jacobs-Reitsma WDT: p. 3, line 12

309. The number of *Campylobacter* organisms per gram of feces is lower for other food animals such as cattle, pigs and sheep, than in poultry. Jacobs-Reitsma WDT: p. 3, lines 13-15

310. Vertical transmission from breeder flocks to their progeny is not regarded to be of major importance. Jacobs-Reitsma WDT: p. 3, lines 26-27

311. The majority of broilers are colonized with *Campylobacter*. Jacobs-Reitsma WDT: p. 3, lines 37-38, and lines 44-45, and lines 48-49

312. *Campylobacter* is generally isolated for the first time in broilers between 3 and 4 weeks of age. Jacobs-Reitsma WDT: p. 3, lines 38-39

313. Colonization in turkeys starts at between 7-15 days of age. Jacobs-Reitsma WDT: p. 4, lines 8-9

314. In turkeys, flocks remain 100% colonized once *Campylobacter* has become established. Jacobs-Reitsma WDT: p. 4, lines 9-11

315. Soon after the first bird(s) becomes colonized by *Campylobacter*, the other broilers or turkeys in the same poultry house become infected very quickly, most likely through ingestion of contaminated fecal droppings (coprophagia) and later also through contaminated water and feed in open systems. Jacobs-Reitsma WDT: p. 4, lines 13-16; G-1415, p. 19-27

316. In an experiment of a small (400 animal) turkey flock, when three experimentally infected seeder birds colonized with *Campylobacter* were introduced into the flock, the remaining turkeys became infected within 9-12 days. Jacobs-Reitsma WDT: p. 4, lines 18-20

317. Coprophagia means ingestion of feces. Jacobs-Reitsma WDT: p. 4, line 15

318. Colonization in commercial poultry flocks can be with more than one *C. jejuni* and/or *C. coli* subtype at the same time, with a succession of strains appearing. Jacobs-Reitsma WDT: p. 4, lines 27-28

319. Transport-induced stress increases the exterior concentration of *Campylobacter* on birds and shedding of *Campylobacter* that may subsequently result in carcass contamination. Jacobs-Reitsma WDT: p. 5, lines 6-8

320. The transport vehicles and crates used in shipping may be an additional source of contamination between batches of birds and farms. Jacobs-Reitsma WDT: p. 5, lines 9-10

321. Contaminated crates may be a serious risk for *Campylobacter* transmission during partial depopulation of broiler houses. Jacobs-Reitsma WDT: p. 5, lines 13-15; G-1663

322. Contamination of retail poultry with *Campylobacter* is higher than contamination of pork or beef. Jacobs-Reitsma WDT: p. 5, lines 18-20; G-444, p. 467-481

323. Zhang found that when fluoroquinolone-resistant and susceptible strains of Campylobacter are given together in equal numbers to chickens, the fluoroquinolone-resistant strains take over, displacing the susceptible ones. Jacobs-Reitsma WDT: p. 6, lines 17-20

324. During 1992 and 1993, Jacobs-Reitsma tested 187 broiler flocks from 160 farms for the presence of *Campylobacter*. 617 isolates from 150 different flocks were tested for susceptibility to nalidixic acid, flumequine, enrofloxacin, and ciprofloxacin by disc diffusion method. In all, 29.3% were found to be cross-resistant to the quinolones tested. These isolates originated from 38% of the flocks tested, indicating even more widespread existence of quinolone resistant *Campylobacter*. Jacobs-Reitsma WDT: p. 6, lines 43-49

325. In 1994, Jacobs-Reitsma led a study to assess the impact of Baytril therapy on the development of quinolone resistance in *Campylobacter*. Jacobs-Reitsma WDT: p. 7, lines 7-9; G-315

326. In her 1994 study, Jacobs-Reitsma inoculated six groups of broilers with a fluoroquinolone-sensitive *Campylobacter jejuni* strain at 19 days of age. At 26 days of age, five groups of broilers were given Flumesol or Baytril in the drinking water for four days. Those inoculated with fluoroquinolone-sensitive *Campylobacter,* then treated with Baytril, rapidly colonized with fluoroquinolone-resistant *Campylobacter*. One group of broilers was given enrofloxacin on days 1-4 of age, well before they were inoculated with *Campylobacter* on day 19. Jacobs-Reitsma found that treatment of broilers with Baytril before the broilers are colonized with *Campylobacter* does not lead to fluoroquinolone-resistant *Campylobacter*. Jacobs-Reitsma WDT: p. 7, lines 9-16; p. 12, Table 1; G-315

327. *Campylobacter* isolates treated with enrofloxacin by Jacobs-Reitsma in her 1994 study were all found to be cross resistant to nalidixic acid, flumequine and enrofloxacin using a disc diffusion method. Jacobs-Reitsma WDT: p. 7, lines 18-21; G-315

328. Once the first chicken or turkey in a flock becomes infected with *Campylobacter* the rest of the flock quickly becomes colonized with *Campylobacter*. Jacobs-Reitsma WDT: p. 7, lines 32-33

329. Once colonized, both chickens and turkeys tend to stay colonized until slaughter. Jacobs-Reitsma WDT: p. 7, line 34

330. The use of fluoroquinolones in poultry that are colonized with *Campylobacter* selects for fluoroquinolone-resistant *Campylobacter* in those poultry. Jacobs-Reitsma WDT: p. 7, lines 35-36

331. Cross contamination of chickens and turkeys occur during the transport and slaughter of commercially raised chickens and turkeys. Jacobs-Reitsma WDT: p. 7, lines 36-38

332. *Campylobacter* are typically present normally and in high numbers in poultry. Jacobs-Reitsma WDT: p. 7, lines 38-39

333. Treatment of *Campylobacter* colonized broilers with Baytril quickly results in the broilers becoming colonized with fluoroquinolone-resistant *Campylobacter*. Jacobs-Reitsma WDT: p. 12, Table 1; G-315

Heidi Kassenborg (G-1460)

334. Dr. Kassenborg is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

335. *Campylobacter* causes a significant amount of diarrheal illness in the United States. Kassenborg WDT: p. 2, lines 10-11

336. *Campylobacter* is the most commonly reported cause of bacterial gastroenteritis in the United States. Kassenborg WDT: p. 2, lines 11-12

337. *Campylobacter* causes an estimated 2.4 million human infections in the United States annually. Kassenborg WDT: p. 2, line 12; G-410

338. When antibiotics are indicated for the treatment of *Campylobacter* gastroenteritis, the drug of choice is either a fluoroquinolone (e.g., ciprofloxacin) or erythromycin. Kassenborg WDT: p. 2, lines 12-14

339. There is an increasing proportion of human *Campylobacter* isolates resistant to fluoroquinolones in most regions of the world. Kassenborg WDT: p. 2, lines 16-18

340. Poultry is the most frequently identified source of *Campylobacter* infections. Kassenborg WDT: p. 2, lines 20-21

341. In 1998-1999, Kassenborg led a 12 month study in FoodNet sites to look at risk factors associated with non-outbreak related fluoroquinolone-resistant *Campylobacter* infections. Kassenborg WDT: p. 3, lines 1-3; G-337

342. FoodNet is an acronym for the Foodborne Diseases Active Surveillance Network. Kassenborg WDT: p. 3, lines 3-4

343. FoodNet was initiated in 1995 as a collaborative effort among the Centers for Disease Control and Prevention (CDC), the U.S. Department of Agriculture, the U.S. Food and Drug Administration, and selected state health departments. Kassenborg WDT: p. 3, lines 4-7

344.   Kassenborg's study enrolled patients from a population of 20,723, 982 people in FoodNet sites (or 7.7% of the U.S. population). Kassenborg WDT: p. 3, lines 11-12; G-337

345.   The purpose of FoodNet is to better determine the burden of foodborne illnesses including *Campylobacter* infections in the United States. Kassenborg WDT: p. 3, lines 7-8

346.   NARMS is a collaborative effort among the FDA, USDA, and CDC to monitor changes in susceptibility of enteric bacteria to antimicrobial drugs used in animals and humans. Kassenborg WDT: p. 3, lines 21-23

347.   There is no official breakpoint for establishing resistance to ciprofloxacin among *Campylobacter* isolates. Kassenborg WDT: p. 4, lines 3-4

348.   The National Committee for Clinical Laboratory Standards (NCCLS) uses an MIC of ≥ 4 µg/ml for ciprofloxacin resistance to *Enterobacteriaceae*. Kassenborg WDT: p. 4, lines 5-6

349.   The Kassenborg study is a case-control study. Kassenborg WDT: p. 4, lines 9-10; G-337

350.   In the Kassenborg study, of the 858 *Campylobacter* isolates from humans tested for susceptibility to fluoroquinolones, 94 (11%) were fluoroquinolone-resistant. Kassenborg WDT: p. 6, line 3; G-337

351.   In the Kassenborg study, taking a fluoroquinolone antibiotic prior to coming down with illness due to *Campylobacter* infection did not account for the fluoroquinolone-resistant strain of *Campylobacter*. Kassenborg WDT: p. 6, line 19- p. 7, line 4; G-337

352.   In the Kassenborg study, patients with fluoroquinolone-resistant *Campylobacter* infections were not more likely to have taken fluoroquinolones in the month before stool specimen collections than were those with susceptible infections. Kassenborg WDT: p. 6 lines 22-23; p 7, lines 1-4; G-337

353.   Of the 27 foreign travel-associated fluoroquinolone-resistant *Campylobacter* cases found in the Kassenborg study, 9 (33%) traveled to Western Europe, seven (26%) traveled to Mexico, five (19%) each traveled to Asia and South America and one (4%) traveled to Central America. Kassenborg WDT: p. 7 lines 14-17; G-337

354.   58% of patients with fluoroquinolone-resistant *Campylobacter* infections in Kassenborg's study had domestically acquired fluoroquinolone-resistant *Campylobacter* infections. Kassenborg WDT: p. 7, lines 19-22; and p. 9, lines 5-6; G-337

355.   In the univariate analysis comparing cases with their age matched well controls in the Kassenborg study, domestically acquired fluoroquinolone-resistant *Campylobacter* infections were associated with eating chicken or turkey cooked at a commercial establishment during the 7 days before illness onset. Kassenborg WDT: p. 8, lines 3-5; G-337

356. A multivariate model is used to see if identified risk factors are independently statistically significant. Kassenborg WDT: p. 8, lines 9-13

357. Using a stepwise conditional logistic regression in Kassenborg's study, eating chicken or turkey at a commercial establishment was the only risk factor that remained independently associated with illness. Kassenborg WDT: p. 8, lines 11-18; G-337

358. In Kassenborg's study, patients with domestically acquired fluoroquinolone-resistant *Campylobacter* infections were 10 times more likely to report having eaten chicken or turkey at a commercial establishment than were well control subjects (MOR, 10; 95% CI, 1.3-78). Kassenborg WDT: p. 8, lines 18-20; G-337

359. Fluoroquinolone-resistant *Campylobacter* is present on chicken products at U.S. grocery stores. Kassenborg WDT: p. 9, lines 19-21

360. A population attributable fraction is the reduction in incidence that would be achieved if the population had been entirely unexposed compared with its current (actual) exposure pattern. Kassenborg WDT: p. 9, lines 1-3

361. Eating chicken or turkey at a commercial establishment account for 38% of the population-attributable fraction for domestically acquired fluoroquinolone-resistant *Campylobacter* infections in the Kassenborg study. Kassenborg WDT: p. 9, lines 3-5; G-337

362. In the Kassenborg study, 22% of all fluoroquinolone-resistant infections could be attributed to eating chicken or turkey in a commercial establishment. Kassenborg WDT: p. 9, lines 7-8; G-337

363. Poultry is the dominant source of domestically acquired fluoroquinolone-resistant *Campylobacter* infections in the United States. Kassenborg WDT: p. 9, lines 21-22

364. Many studies suggest that fluoroquinolone use in poultry is a major contributor to the increase in human fluoroquinolone-resistant *Campylobacter* infections. Kassenborg WDT: p. 10, lines 2-3

365. Many travel-associated *Campylobacter* cases may also be a consequence of fluoroquinolone use in food-producing animals. Kassenborg WDT: p. 10, lines 4-5

366. The average person's risk for fluoroquinolone-resistant *Campylobacter* infection could potentially be reduced 22% if the risk associated with commercially prepared chicken and turkey were eliminated. Kassenborg WDT: p. 10, lines 17-19

367. Fluoroquinolone use in humans did not contribute directly to the observed resistance in Kassenborg's study. Kassenborg WDT: p. 10, line 22; G-337

368. In Kassenborg's study, patients with fluoroquinolone-resistant *Campylobacter* infections were no more likely to have taken a fluoroquinolone before the specimen was collected than

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were patients with fluoroquinolone sensitive infections. Kassenborg WDT: p. 10, line 22 – p. 11, line 2; G-337

369.   Approximately 320,000 fluoroquinolone-resistant *Campylobacter* infections occurred in 1998 in the United States. Kassenborg WDT: p. 11, lines 8-9; G-337

370.   The Kassenborg study defined fluoroquinolone resistance as a MIC greater than or equal to 4 micrograms per milliliter for ciprofloxacin. Kassenborg WDT: p. 4, lines 2-3.

371.   The Kassenborg study used the following methods: (a) a case was defined as diarrheal illness in a person living in a FoodNet site whose stool sample yielded a *Campylobacter* isolate and who was not part of a recognized outbreak; (b) diarrhea was defined as three or more loose stools in a 24-hour period; (c) one control subject was obtained for each infected person; and (d) controls were persons without infection who were matched by age group to the case. Kassenborg WDT: p. 4, lines 7-20; G-337.

372.   The Kassenborg study defined "foreign travel-associated" cases as *Campylobacter* infection in persons who had traveled outside the United States during the week before their illness onset and "domestically acquired" cases as infection in those who did not travel outside the United States during the week before their illness onset. Kassenborg WDT: p. 5, lines 17-20; G-337.

373.   The Kassenborg study excluded potential case and control subjects if they could not speak English, if they did not have a home telephone, if they or a household member had a confirmed case of *Campylobacter* infection in the 28 days before the potential case subject's stool collection date, or if they were otherwise unable to complete the interview. Kassenborg WDT: p. 5, lines 4-8; G-337.

374.   The Kassenborg study also excluded potential case subjects if their diarrhea started more than 10 days before their stool sample was collected, if they were unreachable by telephone within 21 days after their stool collection date, or if they could not recall their illness onset date and also excluded potential control subjects if they had diarrhea in the 28 days before their matching case subject's onset date. Kassenborg WDT: p. 4, line 23 through p. 5, line 4; G-337.

375.   Of the 858 persons whose fluoroquinolone resistance status was known, 646 (75%) were interviewed and enrolled in the Kassenborg study. Kassenborg WDT: p. 6, lines 5-7; G-337.

376.   Of the 646 persons with a *Campylobacter* infection, 64 persons had a fluoroquinolone-resistant *Campylobacter* infection and 582 persons had a fluoroquinolone-sensitive *Campylobacter* infection in the Kassenborg study. Kassenborg WDT: p. 6, lines 7-8; G-337.

Stuart Levy (G-1463)

377.   Dr. Levy is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

378. APUA is a non-profit organization, founded in 1981, dedicated to research and education on antibiotic use and antibiotic resistance. Levy WDT: p. 1, lines 42-43

379. Overuse of antimicrobials can render them ineffective. Levy WDT: p. 2, line 7

380. Infections caused by multi-resistant bacteria can be difficult or impossible to treat. Levy WDT: p. 2, lines 12-13

381. One way that bacterial antibiotic resistance genes appears is through mutation in the target chromosomal gene. Levy WDT: p. 2, lines 39-40

382. The emergence of fluoroquinolone resistance among *Campylobacter* (following fluoroquinolone use in poultry) involves spontaneous mutation in the target gene for the fluoroquinolones (the gyrase or topoisomerase, enzymes essential for bacterial replication) which prevents the drug's inhibition of the enzyme activity. Levy WDT: p. 3, lines 20-25

383. *Campylobacter* is unique in that the single target gene mutation is enough to produce a sufficiently high level of fluoroquinolone resistance to thwart treatment of the bacteria in a clinical disease. Levy WDT: p. 3, lines 26-28

384. The single target gene mutation seen in *Campylobacter* can explain why fluoroquinolone resistance emerges more rapidly in *Campylobacter* following fluoroquinolone use than in other enteric pathogens as *E. coli* and *Salmonella*. Levy WDT: p. 3, lines 32-35

385. The endogenous multidrug efflux system identified in *Campylobacter* (CmeABC) has only been shown to contribute to intrinsic low-level resistance to a fluoroquinolone and not to clinical resistance levels. Levy WDT: p. 3, lines 37-42

386. In *Campylobacter*, the endogenously expressed efflux pump amplifies the effect of a single mutation in the target gene, making these single gene mutants more easily selected and the resistance they specify more clinically relevant than are single target mutations in other bacteria. Levy WDT: p. 3, lines 42-46

387. Fluoroquinolone resistance in *Campylobacter* is attributable to a chromosomal mutation. Levy WDT: p. 4, lines 1-2

388. Fluoroquinolone resistance in *Campylobacter* is not transferable because this organism is not able to transfer DNA from one strain to another by the mechanism of transformation. Levy WDT: p. 4, lines 1-4

389. The increase in the frequency of fluoroquinolone resistance among the *Campylobacter* associated with poultry is the result of multiplication and spread of the original mutant and not the transfer of the resistance gene itself. Levy WDT: p. 4, lines 4-7

390. The emergence, selection, and mechanism of fluoroquinolone resistance in bacteria is characteristic of the bacterium and not the host animal in which resistance is selected. Levy WDT: p. 4, lines 9-11

391. What we observe in the selection of fluoroquinolone resistance in *Campylobacter* from chickens is what we would expect to see emerge in *Campylobacter* associated with people, turkeys, cattle, pigs, and other animals when given fluoroquinolones. Levy WDT: p. 4, lines 13-16

392. Multiple studies demonstrate the ease with which bacteria, including *Campylobacter,* harbored in animals on farms can be passed via food products from animals to people leading to disease. Levy WDT: p. 4, lines 30-33

393. APUA initiated a two-year project called Facts about Antimicrobials in Animals and the Impact on Resistance (FAAIR) and convened a Scientific Advisory Panel, whose charge was to gather evidence and draw conclusions about human health impacts of antimicrobial use in agriculture. Levy WDT: p. 4, lines 41-46

394. Scientific and medical evidence indicates that resistant pathogens may be transferred directly from food animals to humans through the food supply. Levy WDT: p. 6, lines 11-12; G-1350

395. Antimicrobial resistance may limit treatment options, and increase the number, severity and duration of infections in humans and animals. Levy WDT: p. 6, lines 16-18; G-1350

396. Precise figures describing the extent and quantity of antimicrobial use in food animal production and plant agriculture are not publicly available. Levy WDT: p. 6, lines 44-45; G-1350

397. Resistant infections may be more severe than susceptible infections. Levy WDT: p. 7, lines 20; G-1350

398. Infections caused by resistant pathogens may be more difficult to treat because doctors have to try several different drugs before they find one that is effective. Levy WDT: p. 7, lines 21-23; G-1350

399. Resistance accounts for an additional 17,668 *Campylobacter jejuni* infections, resulting in 95 hospitalizations per year in the United States. Levy WDT: p. 7, lines 37-39; G-1350

400. Resistance to fluoroquinolones, the drug of choice for severe food poisoning in humans, results in an estimated 400,000 more days of diarrhea per year in the U.S. Levy WDT: p. 7, lines 44-46; G-1350

401. In the United States, the total amount of antimicrobials administered to animals is comparable to that used in human medicine. Levy WDT: p. 8, lines 17-18; G-1350

402.   Transfer of bacteria from food animals to humans is a common occurrence. Levy WDT: p. 8, line 22; G-1350

403.   Antimicrobial resistance limits treatment options and increases the number, severity and duration of infection in humans. Levy WDT: p. 8, lines 24-25; G-1350

404.   The loss of antibiotics because of resistance severely limits the clinician's choice for treatment and can lead to death of the patient. Levy WDT: p. 9, lines 26-28; G-1350

405.   Some antibiotics, including the fluoroquinolones, are among the most important antibiotics in the clinician's armamentarium because they are last-resort drugs for multidrug resistant bacterial infections. Levy WDT: p. 9, lines 30-33; G-1350

406.   Fluoroquinolone use in poultry contributes to the selection of fluoroquinolone-resistant *Campylobacter* which may be transferred through the food chain to humans. Levy WDT: p. 9, lines 34-36

407.   Because antimicrobial treatment is usually initiated before the antimicrobial susceptibilities of *Campylobacter* are known, the initial choice of antimicrobial must be made empirically. Levy WDT: p. 9, lines 36-38

408.   The emergence of increasing resistance to the fluoroquinolones among *Campylobacter* and other bacterial pathogens seriously compromises human chemotherapy and can lead to increased morbidity and mortality associated with *Campylobacter* infections. Levy WDT: p. 10, lines 1-4

Catherine Logue (G-1464)

409.   Dr. Logue is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

410.   Stresses associated with transporting poultry from farms to commercial slaughter facilities prior to slaughter, such as the actual transport, pre-slaughter holding and feed withdrawal, can increase pathogen populations such as *Salmonella* and *Campylobacter* in the intestinal tract, fecal material and on carcass exteriors. Logue WDT: p. 2, lines 4-7

411.   Poultry presented for processing can have greater bacterial carcass contamination levels than compared to what was on the birds originally at the farm. Logue WDT: p. 2, lines 8-10

412.   Poultry carcasses provide a significant source of bacterial cross contamination (including *Campylobacter spp.)* of other carcasses during commercial processing. Logue WDT: p. 2, lines 11-14

413.   At the processing level, the gut of live birds is the principal source of *Campylobacter spp.* and can be transferred between the birds' skin during slaughter and processing. Logue WDT: p. 2, lines 16-17

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414.   Chill water and the chilling process can be a significant source of pathogen contamination contributing to cross contamination between carcasses during chilling. Logue WDT: p. 2, lines 18-20

415.   A small number of contaminated carcasses may have an impact in spreading contamination. Logue WDT: p. 2, lines 21-22

416.   Handling (during carcass orientation and hanging), defeathering, and evisceration contribute to cross contamination between carcasses. Logue WDT: p. 2, lines 23-24

417.   Logue studied the prevalence of *Campylobacter*, including fluoroquinolone-resistant *Campylobacter,* from turkey carcasses at slaughter. Logue WDT: p. 2, line 27 – p. 3, line 2; G-1677

418.   Logue's study involved two processing plants; one had a processing rate of 800 carcasses per hour, the other 8000 carcasses per hour. Logue WDT: p. 3, lines 27-31

419.   Turkeys stay in the chill tank for approximately 4 hours. Logue WDT: p. 3, line 27 – p. 4, line 1

420.   Over 1/3 of 2412 turkey carcasses sampled by Logue were positive for *Campylobacter spp.* Logue WDT: p 5, L 22-23; p. 6, lines 27-29; p. 7, lines 9-10

421.   841 of 2412 (34.9%) turkey carcass sampled by Logue were positive for *Campylobacter spp.*

422.   Logue WDT: p. 5, L 22-23; p. 6, lines 27-29; p. 7, lines 9-10

423.   *Campylobacter* isolates recovered and tested from one turkey slaughter plant (processing 800 turkeys/hour) had 20% resistance to erythromycin, 8.8% resistance to ciprofloxacin and 6.6% resistance to nalidixic acid. Logue WDT: p. 6, lines 14-17

424.   *Campylobacter* isolates recovered from one turkey slaughter plant (processing 8000 turkeys/hour) exhibited resistance to nalidixic acid 77.6%, ciprofloxacin 65.3%, and erythromycin 20.4%. Logue WDT: p. 6, lines 17-20

425.   Organic material in chill tanks reduces the effectiveness of chlorine compounds in the chill tanks. Logue WDT: p. 7, lines 20-21

426.   The rate of production influences *Campylobacter* contamination rates of turkey carcasses. Logue WDT: p. 7, lines 23-24

427.   Size and processing line speed are factors influencing overall carcass contamination rates. Logue WDT: p. 7, line 31 – p. 8, line 1

428. *C. jejuni* and *C. coli* are the most common species of *Campylobacter* recovered from turkey carcasses. Logue WDT: p. 8, lines 4-5

429. Logue's study observed multiple-drug resistant *Campylobacter* strains. Logue WDT: p. 8, lines 15-16

430. The high isolation rate of fluoroquinolone-resistant *Campylobacter* from turkey carcasses indicates that fluoroquinolone use in turkey production is selecting for drug resistant variants that could result in fluoroquinolone-resistant *Campylobacter* infections in humans associated with contaminated turkey. Logue WDT: p. 8, line 31- p. 9, line 2

431. Fluoroquinolone-resistant *Campylobacter* infections may not respond to human fluoroquinolone antimicrobials. Logue WDT: p. 9, line 3

432. The use of antimicrobials (i.e., fluoroquinolones) at the farm level is an influencing factor in promoting the selection of antimicrobial resistant *Campylobacter*. Logue WDT: p. 9, lines 4-6

Patrick McDermott (G-1465)

433. Dr. McDermott is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

434. Fluoroquinolones are a class of highly potent antibacterial agents. McDermott WDT: p. 1, lines 45

435. Fluoroquinolone compounds include human agents such as ciprofloxacin and levofloxacin, and the animal drugs, enrofloxacin and sarafloxacin. McDermott WDT: p. 2, lines 1-2

436. Fluoroquinolones are easy to use, have good distribution in the body and are effective against a broad range of bacteria. McDermott WDT: p. 2, lines 2-4

437. Given their ease of use, good distribution in the body, and effectiveness against a broad range of bacteria, fluoroquinolones are a valuable group of compounds for treating bacterial infections. McDermott WDT: p. 2, lines 2-5

438. Currently, the only fluoroquinolone approved for use in poultry in the United States is Baytril™ (enrofloxacin). McDermott WDT: p. 2, lines 5-6

439. Saraflox™ (sarafloxacin) was approved for poultry use in 1995 in the United States, but has since been voluntarily withdrawn from the market by the manufacturer. McDermott WDT: p. 2, lines 6-8

440. As labeled, Baytril antimicrobial solution (3.23%) is approved for treatment of *E. coli* infections in chickens and for *E. coli* and *Pasteurella* infections in turkeys. McDermott WDT: p. 2, lines 11-13

441. Baytril is a water soluble product administered at a final concentration of 25-50 ppm in drinking water, as the sole source of drinking water, for 3 to 7 days. McDermott WDT: p. 2, lines 13-15

442. Like other fluoroquinolones, Baytril acts by binding to DNA gyrase, a gene involved in DNA metabolism. McDermott WDT: p. 2, lines 17-18

443. Mutations in this gene (gyrA) has been linked to fluoroquinolone resistance in *Campylobacter* and many other bacteria. McDermott WDT: p. 2, lines 18-19

444. McDermott conducted an experiment to measure the impact of Baytril when used according to label indications on the development of fluoroquinolone resistance in *Campylobacter jejuni* present in the gut of broiler chickens. McDermott WDT: p. 2, lines 21-24; B-868

445. McDermott's experiment used the following methods: (a) chicks were purchased from a commercial supplier; (b) intestinal colonization was established by inoculating the birds orally at 24 days of age with *C. jejuni*, which had a ciprofloxacin MIC of 0.250 µg/mL; (c) two groups of *Campylobacter*-infected chickens were examined: one control group of infected, non-treated chickens and one group of chickens that were treated with Baytril at the highest dose stipulated on the product label (50 ppm) for 5 consecutive days; (d) fecal samples were collected just prior to treatment and 1, 3, 5, 12 and 21 days after starting fluoroquinolone treatment; and (e) *C. jejuni* isolates were tested for susceptibility to ciprofloxacin and to enrofloxacin to assess development of resistance in the antibiotic-treated groups. McDermott WDT: p. 2, lines 21-40; B-868.

446. In McDermott's experiment, within 24 hours of Baytril treatment, the *C. jejuni* present in the chicken gut were seven-fold more resistant to ciprofloxacin than before treatment. McDermott WDT: p. 3, lines 1-3; B-868

447. In McDermott's experiment, within 24 hours of Baytril treatment, ciprofloxacin minimum inhibitory concentrations (MICs) increased from a base of 0.250 µg/mL to 32 µg/mL; enrofloxacin MICs increased from 0.06 µg/mL to 8 µg/mL. McDermott WDT: p. 3, lines 3-5; B-868

448. In McDermott's experiment, resistant bacteria remained in the birds throughout the life span of production birds (up to 7 weeks of age). McDermott WDT: p. 3, lines 6-7; B-868

449. In McDermott's experiment, fluoroquinolone-resistant organisms appeared rapidly and did not go away. McDermott WDT: p. 3, lines 8-9; B-868

450. In McDermott's experiment, no resistant *Campylobacter* isolates were detected in the non-Baytril treated control group. McDermott WDT: p. 3, lines 9-10; B-868

451. In McDermott's experiment, the action of Baytril itself was responsible for causing the observed resistance. McDermott WDT: p. 3, lines 10-11; B-868

452. In McDermott's experiment, in the Baytril-treated group, 100% of isolates displayed high-level resistance to ciprofloxacin ($\geq$32 µg/mL). McDermott WDT: p. 3, lines 11-12; B-868

453. McDermott's study was published in the Journal of Infectious Diseases. McDermott WDT: p. 3, lines 12-13; B-868

454. McDermott found that isolates with high level MICS ($\geq$ 32 µg/ml) contain a single gyrA mutation resulting in an amino acid substitution at position 86 from threonine to isoleucine. McDermott WDT: p. 4, lines 5-7; B-868

455. Only a single *gyrA* mutation is necessary to confer fluoroquinolone resistance in *Campylobacter*. McDermott WDT: p. 4, lines 8-9

456. Jacobs-Reitsma found fluoroquinolone-resistant *Campylobacter* emerging during enrofloxacin treatment of broilers. McDermott WDT: p. 4, lines 13-14; G-315

457. Jacobs-Reitsma observed fluoroquinolone-resistant *Campylobacter* organisms persisted for two weeks following enrofloxacin treatment. McDermott WDT: p. 4, line 15; G-315

458. Fluoroquinolone treatment does not eliminate *Campylobacter* from the intestinal tract of chickens, but rather, rapidly selects for fluoroquinolone-resistant isolates. McDermott WDT: p. 4, lines 21-23; G-315; B-868

459. Fluoroquinolone-resistant isolates remain weeks after stopping exposure to the drug. McDermott WDT: p. 4, lines 21-23; G-315; B-868

460. Zhang found fluoroquinolone-resistant *Campylobacter* in chickens emerging within 24-48 hours after Baytril treatment. McDermott WDT: p. 4, lines 27-30; A-190

461. Zhang found fluoroquinolone-resistant *Campylobacter* in chickens persisted after ending Baytril treatment. McDermott WDT: p. 4, lines 27-31; A-190

462. A single genetic change in the gyrase gene (*gyrA*) confers high-level fluoroquinolone resistance. McDermott WDT: p. 4, lines 32-33

463. There is emergence of high-level MICs in *C. jejuni* following Baytril treatment. McDermott WDT: p. 4, lines 36-37

464.   Newell observed resistance to enrofloxacin and ciprofloxacin measured by a 7-8 fold increase in MICs in all *C. jejuni* recovered 48 hours after starting Baytril treatment of the chickens. McDermott WDT: p. 4, lines 38-39; G-1465 Attachment, p. 25

465.   Newell found a single mutation in the gyrase gene (gyrA) conferred fluoroquinolone-resistance in *Campylobacter*. McDermott WDT: p. 4, lines 39-41

466.   There is a bimodal MIC distribution of fluoroquinolone-resistant *Campylobacter* isolates in both chickens and humans. McDermott WDT: p. 4, line 44-p. 5, line 5; G-1517; G-99; B-868

467.   McDermott found that *Campylobacter* isolates from chickens are either susceptible to fluoroquinolones or highly resistant to fluoroquinolones. McDermott WDT: p. 5, line 6

468.   A single point mutation in the gyrase gene (gyrA) occurs in approximately 1 to 5 in 100 million cells (i.e., 1-5 in 10^8 cells). McDermott WDT: p. 5, lines 9-10

469.   The single point mutation in the gyrase gene (gyrA) in *Campylobacter* leads to high level fluoroquinolone resistance. McDermott WDT: p. 5, lines 9-11

470.   The cells involved in the point mutation in the gyrase gene in *Campylobacter* are also cross resistant to other fluoroquinolones. McDermott WDT: p. 5, lines 10-12

471.   In *E. coli*, two genetic mutations are necessary for high level resistance in *Campylobacter*. McDermott WDT: p. 5, lines 13-14

472.   The probability of high level resistance appearing in a fully susceptible *E. coli* cell is much lower than in a *Campylobacter* cell. McDermott WDT: p. 5, lines 15-16

473.   *Campylobacter* are present in the chicken gut at approximately $10^5 - 10^9$ organisms per gram of fecal material. McDermott WDT: p. 5, lines 19-20

474.   Whenever chickens are exposed to a fluoroquinolone in the manner indicated on the Baytril product label, the susceptible cells rapidly die off allowing the naturally resistant variants to quickly take over and multiply, colonizing the chicken with fluoroquinolone-resistant *Campylobacter*. McDermott WDT: p. 5, lines 21-24

475.   The prevalence of fluoroquinolone-resistance among *Campylobacter jejuni* is high compared to other intestinal organisms such as *E. coli*. McDermott WDT: p. 5, lines 28-29

476.   *Campylobacter* are intrinsically less susceptible to fluoroquinolones than are other enteric organisms. McDermott WDT: p. 6, lines 2-3

477.   In *Campylobacter*, a continuously-active efflux pump encoded by the *cmeB* gene has been shown to contribute much of the baseline fluoroquinolone resistance in this organism. McDermott WDT: p. 6, lines 4-6

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478. Mutations in the gyrA genes are essential to impart clinically significant resistance. McDermott WDT: p. 6, lines 6-7

479. The widespread dissemination of fluoroquinolone resistance does not emerge in the absence of direct selection pressure brought about by fluoroquinolone exposure. McDermott WDT: p. 6, lines 9-11

480. In the poultry production environment, the multiplication of resistant *Campylobacter* under fluoroquinolone selection pressure is the major means of the emergence and dissemination of fluoroquinolone-resistant *Campylobacter* in chickens and turkeys. McDermott WDT: p. 6, lines 13-16

481. In one study, *Campylobacter* was found in both organic and conventionally raised chickens, but fluoroquinolone-resistant *Campylobacter* was found in very low levels (i.e. 1%) in organic flocks compared to very high levels (up to 90%) in conventionally raised flocks. McDermott WDT: p. 6, lines 18-21

482. Zhang observed that when a mixture containing equal numbers of fluoroquinolone-resistant and fluoroquinolone-susceptible stains are introduced into a chicken, the fluoroquinolone-resistant strains consistently out-compete the susceptible strains. McDermott WDT: p 6, lines 23-26; G-1746; G-1465 Attachment, p. 26-39

483. Zhang's results suggest that once a fluoroquinolone drug is introduced into a poultry house, a resistant strain has an advantage over susceptible strains in colonizing other birds, even in the absence of concurrent drug exposure. McDermott WDT: p. 6, lines 27-29

484. Fluoroquinolone antibacterial activity is based on dose-dependent pharmacokinetics. McDermott WDT: p 6, lines 38-39

485. Dose-dependent pharmacokinetics means the peak concentration of the drug at the infected site, rather than the time of the drug at the infected site, is the parameter that predicts efficacy. McDermott WDT: p. 6, lines 39-41

486. Ideally, a peak concentration 8 - 10 times the MIC is needed to kill *Campylobacter*. McDermott WDT: p. 6, lines 41-42

487. Fluoroquinolone concentrations near or below the MIC are more apt to select for fluoroquinolone-resistant bacteria. McDermott WDT: p. 6, lines 42-43

488. The current method of medicating chickens is by treating the entire house via water, even though relatively few birds may be ill at the time. McDermott WDT: p. 6, line 46- p 7, lines 2

489. The practice of treating the entire poultry house via water exposes more organisms to the antimicrobial and is therefore more likely to result in the emergence of resistance. McDermott WDT: p. 7, lines 2-3

490. Medication of poultry via the drinking water does not always ensure an adequate dose of active enrofloxacin is taken up by the treated birds. McDermott WDT: p. 7, lines 6-8; G-52

491. Lack of control over the amount of water consumed by the chickens, especially older birds, may result in sub-optimal dosing (*i.e.,* doses <8 - 10 times the MIC). McDermott WDT: p. 7, lines 8-10

492. Suboptimal dosing increases the probability of selecting for resistant *Campylobacter* in both healthy and diseased birds. McDermott WDT: p. lines 10-11

493. Resistant *Campylobacter* persist long after stopping fluoroquinolone treatment. McDermott WDT: p. 7, lines 13-14

494. Animals previously medicated with fluoroquinolones will carry fluoroquinolone-resistant *Campylobacter* in their intestines at slaughter. McDermott WDT: p. 7, lines 14-15

495. Retail meat surveillance studies regularly find that 70 - 80% of retail chicken is contaminated with *Campylobacter*. McDermott WDT: p. 7, lines 18-19

496. Approximately one quarter to one third of *Campylobacter*-contaminated retail chicken meat products carry a fluoroquinolone-resistant strain. McDermott WDT: p. 7, lines 20-21

497. The use of nalidixic acid in *Campylobacter* speciation has likely resulted in a substantial under-estimation of fluoroquinolone resistance among *C. jejuni/coli* reported to local and national surveillance systems. McDermott WDT: p. 7, lines 32-34

498. The use of Baytril in chickens rapidly produces high-level fluoroquinolone resistance in *Campylobacters* residing in the chicken intestine. McDermott WDT: p. 7, lines 38-40

499. The use of fluoroquinolones in poultry is the leading cause of the emergence and dissemination of fluoroquinolone-resistant *Campylobacter* in poultry. McDermott WDT: p. 7, lines 41-43

500. The use of fluoroquinolones in poultry is a significant cause of fluoroquinolone-resistant foodborne *Campylobacter* infections in humans. McDermott WDT: p. 7, lines 44-45

Jianghong Meng (G-1466)

501. Dr. Meng is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

502. Leakage of intestinal contents during the slaughtering process almost inevitably contaminates poultry carcasses with *Campylobacter*. Meng WDT: p. 1, lines 38-40

503.	Most retail fresh chicken carcasses and some turkey carcasses are contaminated with *Campylobacter*. Meng WDT: p. 1, lines 41-42

504.	In Zhao's survey of 184 chicken carcasses and 172 turkey breasts bought from retail stores in the Washington, D.C. area between June 1999 and July 2000, the prevalence of *Campylobacter* was 70.7% in chickens and 14.5% in turkeys. Meng WDT: p. 2, lines 26-34; G-727

505.	In Zhao 's study, approximately half (53.6%) of the isolates were identified as *C. jejuni*, 41.3% as *C. coli*, and 5.1% as other species. Meng WDT: p. 3, lines 14-16; G-727

506.	In Zhao's study, both *C. jejuni* and *C. coli* were isolated more frequently from retail chicken than from turkey, pork, or beef. Meng WDT: p. 3, lines 16-17; G-727

507.	In Zhao's study, *C. coli* was more often recovered from retail turkey samples than *C. jejuni*. Meng WDT: p. 3, lines 17-18; G-727

508.	In vitro antimicrobial susceptibilities of 378 *Campylobacter jejuni* and *coli* isolates from 159 contaminated retail raw meats (130 chicken, 25 turkey, 3 pork, and 1 beef) analyzed by Ge showed resistance among the *Campylobacter* poultry isolates to erythromycin (54%), nalidixic acid (41%), and ciprofloxacin (35%). Meng WDT: p. 3, lines 24-29; G-1778

509.	*C. coli* isolates displayed significantly higher resistance rates (p<0.05) to ciprofloxacin and erythromycin than *C. jejuni* in Ge's study. Meng WDT: p. 3, lines 31-32; G-1778

510.	Turkey isolates, from either *Campylobacter* species, showed significantly higher resistance rates (p<0.05) to ciprofloxacin and erythromycin than *Campylobacter* isolates from retail chickens in Ge's study. Meng WDT: p. 3, lines 32-35; G-1778

511.	Ge found that multi-drug resistant *Campylobacter* were commonly present in poultry products. Meng WDT: p. 3, lines 35-36; G-1778

512.	All the ciprofloxacin-resistant *Campylobacter* analyzed by Ge were also resistant to nalidixic acid. Meng WDT: p. 3, lines 39-40; G-1778

513.	Ge found co-resistance to ciprofloxacin and erythromycin in *Campylobacter* from 41 (26%) of 159 contaminated meat samples. Meng WDT: p. 3, lines 43-44; G-1778

514.	Multi-drug resistance, including co-resistance to fluoroquinolones and erythromycin (a macrolide antimicrobial), has been identified in *Campylobacter* isolated from retail meat products and from humans. Meng WDT: p. 3, lines 44-47G-549; G-191; G-1778

515.	Co-resistance to fluoroquinolones and erythromycin in *Campylobacter* is highly undesirable because those two antimicrobials are generally advocated as first-line drugs for treatment of human campylobacteriosis. Meng WDT: p. 4, lines 1-4

49

516.   In *Campylobacter,* acquired resistance to fluoroquinolones appears to be due mostly to mutations in genes *(gyrA)* encoding DNA gyrase. Meng WDT: p. 4, lines 10-11

517.   Cloning and sequencing of the *gyrA* gene show that mutations in *gyrA* at positions Thr-86, Asp-90, and Ala-70 can be detected in fluoroquinolone-resistant isolates. Meng WDT: p. 4, lines 12-14

518.   Point mutations in *gyrA* occur frequently. Meng WDT: p. 4, line 32

519.   Contamination with *C. jejuni* and *C. coli* is widespread in poultry. Meng WDT: p. 4, lines 42-43

520.   Poultry products often become contaminated during processing. Meng WDT: p. 4, lines 43-44

521.   *Campylobacter* often survives food processing and storage. Meng WDT: p. 4, lines 44-45

522.   *Campylobacter* are present in most retail chicken meats and some retail turkey meats. Meng WDT: p. 4, lines 44-46

523.   Many *Campylobacter* isolates recovered from retail poultry carcasses are resistant to antimicrobials. Meng WDT: p. 5, lines 1-3

524.   Fluoroquinolone use in poultry has contributed to an increase of fluoroquinolone-resistant *Campylobacter* on poultry carcasses. Meng WDT: p. 5, lines 4-6

525.   Retail poultry meat is a source of fluoroquinolone-resistant *Campylobacter* and subsequent human campylobacteriosis infections. Meng WDT: p. 5, lines 6-8

Carolyn Minnich (G-1467)

526.   Dr. Minnich is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

527.   The average chicken plant slaughters over 60,000 birds per shift. Minnich WDT: p. 2, lines 27-28

528.   Chicken plants usually operate 1 - 2 shifts per day, 5 days per week. Minnich WDT: p. 2, line 28

529.   Live chickens arrive at slaughter plants in crates which are stacked on top of each other on the back of tractor trailers. Minnich WDT: p. 2, line 32-33

530.   A tractor trailer contains between 20-1000 chicken crates, depending on the size of the crates. Minnich WDT: p. 2, lines 34-35

531.   A plant may slaughter up to 14 or more trucks of chickens per shift. Minnich WDT: p. 2, lines 35-36

532.   Chickens remain on the trucks after arrival at the plant for 1-8 hours. Minnich WDT: p. 2, lines 37-38

533.   Chickens are unloaded from the crates onto conveyor belts that transport them inside of the plant. Minnich WDT: p. 2, lines 38-39

534.   Chickens are slaughtered by cutting their necks (by hand or through the use of a mechanical blade). Minnich WDT: p. 2, lines 41-42

535.   The chicken scalder tank is between 60-120 feet long and contains over 2000 gallons of water. Minnich WDT: p. 2, lines 42-43

536.   The water in the chicken scalder tank is 130°F or greater. Minnich WDT: p. 2, lines 43-44

537.   The purpose of the scalder is to allow the chicken's feathers to be more easily removed. Minnich WDT: p. 2, lines 44-45

538.   Fresh water is added to the scalder constantly and it takes approximately 1 - 3 hours for the water in the chicken scalder to completely exchange. Minnich WDT: p. 3, lines 1-2

539.   There are 300 or more chickens in the scalder at any given time and they remain there for 1-3 minutes. Minnich WDT: p. 3, lines 2-3

540.   Mechanical picking machines remove feathers from chicken carcasses. Minnich WDT: p. 3, lines 5-6

541.   Picking machines are metal cabinets with rubber projections that vibrate. Minnich WDT: p. 3, lines 6-7

542.   During evisceration, chickens' body cavities are opened mechanically. Minnich WDT: p. 3, lines 21-22

543.   Neither water nor antimicrobial washes eliminate all bacteria from chicken carcasses. Minnich WDT: p. 4, lines 1-2

544.   Chicken chilling tanks are metal structures that are 125-140 feet or more in length. Minnich WDT: p. 5, lines 4-5

545.   Chicken chilling tanks hold over 20,000 gallons of water when full. Minnich WDT: p. 5, lines 5-6

546. Chickens remain in the chill tank for approximately 1 to 2 hours. Minnich WDT: p. 5, lines 6-7

547. Water in the chicken chill tank is exchanged every 3-5 hours. Minnich WDT: p. 5, lines 10-11

548. Live turkeys are transported to the slaughter plant in crates approximately 18 cubic feet in size, containing 8 - 24 turkeys each. Minnich WDT: p. 6, lines 22-23

549. An average turkey transport truck will hold 32 or more crates. Minnich WDT: p. 6, lines 23-24

550. A turkey slaughter plant may slaughter up to 20 - 30 trucks of turkeys per shift. Minnich WDT: p. 6, line 24

551. The average turkey slaughter plant operates 1 - 2 shifts per day, 5 days per week. Minnich WDT: p. 6, lines 24-25

552. About 23,000 – 60,000 turkeys or more are slaughtered per day at the average turkey slaughtering plant. Minnich WDT: p. 6, lines 25-26

553. The turkey scalder used is about 40-65 feet long and contains over 7000 gallons of water. Minnich WDT: p. 6, lines 28-30

554. It takes approximately 3 hours for the water in the turkey scalder plant to completely exchange. Minnich WDT: p. 6, lines 30-31

555. There are over 100 turkeys in the scalder at any given time and they remain in the scalder for 1-3 minutes. Minnich WDT: p. 6, lines 31-32

556. Turkey slaughter plants have less mechanical equipment than seen in chicken slaughter plants. Minnich WDT: p. 6, line 12-13

557. Variation in turkey process size makes it more practical to manually process turkeys rather than try to fit and adjust equipment to a variety of bird sizes. Minnich WDT: p.6, lines 18-19

558. Evisceration of turkey carcasses is accomplished manually rather than mechanically. Minnich WDT: p. 6, line 37

559. Chill tanks in turkey slaughter plants are over 160 feet in total length. Minnich WDT: p. 7, line 2

560. Chill tanks in turkey slaughter plants hold over 120,000 gallons of water when full. Minnich WDT: p. 7, lines 2-3

561. Turkey carcasses remain in the chiller for approximately 3 - 6 hours. Minnich WDT: p. 7, line 3

562. The water in the turkey chill tank is exchanged approximately once per shift. Minnich WDT: p. 7, lines 7-9

563. Line speeds at chicken slaughter plants range from 70 - 175 chickens per minute. Minnich WDT: p. 5, line 43- p. 6, line 8

564. Line speeds at turkey slaughter plants range from 30 - 51 turkeys per minute. Minnich WDT: p. 7, lines 16-19

565. It is possible for chickens and turkeys that were free from *Campylobacter* at the farm to become contaminated with *Campylobacter* during the transportation and slaughter process. Minnich WDT: p. 7, lines 23-25

566. There are numerous places where cross-contamination may occur between chickens/turkeys or chicken/turkey carcasses with *Campylobacter* and, if present, fluoroquinolone-resistant *Campylobacter*, and those carcasses without *Campylobacter* in the slaughter plant. Minnich WDT: p. 7, lines 25-28

567. Even when equipment is washed in water containing an antimicrobial agent such as chlorine, when equipment is not completely rinsed clean of debris between carcasses or when heavily contaminated carcasses are processed, it is possible that cross-contamination between carcasses with bacteria may occur. Minnich WDT: p. 7, lines 28-31

568. Washing equipment with chlorine rinse does not necessarily eliminate bacteria on the equipment. Minnich WDT: p. 7, lines 28-31

569. Product-contact parts of machines are only exposed to antimicrobials for less than 10 seconds before the next carcass comes into contact with the equipment, making it still possible to spread bacteria, such as *Campylobacter*, between carcasses. Minnich WDT: p. 7, lines 33-35

570. Plants operate using equipment for up to 20 hours per day depending on the size of the establishment and number of shifts without cleaning the equipment with soap and water. Minnich WDT: p. 7, lines 36-37

571. Equipment is generally cleaned with chemicals (e.g., soap) once every 24 hours. Minnich WDT: p.7, line 38

572. Tap water rinse is available to plant employees and inspection personnel at evisceration line positions. Minnich WDT: p. 7, lines 38-40

573. Soap is generally not available to plant employees or inspection personnel at evisceration line positions. Minnich WDT: p. 7, line 41

574. Personnel rarely rinse their hands in tap water between each carcass. Minnich WDT: p. 7, lines 41-42

575. Line personnel cannot reach hand sinks equipped with soap without leaving their posted positions. Minnich WDT: p. 7, lines 44-46

576. In order to leave the line, an employee (either USDA or plant) would either have to be replaced on the line or the line would have to be stopped until they returned. Minnich WDT: p. 7, line 46 – p. 8, line 2

577. If a utensil is contaminated (either with gastrointestinal contents or by being dropped on the floor), it is usually rinsed in the tap water (without antimicrobials) at the line position before being reused. Minnich WDT: p. 8, lines 4-6

578. When chickens and turkeys are transported to the slaughter plant, and while the animals remain on the trucks awaiting slaughter, the animals are kept in crates stacked on top of each other. Minnich WDT: p. 8, lines 12-14

579. Poultry transport crates have openings on the top, bottom and sides. Minnich WDT: p. 8, line 15

580. It is very easy for feces that may contain bacteria such as *Campylobacter* to spread or drop from one animal to another during the transportation of chickens and turkeys to the slaughter house. Minnich WDT: p. 8, line 15-16

581. The transportation of chickens and turkeys represent a source of contamination and cross-contamination with bacteria, including *Campylobacter* and/or fluoroquinolone-resistant *Campylobacter*. Minnich WDT: p. 8, lines 8-16

582. Conveyer belts used to transport chicken and turkey from the transportation crates into the plant can become contaminated with chicken or turkey feces and contaminate the exterior of the animals with bacteria. Minnich WDT: p. 8, lines 18-20

583. The conveyor belts used to transport chickens and turkeys from transport crates into the plant represents a source of cross-contamination with *Campylobacter* and/or fluoroquinolone-resistant *Campylobacter*. Minnich WDT: p. 8, lines 8-20

584. Mechanical blades or hand held knives represent a source of contamination and cross-contamination with *Campylobacter* and/or fluoroquinolone-resistant *Campylobacter*. Minnich WDT: p. 8, lines 22-27

585. External contamination on the chicken or turkey carcass frequently comes off in the scalding tank. Minnich WDT: p. 8, lines 29-30

586.   Scalder water at chicken plants stays murky brown throughout each day from the dirt and feces in it. Minnich WDT: p. 8, lines 32-33

587.   Scalder water represents a potential for bacterial cross-contamination between chickens. Minnich WDT: p. 8, lines 33-34

588.   The picking machine equipment is not washed between carcasses and represents a source of contamination and cross-contamination with *Campylobacter* and/or fluoroquinolone-resistant *Campylobacter*. Minnich WDT: p. 8, lines 36-38

589.   The head puller bars (used to remove chickens' heads) are not washed in between each carcass and represent a source of contamination and cross-contamination with *Campylobacter* and/or fluoroquinolone-resistant *Campylobacter*. Minnich WDT: p. 8, lines 40-43

590.   The machinery used to detach the feet from the chickens and turkeys present a vehicle for cross-contamination. Minnich WDT: p. 9, lines 1-2

591.   Mechanical equipment used to transfer chickens from the kill line to the evisceration line may present a point of cross-contamination. Minnich WDT: p. 9, lines 5-6

592.   At both the opening/venting and the evisceration steps in chicken and turkey processing, there is a risk of cross-contamination due to the breakage of intestinal contents by plant employees or their equipment. Minnich WDT: p. 9, lines 8-10

593.   Most chicken plants have a visible contamination rate following venting, opening, and evisceration of 5% or more. Minnich WDT: p. 8, lines 12-13

594.   During the viscera removal and separation process in both chicken and turkey processing, scissors or mechanical equipment may transfer bacteria from one carcass to another. Minnich WDT: p. 9, lines 15-17

595.   The chilling tanks for chilling chicken and turkey giblets may be a point of cross-contamination of giblets with bacteria. Minnich WDT: p. 9, lines 19-20

596.   Bacteria from the knife and from employee's hands during final trimming in chicken and turkey processing represents a point of cross-contamination. Minnich WDT: p. 9, lines 26-28

597.   Mechanical or hand held blades during oil gland removal represents a source of contamination or cross-contamination with *Campylobacter* or fluoroquinolone-resistant *Campylobacter*. Minnich WDT: p. 9, lines 30-32

598.   Water in the chill tank represents a point for cross-contamination for chickens and turkeys due to the number of carcasses within it at any given time. Minnich WDT: p. 9, lines 34-35

599. Chill tank water facilitates the spread of bacteria. Minnich WDT: p. 9, lines 34-36

600. Packing and further processing (cut-up and deboning) areas represent a point of cross-contamination. Minnich WDT: p. 9, lines 38-39

Kare Molbak (G-1468)

601. Dr. Molbak is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

602. *Campylobacter spp.* is one of the most common causes of gastrointestinal infections in humans. Molbak WDT: page 2, line 21

603. Gastrointestinal infections are frequently acquired during foreign travel, i.e., traveler's diarrhea. The risk of a gastrointestinal infection is highest for persons traveling from an industrialized country to a less developed region of the world. Traveler's diarrhea is, however, not any different from other gastrointestinal infection; it is usually a food- or waterborne infection acquired away from home. A higher prevalence of fluoroquinolone-resistant *Campylobacter* in isolates from individuals with travel acquired infection than in domestically acquired infections reflects that there is a higher prevalence of resistance in the foreign sources than the indigenous. Molbak WDT: p. 2, line 31 – p. 3, line 6

604. In patients who have moderate-to-severe dysentery (diarrhea with blood), who are elderly, who are presumed to be bacteremic with chills and systemic symptoms, or who are at increased risk of complications such as immunocompromised patients, patients with underlying disease, or pregnant women, antimicrobial treatment may be of significant benefit. Molbak WDT: p. 3, lines 21- 26

605. It is essential to be able to treat *Campylobacter* with antibiotics, and critical to preserve the efficacy of fluoroquinolones. Molbak WDT: p. 3, lines 28-29

606. Campylobacteriosis in the industrialized countries is primarily a foodborne disease, with poultry as a principle source. Molbak WDT: p. 3, lines 32-33

607. Human-to-human transmission of campylobacteriosis is uncommon, and it is therefore the contribution from the poultry reservoir that pays the lead role in the emergence of fluoroquinolone resistance in *Campylobacter*. Molbak WDT: p. 3, lines 34-36

608. The Emerging Infections Program (EIP) Foodborne Diseases Active Surveillance Network (FoodNet) is a collaborative project with the CDC, nine EIP state health departments, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA), and the United States Food and Drug Administration (FDA). FoodNet currently collects data on ten foodborne diseases in the nine sites to quantify and monitor foodborne illnesses in the United States. Molbak WDT: p. 3, lines 42-47

609. Between 1997 and 2001, the prevalence of fluoroquinolone resistance in the United States increased significantly. Molbak WDT: p. 8, line 25

610. While the consumer in the United States had a lower risk of getting a *Campylobacter* infection in 2001 compared with 1996, the risk of getting an infection with a fluoroquinolone-resistant infection had increased. Thus, the 27% decrease in the incidence is more than outweighed by the 61% to 98% increase in proportion of isolates that are resistant. Molbak WDT: p. 8, lines 39-42

611. In Iceland, the incidence of *Campylobacter* infection increased from 15 in 1995 to 157 per 100,000 in 1999. The incidence in 2000 was 87 and in 2001, 78 per 100,000 population. This marked decrease was due to an intervention program that was based on a screening procedure, which ensured that *Campylobacter* positive flocks were diverted to frozen poultry products and *Campylobacter* negative flocks primarily were used for chilled products. Molbak WDT: p. 12, lines 9-13

612. In Belgium, June 1999, the dioxin crisis, caused by dioxin-contaminated feed components resulted in withdrawal of chicken and eggs from the market. Through the sentinel surveillance system, a decrease in *Campylobacter* infections during June 1999 was noticed. A statistical analysis showed a significant decline (40%) in the number of infections, mainly because of the withdrawal of poultry. Molbak WDT: p. 12, lines 15-19, G-672

613. In a Danish study to compare the mortality of patients with a group of individuals without known bacterial gastrointestinal infections (the reference group), 10 persons matched by age, gender and county of residence were randomly selected for every patient with culture-confirmed *Campylobacter*. These persons were all alive on the date of receipt of sample. The researchers obtained information on vital status, date of change of vital status (i.e., date of death or emigration) and county of residence for patients and individuals included in the reference group. Finally, from the National Registry of Patients and the Cancer Registry, the researchers obtained data on all hospital discharges, outpatient attendances (since January 1995) and cancer diagnoses up to 5 years prior to entry in the study, allowing the researchers to control for pre-existing illness (comorbidity). Molbak WDT: p. 12, line 44 – p. 13, line 4. G –1799.

614. The Danish study's analysis included 16,180 *Campylobacter* patients, of which 190 (1.2%) died within one year after the diagnosis of *Campylobacter*. This mortality rate was 2.33 times higher than the background population (95% CI 1.98 to 2.73). In other words, 57% of these deaths were in excess of the background mortality. Among the patients, 695 were identified with one or more diseases included in the comorbidity index. Molbak WDT: p. 13, lines 26-29

615. After the adjustment for underlying conditions, the relative rate decreased from 2.33 to 1.86 (95% CI 1.56 to 2.20). In other words, of 100 deaths occurring after a *Campylobacter* infection, 46 are caused by the bacterial infection, 11 by underlying illness, and 43 are coincidental and may be explained by the general mortality. We estimate, at the current level

of incidence, may be some 25 annual *Campylobacter* deaths in Denmark. Molbak WDT: p. 13, lines 40-44

616.   The relative mortality was highest in the acute phase of *Campylobacter* infection, defined 30 days after episode date. The Danish study found an excess mortality up to one year after *Campylobacter* infection. Molbak WDT: p. 13, lines 47 – 48, and p. 14, Tables 7 and 8

617.   To determine the relative rate of intestinal, extra-intestinal and late-onset complications of *Campylobacter* infections Dr. Molbak and others determined the rate of these diagnoses in the National Registry of Patients, and compared these rates with the rate in the reference population. Of the 16,180 patients with *Campylobacter* infection, one or more diagnoses were found in 269 (1.7%), compared with 1363 (0.8%) of 161,967 persons from the Danish background population. Molbak WDT: p. 15, lines 3-7

618.   In the determination of the relative rates in the Danish study, these diagnoses groups were combined into groups as shown in table 10 of Dr. Molbak's testimony. The scientists found, in particular, elevated risks of Guillain-Barré syndrome, inflammatory bowel disease, acute abdominal conditions, pancreatitis, unexpected death, and reactive or rheumatoid arthritis (Table 10). The elevated risk of acute abdominal conditions, invasive illness, inflammatory bowel disease and arthritis were statistically significant even in the period 91 to 360 days after the *Campylobacter* infection. Molbak WDT: p. 15, lines 10-15 and p.16, Table 10

619.   The findings of the Danish study underscore that *Campylobacter* infection is associated with an excess risk of complications. While the absolute risk for the individual patient may be small, the public health burden is considerable due to the high incidence of *Campylobacter* infections. Molbak WDT: p. 16, lines 25-27

620.   There are data that suggest that infections with fluoroquinolone-resistant *Campylobacter* are associated with an increased morbidity compared with sensitive strains. The a priori expectation is that a detrimental effect of resistance can be demonstrated in patients treated with fluoroquinolones. Among those, the drug may be harmful because it suppresses the normal gut flora (and possibly other side effects), while the patients do not benefit from the drug because the *Campylobacter* is resistant. This scenario will result in longer disease duration among treated patients. Molbak WDT: p. 19, lines 15-21

621.   Smith, Marano, Neimann, and McClellan all showed an increased duration of diarrhea in fluoroquinolone treated patients infected with fluoroquinolone-resistant *Campylobacter* strains compared to patients with fluoroquinolone-susceptible *Campylobacter* strains. Molbak WDT: p. 19, Table 12

622.   Molbak's Table 12 reflects four different studies showing an increased duration of diarrhea in fluoroquinolone-treated patients infected with fluoroquinolone-resistant *Campylobacter* strains. The increased duration of diarrhea of the four studies ranged from 2 additional days of diarrhea to 5 additional days of diarrhea. Molbak WDT: p. 19, Table 12

623. In the Danish study, the risk of a complication was 3.7 times higher in patients with a resistant *Campylobacter* isolate compared to patients with a sensitive *Campylobacter* (95% CI 1.5 to 8.9, p=0.004). Molbak WDT: p. 21, lines 6-7

624. In the period up to one year after *Campylobacter* infection a total of 48 (0.9%) deaths were registered among 5,393 *Campylobacter* patients and 212 (0.4%) deaths among 53,874 referents. Median age among the 48 deaths was 73.4 years (range 10.4-92.3). Overall, patients with *Campylobacter* were 2.42 times (95% CI 1.77 to 3.32) more likely to die than referents in the one year following infection. After adjusting for co-morbidity, the relative rate was 2.41 (95% CI 1.73 to 3.34). Molbak WDT: p. 21, lines 19-24

625. The one-year mortality rate for patients infected with fluoroquinolone-resistant *Campylobacter* strains was 3.73 (95% CI 2.10-6.64) times higher than the general population, compared with a relative rate of 2.01 (95% CI 1.34-3.00) among those with resistant strains (all estimates adjusted for comorbidity). The p-value for homogeneity of the relative rates was 0.08. Molbak WDT: p. 21, lines 26-29

626. Data from Denmark suggest that the detrimental effects of fluoroquinolone resistance in *Campylobacter* is not limited to an increased duration of disease, but that there is an increased risk of intestinal and extraintestinal complications. Molbak WDT: p. 21, line 40 -p. 22, line 1

627. The data from the Danish study corroborates the hypothesis that fluoroquinolone resistance in *Campylobacter,* at the current level of resistance, has a negative impact on public health. Molbak WDT: p. 21, line 40 – p. 22, line 6.

628. It is essential to preserve fluoroquinolone sensitivity in *Campylobacter,* in particular in a global scenario where larger segments of the population have chronic diseases, are elderly, or otherwise vulnerable to severe outcomes after *Campylobacter* infection. Molbak WDT: p. 22, lines 10-12

629. The marked decline in the incidence of *Campylobacter* in the US has in part been set back by an increase in fluoroquinolone resistance in *Campylobacter.* Molbak WDT: p. 22, lines 14-15

630. In Denmark where only very small amounts of fluoroquinolones are used in food production, the prevalence of fluoroquinolone resistance is relatively lower in indigenous infections compared with infections acquired abroad. Molbak WDT: p. 22, lines 17-19

631. Data from Denmark suggest that the mortality of *Campylobacter* infections is underestimated, and confirms that *Campylobacter* infection may be associated with serious late onset complications. The detrimental effects of fluoroquinolone resistance in *Campylobacter* is not limited to an increased duration of disease, but is also associated with an increased risk of intestinal and extraintestinal complications, and possibly also an increased mortality. Molbak WDT: p. 22, lines 21-25

59

J. Glenn Morris (G-1469)

632. Dr. Morris is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

633. There has been a rapid increase in antimicrobial resistance among the bacteria that cause illness in humans. Morris WDT: p. 3, lines 11-12

634. Rates of quinolone resistance among clinical *Campylobacter* isolates are rising. Morris WDT: p. 3, lines 13-14

635. *Campylobacter* species cause approximately 2 million foodborne cases, 10,539 hospitalizations, and 99 deaths each year. Morris WDT: p. 3, lines 17-18

636. *Campylobacter* is the most common bacterial cause of foodborne illness in the United States. Morris WDT: p. 3, lines 18-19

637. *Campylobacter* is the most common bacterial cause of severe diarrheal illness in adults. Morris WDT: p. 3, lines 19-20

638. Diminution of the ability to effectively treat infections due to *Campylobacter* is a major public health concern. Morris WDT: p. 4, lines 1-2

639. *Campylobacter jejuni* causes a gastroenteritis syndrome that may include diarrhea, vomiting, abdominal pain, and fever. Morris WDT: p. 4, lines 3-5

640. Up to 20% of persons with *Campylobacter* infections have grossly bloody diarrhea, making it the most common cause of bloody diarrhea among adults. Morris WDT: p. 4, lines 5-7

641. Severe *Campylobacter* infections can include fever, diarrhea filled with blood and mucus, abdominal pain, muscle aches, and headache. Morris WDT: p. 4, lines 7-9

642. Otherwise healthy adults with *Campylobacter* infections can be totally incapacitated for well over a week. Morris WDT: p. 4, lines 10-12

643. *Campylobacter* has been linked with occurrence of Guillian-Barre syndrome. Morris WDT: p. 4, lines 12-13

644. A clear clinical response has been observed in persons infected with *Campylobacter* who are treated with appropriate antibiotics. Morris WDT: p. 4, lines 18-19

645. Antibiotic therapy early in the course of illness is efficacious. Morris WDT: p. 4, lines 21-22

646. Quinolones have been shown to have clinical efficacy in *Campylobacter* infections. Morris WDT: p. 4, line 23 – p. 5, line 1

647. Fluoroquinolone therapy must be started as soon as possible after onset of illness if it is to have an optimal effect. Morris WDT: p. 5, lines 2-3

648. It usually takes three to four days after a patient sees the doctor for a definitive diagnosis of *Campylobacter* infection to be made, based on the time required for the organism to grow and be identified on a stool culture. Morris WDT: p. 5, lines 6-8

649. Physicians generally resort to empiric therapy while awaiting culture results. Morris WDT: p. 5, lines 8-10

650. The average stool culture costs in excess of $100. Morris WDT: p. 5, lines 10-11

651. Physicians often initiate empiric therapy, and skip the culture. Morris WDT: p. 5, lines 11-12

652. Conn's Current Therapy, published annually, is widely used by clinicians as a practical guide to management of medical problems seen in their day-to-day practice. Morris WDT: p. 5, lines 18-19

653. Conn's Current Therapy recommends ciprofloxacin empiric therapy for diarrheal disease cases in which a bacterial etiology is suspected. Morris WDT: p. 5, lines 21-22

654. Ciprofloxacin has a broad-spectrum clinical activity against a number of enteric pathogens. Morris WDT: p. 5, line 23

655. Erythromycin has limited activity against some enteric pathogens other than *Campylobacter*. Morris WDT: p. 6, lines 1-2

656. Erythromycin is a poor choice for empiric therapy. Morris WDT: p. 6, lines 1-2

657. Kelly's Textbook of Internal Medicine recommends ciprofloxacin for use in patients before culture results are known because of its broad-spectrum activity against a number of bacterial enteric pathogens. Morris WDT: p. 6, lines 3-5

658. Ciprofloxacin tends to be better tolerated by patients than erythromycin. Morris WDT: p. 6, line 6

659. Erythromycin can cause gastrointestinal discomfort. Morris WDT: p. 6, lines 6-7

660. The recent rapid emergence of ciprofloxacin resistance in *Campylobacter* is of clear concern. Morris WDT: p. 6, lines 11-12

661. Ciprofloxacin has demonstrated great utility in management of diarrheal illness. Morris WDT: p. 6, line 17 – p. 7, line 1

Irving Nachamkin (G-1470)

662. Dr. Nachamkin is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

663. *Campylobacter jejuni* is the most common cause of bacterial diarrhea in the United States. Nachamkin WDT: p. 2, lines 31-32

664. *Campylobacter jejuni* is one of the most common causes of bacterial diarrhea worldwide. Nachamkin WDT: p. 2, lines 32-33

665. People acquire *Campylobacter* from contaminated food. Nachamkin WDT: p 2, L 34-35

666. Symptoms of *Campylobacteriosis* include fever, abdominal pain and diarrhea (bloody or watery). Nachamkin WDT: p. 2, lines 35-36

667. 5% - 10% of untreated patients with campylobacteriosis may experience relapse of illness. Nachamkin WDT: p. 2, lines 40-41

668. Fluoroquinolones such as ciprofloxacin are widely used to treat *Campylobacter* infections. Nachamkin WDT: p. 2, lines 44-45

669. Some patients with *Campylobacter* may go on to develop reactive arthritis and Guillain-Barre syndrome, both the result of the body's immune response to the *Campylobacter* infection. Nachamkin WDT: p. 3, lines 6-7

670. In some patients, reactive arthritis, with pain and joint swelling, occurs within 2 weeks following *Campylobacter* infections. Nachamkin WDT: p. 3, lines 8-9

671. It is estimated that 1 per 1000 *Campylobacter* infections results in Guillain-Barre syndrome. Nachamkin WDT: p. 3, lines 9-10

672. Guillain-Barre syndrome is characterized by a sudden onset of paralysis (polio like). Most patients, however, recover from the paralysis and return to a normal life function within 1 year of the onset of the disease. Nachamkin WDT: p. 3, lines 10-13

673. The overall health burden from *Campylobacter* infection is considerable. Nachamkin WDT: p. 3, line 15

674. A recent analysis of more than 16,000 patients with *Campylobacter* infection in Denmark suggests mortality following *Campylobacter* infection is 4/1000 within 30 days with a 1.9 times excess mortality within 2 years. Nachamkin WDT: p. 3, lines 17-23

675.   People can die from *Campylobacteriosis*. Nachamkin WDT: p. 3, lines 21-26

676.   *Campylobacter jejuni* is a gram-negative bacteria. Nachamkin WDT: p. 3, lines 28

677.   *Campylobacter* is microaerophillic. It requires about 5% oxygen to grow and does not grow well in the presence of atmospheric levels of oxygen. Nachamkin WDT: p. 3, lines 31-33

678.   *Campylobacter jejuni* is the major *Campylobacter* species to cause human infection. Nachamkin WDT: p. 3, lines 37-38

679.   Approximately 90 – 95% of human *Campylobacter* infections are caused by *C. jejuni*. Nachamkin WDT: p. 3, lines 39-40

680.   *Campylobacter coli* is thought to cause about 5 - 10% of the infections reported as *Campylobacter jejuni*. Nachamkin WDT: p. 3, lines 40-41

681.   *C. coli* causes a disease identical to *C. jejuni* in terms of gastroenteritis. Nachamkin WDT: p. 3, lines 42-43

682.   *Campylobacter jejuni* is primarily associated with poultry. Nachamkin WDT: p. 4, lines 8-9

683.   Handling raw poultry is a risk factor for sporadic cases of campylobacteriosis. Nachamkin WDT: p. 4, lines 16-17

684.   Ingestion of contaminated poultry/poultry products is a risk factor for sporadic cases of campylobacteriosis. Nachamkin WDT: p. 4, lines 16-17

685.   Eating poultry at restaurants is a risk factor for campylobacteriosis. Nachamkin WDT: p. 4, lines 19-20

686.   Contamination of food products via cross-contamination (i.e., cutting boards) is also a risk factor for infection. This cross contamination with poultry may account for 10 – 50% of human *Campylobacter* infections. Nachamkin WDT: p. 4, lines 20-22

687.   Since January 2000, in Iceland, *Campylobacter* culture-positive poultry were frozen before sale to the general public and culture-negative poultry were allowed to be sold as fresh. Nachamkin WDT: p. 4, lines 34-36

688.   Iceland has experienced a 60% reduction in campylobacteriosis cases from 1999 levels. Nachamkin WDT: p. 4, lines 36-38

689.   Since January 2000, in Norway, *Campylobacter* culture-positive poultry were frozen before sale to the general public and culture-negative poultry were allowed to be sold as fresh. Nachamkin WDT: p. 4, lines 34-36

690. In Norway, cases of campylobacteriosis in 2002 were reduced to 50% of the levels of campylobacteriosis in 2001. Nachamkin WDT: p. 4, lines 39-41

691. Poultry consumption is one of the most important sources for human *Campylobacter* infection. Nachamkin WDT: p. 4, lines 40-41

692. Estimates from experimental human *Campylobacter* infection suggest an infective dose of as few as 500 - 800 *Campylobacter* organisms. Nachamkin WDT: p. 4, lines 45-46; G-67

693. Dose does not appear to be the only factor contributing to campylobacteriosis. Nachamkin WDT: p. 5, lines 1-3

694. In *Campylobacter*, there appears to be strain to strain variation in virulence to humans. Nachamkin WDT: p. 5, line 3

695. In industrialized nations, such as the U.S., there does not appear to be a carrier state for *Campylobacter jejuni*. Nachamkin WDT: p. 5, lines 10-11

696. "Carrier state" refers to a situation in which infected individuals harbor the organisms and may shed them in their feces, but are not ill. Nachamkin WDT: p. 5, lines 11-13

697. In the U.S. and other developed countries, there is a low occurrence of human-to-human transmission of infection. Nachamkin WDT: p. 5, lines 13-15

698. Patients with untreated campylobacteriosis may shed *Campylobacter* organisms in their stool for 2-3 weeks following infection. Nachamkin WDT: p. 5, lines 17-18

699. Diagnosis of campylobacteriosis is made by stool culture. Nachamkin WDT: p. 5, lines 37-38

700. The number of cases of campylobacteriosis is grossly underestimated. Nachamkin WDT: p. 5, lines 43-44

701. Stool cultures are probably performed on only 1 patient for every 20 with infection. Nachamkin WDT: p. 5, lines 44-45

702. Erythromycin may not be tolerated well by patients. Nachamkin WDT: p. 6, lines 3-4

703. Fluoroquinolone agents, such as ciprofloxacin, have very good activity against susceptible *C. jejuni*, as well as for the other major causes of gastroenteritis. Nachamkin WDT: p. 6, lines 5-7

704. Fluoroquinolone treatment is a standard to treating patients with suspected bacterial gastroenteritis because of good tolerance to the drug and its effectiveness against a broad range of bacteria capable of causing diarrhea. Nachamkin WDT: p. 6, lines 7-10

705.　Fluoroquinolones are commonly used to treat serious *Campylobacter* infections and are also used as empiric therapy for travelers' diarrhea and diarrhea of unknown etiology. Nachamkin WDT: p. 6, lines 11-13

706.　From 1982-1992, no fluoroquinolone-resistant *Campylobacter jejuni* were detected at the University of Pennsylvania Medical Center. Nachamkin WDT: p. 6, lines 33-34; G-440

707.　From 1982-1992, erythromycin resistance in *Campylobacter* was 2% overall. Nachamkin WDT: p. 6, line 36; G-440

708.　At the University of Pennsylvania Medical Center fluoroquinolone resistance rates ranged from a low of 8.3% in 1996 to a high of 40.5% in 2001. Nachamkin WDT: p. 6, lines 42-43;G-1517

709.　All but one of the fluoroquinolone-resistant isolates studies at the University of Pennsylvania Medical Center between 1996 – 2001 had a ciprofloxacin MIC $\geq$ 32 μ/mL. Nachamkin WDT: p. 6, lines 43-45;G-1517

710.　There is a temporal relationship between the approval of fluoroquinolones for use in poultry in the U.S. and the rise in the level of fluoroquinolone-resistant *Campylobacter* in humans in the United States. Nachamkin WDT: p. 7, lines 12-14

711.　Fluoroquinolone resistance has not been appreciably observed in New South Wales, Australia, where fluoroquinolones are not permitted for veterinary use. Nachamkin WDT: p. 7, lines 16-19

712.　In genetic fingerprinting to determine bacterial strain similarity, it is impractical to determine the DNA sequence of the enteric bacterial chromosome of each strain for comparison. Nachamkin WDT: p. 7, lines 28-32

713.　Methods successfully used for fingerprinting *Campylobacter* include pulsed-field gel electrophoresis (PFGE), restriction-fragment length polymorphism (RFLP), ribotyping, multilocus sequence typing (MLST), and restriction endonuclease digestion analysis (REA). Nachamkin WDT: p. 7, lines 34-38

714.　PFGE and RFLP have been used to show a link between *Campylobacter jejuni* strains found in turkeys and in humans. Nachamkin WDT: p. 8, lines 1-4

715.　RFLP-fla typing is accurate in identifying *Campylobacter* strains. Nachamkin WDT: p. 6, lines 6-8

716.　In *Campylobacter*, the fla gene is highly variable and can be used to discriminate between strains. Nachamkin WDT: p. 8, line 14

65

717. Studies have shown that human and poultry *Campylobacter* isolates share similar biochemical and genetic characteristics. Nachamkin WDT: p. 8, line 36

718. Piffaretti showed human and poultry isolates were similar. Nachamkin WD: p. 8, lines 38-39

719. Nachamkin found poultry isolates showed strong similarity to strains from humans. Nachamkin WDT: p. 8, lines 42-45

720. K. Smith found retail poultry isolates that exhibited fluoroquinolone resistance were also of the same type as from humans. Nachamkin WDT: p. 9, lines 4-7; G-589

721. Fitzgerald found turkeys are a reservoir for similar *Campylobacter* strains found among human clinical *Campylobacter* isolates. Nachamkin WDT: p. 9, lines 8-11; G-218

722. Stern found some *Campylobacter* isolates from poultry in the production houses had the same fla types as commonly identified in human strains. Nachamkin WDT: p. 9, lines 12-15; B-715

723. Poultry is a major source of human campylobacteriosis. Nachamkin WDT: p. 9, line 18

724. *Campylobacter* strains isolated from humans are similar to those isolated from contaminated poultry. Nachamkin WDT: p. 9, line 24-25

725. Fluoroquinolone-resistant *Campylobacter jejuni* causing human infections has increased dramatically in the United States and is temporally associated with the introduction of fluoroquinolones for use in poultry in the United States. Nachamkin WDT: p. 9, lines 25-28

Geraldine Ransom (G-1472)

726. Ms. Ransom is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

727. USDA Food Safety and Inspection Service conducted two one-year baseline studies on poultry carcass rinsates to determine the prevalence and levels of *Campylobacter jejuni/coli* on young chicken carcasses sampled in USDA-inspected poultry establishments. Ransom WDT: p. 2, line 8-11.

728. In the 1994-1995 FSIS baseline study in chickens, the in-plant FSIS inspector randomly selected post-chiller young chicken carcasses for submission to one of three pre-assigned FSIS field laboratories (Athens, GA, St. Louis, MO and Alameda, CA); the entire carcass was shipped via FedEx and analysis was initiated the next day; and the laboratory aseptically rinsed the carcass in 400 ml of Buffered Peptone Water and analyzed the rinsate sample. Ransom WDT: p. 2, line 17-22.

729. In the 1999-2000 FSIS baseline study in chickens, the in-plant FSIS inspector rinsed the carcass; a portion of the rinsate was submitted via FedEx to assigned laboratories (i.e., the same laboratories used in the 1994-1995 FSIS baseline study) for testing. Ransom WDT: p. 2, line 22-24.

730. In the 1994-1995 and 1999-2000 FSIS baseline studies in chickens, the rinsate samples were tested using a Most Probable Number format of the FSIS *Campylobacter* method. Ransom WDT: p. 2, line 26-28; G-1472, Attachments 1, 2, 6.

731. In the 1994-1995 and 1999-2000 FSIS baseline studies in chickens, the FSIS *Campylobacter* method consists of two-stage enrichment in Hunt Broth followed by plating onto Modified *Campylobacter* Charcoal Differential Agar where suspect *Campylobacter* colonies were then confirmed based on the following criteria: typical morphology and motility by microscopic examination, oxidase positive, catalase positive, glucose non-fermentative, resistance to cephalothin, and susceptible to nalidixic acid. Ransom WDT: p. 2, line 31-36.

732. Suspect *Campylobacter* colonies that were resistant to nalidixic acid were not identified as C. *jejuni/coli* in the 1994-1995 or 1999-2000 FSIS baseline studies in chickens. Ransom WDT: p. 2, line 36-38.

733. In the 1999-2000 FSIS baseline study in chickens, laboratories were asked to submit suspect nalidixic acid resistant colonies that were encountered to the FSIS Special Projects and Outbreak Support Laboratory in Athens, GA, where they were passed to Dr. Paula Cray (USDA/Agricultural Research Service, Athens, GA) for speciation and antibiotic resistance profiling. Ransom WDT: p. 2, line 38-43; G-1472, Attachments 3, 4, 5.

734. As part of the 1999-2000 FSIS baseline study in chickens, all samples were also analyzed using a method developed by Dr. Eric Line (USDA/Agricultural Research Service, Athens, GA), i.e., the modified ARS method; this part of the study was called "The *Campylobacter* Methods Comparative Study." Ransom WDT: p. 3, line 1-4.

735. The *Campylobacter* Methods Comparative Study consisted of direct plating onto Campy-Line Agar for enumeration of C. *jejuni/coli*, with backup qualitative test (i.e., enrichment in Bolton Broth followed by plating onto Campy-Line Agar) and colonies were identified as C. *jejuni/coli* using the FSIS confirmation protocol where suspect *Campylobacter* colonies were then confirmed based on the following criteria: typical morphology and motility by microscopic examination, oxidase positive, catalase positive, glucose non-fermentative, resistance to cephalothin, and susceptible to nalidixic acid. Ransom WDT: p. 2 line 33-36; p. 3, line 4-8; G-1472, Attachments 3, 4, 5.

736. Laboratories were instructed to submit suspect nalidixic acid resistant *Campylobacter* colonies from Campy-Line Agar to the FSIS Special Projects and Outbreak Support Laboratory in Athens, GA, where they were passed to Dr. Paula Cray (USDA/Agricultural Research Service, Athens, GA) for speciation and antibiotic resistance profiling. Ransom WDT: p. 3, line 8-11.

737. From August 1996 through July 1997, FSIS conducted a baseline study on young turkey carcasses. Ransom WDT: p. 3, line 15-16.

738. In the August 1996 – July 1997 FSIS baseline study in turkeys, whole carcasses were shipped to FSIS laboratories where rinsate samples were prepared using 600 ml of Butterfields Phosphate Diluent and quantitative C. *jejuni/coli* analyses were conducted. Ransom WDT: p. 3, line 16-19; G-651.

Kirk Smith (G-1473)

739. Dr. Smith is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

740. K. Smith's study evaluated 91 percent of all *Campylobacter* isolates from cases of clinical illness in humans from Minnesota in 1996 through 1998. K. Smith WDT: p. 3, lines 3-5; G-589

741. The 1996-1998 sample in K. Smith's study was population-based. K. Smith WDT: p. 3, line 6; G-589

742. In the K. Smith case-comparison study, the comparison group was selected from Minnesota residents infected with *C. jejuni* isolates that were sensitive to quinolones. To be eligible to be chosen as one of the two persons with sensitive *C. jejuni* to be matched to a person with resistant *C. jejuni*, the person with sensitive *C. jejuni* had to meet three criteria: (a) be within ten years of age of the person with resistant *C. jejuni*; (b) live in the same region of Minnesota as the person with resistant *C. jejuni* (either in the seven county Minneapolis St. Paul metropolitan area or elsewhere in Minnesota); and (c) have a date of stool collection that yielded their *C. jejuni* isolate that was as close as possible to the date of stool collection to the person with resistant *C. jejuni*. K. Smith WDT: p. 5, lines 5-17.

743. Nalidixic acid is an antibiotic which belongs to the quinolone family. K. Smith WDT: p. 3, line 20

744. Nalidixic acid is not a fluoroquinolone like ciprofloxacin, but rather is the building block upon which fluoroquinolones are built, i.e., all of the fluoroquinolones are also quinolones. K. Smith WDT: p. 3, lines 20-23

745. If bacteria develop resistance to a fluoroquinolone they are exposed to, they will usually also be resistant to nalidixic acid, the building block base component of all fluoroquinolones. K. Smith WDT: p. 3, lines 23-25

746. Resistance to nalidixic acid was a reliable marker for resistance to fluoroquinolones in the K. Smith study. K. Smith WDT: p. 3, lines 32-33; G-589

747. There are no official interpretive criteria for what constitutes resistance to fluoroquinolones for *Campylobacter*. K. Smith WDT: p. 4, lines 4-5

748. K. Smith used an MIC of 4 μg/ml to define resistance to ciprofloxacin, enrofloxacin and sarafloxacin in *Campylobacter*. K. Smith WDT: p. 4, lines 10-12; G-589

749.　In the K. Smith study, the vast majority (96%) of isolates that were resistant to fluoroquinolones (MIC of ≥ 4 µg per milliliter) actually had MICs of ≥ 32 µg per milliliter. K. Smith WDT: p. 4, lines 19-21; G-589

750.　An MIC of 32 µg/ml represents a stronger resistance than 4 µg/ml. K. Smith WDT: p. 4, lines 23-25

751.　*C. jejuni* is by far the most common species causing human illness. K. Smith WDT: p. 4, lines 32-33

752.　In Minnesota, *Campylobacter jejuni* accounts for 95% of human *Campylobacter* infections. K. Smith WDT: p. 4, lines 33-34

753.　K. Smith's case-comparison study enrolled 96% of Minnesota residents with a quinolone-resistant *Campylobacter jejuni* during 1996-1997. K. Smith WDT: p. 4, line 36-40; G-589

754.　Poultry is a major food reservoir of *Campylobacter* for humans. K. Smith WDT: p. 5, lines 26-27

755.　K. Smith's retail chicken study tested 91 domestic chicken products purchased in Minnesota between September 8, 1997 to November 3, 1997 from 16 retail markets representing 11 franchises. The chicken products came from 15 poultry processing plants in nine states. K. Smith WDT: p. 5, lines 39-42; G-589

756.　K. Smith performed molecular subtyping on 91 *C. jejuni* isolates from retail chicken products to compare them with molecular subtyping on human *C. jejuni* isolates to see if chicken was a source of quinolone-resistant *Campylobacter jejuni* infections in humans. K. Smith WDT: p. 6, lines 16-19; G-589

757.　The DNA fingerprinting method used to evaluate *C. jejuni* from retail chicken in the K. Smith study is called restriction-fragment-length polymorphism of the flagellin gene amplified by polymerase chain reaction (PCR-RFLP). K. Smith WDT: p. 6, lines 19-21; G-589

758.　PCR-RFLP involves a single gene from the bacteria, the flagellin gene, which is amplified and then cut up with an enzyme; the resultant pieces of the gene are spread out using a process known as gel electrophoresis, creating a pattern of bands that represents a DNA fingerprint. K. Smith WDT: p. 6, lines 21-25

759.　In 1997, the PCR-RFLP method was the most widely accepted method of molecular subtyping of *Campylobacter*. K. Smith WDT: p. 6, lines 25-26

760.　K. Smith's study found that the percentage of *C. jejuni* isolates from Minnesota residents that were resistant to nalidixic acid increased from a low of 1.3% in 1992 to 10.2% in 1998;

this increase was statistically significant using a chi-square test for linear trend. K. Smith WDT: p. 7, lines 9-12; G-589

761.   K. Smith found that if an isolate was resistant to nalidixic acid, it is almost always was resistant to ciprofloxacin as well, and vice versa. K. Smith WDT: p. 8, lines 7-9

762.   In the K. Smith study, 285 nalidixic acid resistant isolates were confirmed to be resistant to ciprofloxacin: 1 in 1993, 16 in 1994, 41 in 1995, 44 in 1996, 98 in 1997, and 85 in 1998. K. Smith WDT: p. 8, lines 13-15; G-589

763.   In the K. Smith study, 20 randomly selected ciprofloxacin-resistant isolates from humans were tested for resistance to a variety of fluoroquinolones, including enrofloxacin and sarafloxacin (the two veterinary fluoroquinolones in use in the United States at that time); of the 20 isolates, all were also resistant to enrofloxacin, sarafloxacin, grepafloxacin, and trovafloxacin. K. Smith WDT: p. 8, line 33 – p. 9, line 1; G-589

764.   During K. Smith's study period in Minnesota, fluoroquinolones are the most popular choice of antibiotics for treating patients with *Campylobacter* infections. K. Smith WDT: p. 10, lines 21-22; G-589

765.   Over 80% of people with *Campylobacter jejuni* infections (both fluoroquinolone-resistant and fluoroquinolone sensitive) were treated with an antibiotic in the K. Smith case-comparison study. K. Smith WDT: p. 10, lines 23-25; G-589

766.   Over 60% of people in Minnesota with *Campylobacter jejuni* infections (either fluoroquinolone-resistant or fluoroquinolone sensitive) who received an antibiotic received a fluoroquinolone in the K. Smith case-comparison study. K. Smith WDT: p. 10, lines 25-28; G-589

767.   Travel to Mexico, Spain, and Asia were risk factors for acquiring fluoroquinolone-resistant *Campylobacter* infections in K. Smith's study. K. Smith WDT: p. 9, lines 23-42; G-589

768.   Among the patients from 1997 in the K. Smith case-comparison study who were treated with a fluoroquinolone after the collection of stool specimens, the duration of diarrhea was statistically significantly longer for the patients with quinolone-resistant *C. jejuni* infections (median, 10 days) than for the patients with quinolone-sensitive *C. jejuni* infections (median, 7 days). K. Smith WDT: p. 10, lines 31-35; G-589

769.   K. Smith found that fluoroquinolones were not as effective in treating patients with quinolone-resistant infections as they are in treating patients with quinolone-sensitive infections. K. Smith WDT: p. 10, lines 35-37

770.   In K. Smith's study, patients with quinolone-resistant infections suffered a significantly longer course of illness because the antibiotic provided to them (a fluoroquinolone) did not work against the resistant *C. jejuni*. K. Smith WDT: p. 10, lines 38-40; G-589

771.	In the Goodman randomized double-blinded study, treatment at the time of presentation with ciprofloxacin compared with placebo shortened the duration of diarrhea (2.4 vs. 3.4 days), and increased the percentage of patients cured or improved by treatment days 1, 3, 4, and 5. K. Smith WDT: p. 11, lines 8-11; G-250

772.	In the Wistrom randomized, double-blinded, multicenter clinical trial, a significant difference was noted between norfloxacin and placebo in median time to cure patients with campylobacteriosis (three days compared with five days, p=0.05). K. Smith WDT: p. 11, lines 30-34; G-705

773.	The percentage of all laboratory-confirmed *C. jejuni* infections in Minnesota residents that were quinolone-resistant and domestically acquired increased from 0.8% in 1996 to 4.5% in 1999. K. Smith WDT: p. 12, lines 20-24; G-589

774.	The increase of domestically acquired fluoroquinolone-resistant *Campylobacter jejuni* in Minnesota from 1996-1998 was a statistically significant increase. K. Smith WDT: p. 12, lines 19-24; G-589

775.	In K. Smith's study, 85% of patients with fluoroquinolone-resistant *Campylobacter jejuni* infections between 1996-1998 did not use a quinolone before culture. K. Smith WDT: p. 13, lines 4-7; G-589

776.	In K. Smith's retail chicken study *Campylobacter* was obtained from 80 (88%), including *C. jejuni* from 67 (74%) and *C. coli* from 19 (21%) of the 91 retail chicken products tested. K. Smith WDT: p. 13, lines 13-15; G-589

777.	In K. Smith's retail chicken study, ciprofloxacin-resistant *Campylobacter* was isolated from 18 products (20%), including resistant *C. jejuni* from 13 (14%) and resistant *C. coli* from five (5%). K. Smith WDT: p. 13, lines 16-18; G-589

778.	*Campylobacter* from retail chicken products in K. Smith's retail chicken study had a MIC for ciprofloxacin of ≥ 32 µg per milliliter for all resistant isolates, indicating very strong resistance. K. Smith WDT: p. 13, lines 21-23; G-589

779.	Of eight *Campylobacter* isolates from retail chicken that were tested by K. Smith for resistance to other fluoroquinolones, all eight were resistant to enrofloxacin, sarafloxacin, grepafloxacin, and trovafloxacin; six of the eight were resistant to levofloxacin, and the other two had intermediate resistance to levofloxacin. K. Smith WDT: p. 13, lines 23-26; G-589

780.	Six of the seven subtypes of quinolone-resistant *C. jejuni* recovered from retail chicken products by K. Smith were also identified among quinolone-resistant *C. jejuni* isolates from humans; 6 of 7 had an identical DNA fingerprint as strains found in humans. K. Smith WDT: p. 13, lines 38-41; G-589

71

781. K. Smith found that, in human isolates from 1997 in Minnesota (the same year the poultry products were collected in Minnesota), excluding patients who had taken a quinolone prior to culture, 12 of 13 human *Campylobacter jejuni* isolates were of the same subtype found among *Campylobacter jejuni* from the chickens. K. Smith WDT: p. 13, lines 41-45; G-589

782. Identical subtypes/DNA fingerprints of quinolone-resistant *C. jejuni* were found by K. Smith in domestically acquired human campylobacteriosis cases and domestic retail chicken products in Minnesota. K. Smith WDT: p. 13, line 46 – p. 14, line 1; G-589

783. Chicken is a source of quinolone-resistant *C. jejuni* for humans in Minnesota. K. Smith WDT: p. 14, lines 2-4

784. In K. Smith's study, patients with domestically acquired quinolone-resistant *C. jejuni* infections were 15 times more likely to have a *C. jejuni* subtype that was also found among quinolone-resistant *C. jejuni* isolates from domestic chicken products collected in 1997 than were patients with domestically acquired quinolone-sensitive *C. jejuni* isolates. This link is statistically significant. K. Smith WDT: p. 14, lines 8-12 and 16-18; G-589

785. In K. Smith's study, patients with domestically acquired resistant *C. jejuni* infections were 22.3 times more likely to have a *C. jejuni* subtype that was also found among resistant *C. jejuni* isolates from domestic chicken products than were patients with foreign travel-associated quinolone-sensitive *C. jejuni* isolates. This link is statistically significant. K. Smith WDT: p. 14, lines 12-16; G-589

786. When a large number of subtypes are generated by a subtyping method, two isolates that share an identical subtype are more likely to be related to a common source than if the method yields a small number of subtypes. K. Smith WDT: p. 14, lines 22-25

787. The use of comparison groups to statistically link domestically acquired quinolone-resistant human cases and retail chicken products renders the exact method of subtyping unimportant. K. Smith WDT: p. 14, lines 26-28

788. If the link between domestically acquired quinolone-resistant human cases and retail chicken products was an artifact of the subtyping method used, then the statistically significant finding that domestically acquired sensitive human isolates and foreign travel-associated resistant human isolates were less similar to domestic resistant chicken isolates would not be present. K. Smith WDT: p. 14, lines 28-33

789. Smith found that in Minnesota, there was an increase in quinolone resistance among human *Campylobacter jejuni* isolates from 1.3% in 1992 to 10.2% in 1998. K. Smith WDT: p. 14, lines 35-37; G-589

790. Smith found that domestically acquired resistant infections increased in statistically significant (i.e., the increase likely was not due to chance) fashion from 1996 to 1998 in Minnesota. K. Smith WDT: p. 14, lines 43-45; G-589

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791.   Smith found that domestic chicken products obtained from Minnesota retail markets in 1997 had high rates of contamination with ciprofloxacin-resistant *C. jejuni*. K. Smith WDT: p. 14, lines 46 – p. 15, line 1; G-589

792.   Smith found that the vast majority of resistant strains from domestically acquired human cases in Minnesota in 1997 were identical to resistant strains from the chicken products using a DNA fingerprinting method. K. Smith WDT: p. 15, lines 2-4; G-589

793.   Chicken is a major source of resistant *C. jejuni* for people. K. Smith WDT: p. 15, line 4-13; G-589

794.   K. Smith found a statistically significant link between domestically acquired fluoroquinolone-resistant *Campylobacter jejuni* in human and chicken strains. K. Smith WDT: p. 15, lines 6-7; G-589

795.   Chicken is an important source of fluoroquinolone-resistant *C. jejuni*. K. Smith WDT: p. 15, line 13 and lines 36-37

796.   The use of fluoroquinolones in poultry has had a primary role in increasing resistance to quinolones among *C. jejuni* isolates from humans. K. Smith WDT: p. 15, lines 39-40

797.   Treatment with enrofloxacin of broiler chickens infected with quinolone-sensitive *C. jejuni* does not eradicate these bacteria; rather, it readily selects for quinolone-resistant strains of *C. jejuni*. K. Smith WDT: p. 15, lines 42-45

798.   At most, 15% of domestically acquired fluoroquinolone-resistant *Campylobacter jejuni* infections were due to prior fluoroquinolone therapy in humans during 1996-1998 in K. Smith's study. K. Smith WDT: p. 15, lines 25-26; G-589

799.   There has been a temporal relationship between the licensure of fluoroquinolones for use in food animals, particularly poultry, and a subsequent increase in quinolone-resistant *Campylobacter* isolates from humans in the United States, the Netherlands, Spain, the United Kingdom, Taiwan and Mexico. K. Smith WDT: p. 15, line 45 – p. 16, line 4

800.   Quinolone resistance in *Campylobacter* from humans follows closely after the use of fluoroquinolones in veterinary medicine. K. Smith WDT: p. 16, lines 5-7

801.   Fluoroquinolones had been used in human medicine for years, but significant increases in resistant *Campylobacter* infections in humans did not happen following this use. K. Smith WDT: p. 16, lines 8-10

802.   The increase in fluoroquinolone resistance is particularly striking in Spain, where the percentage of ciprofloxacin-resistant *Campylobacter* isolates increased from 0-3% in 1989 to 30-50% in 1991 following the licensure of enrofloxacin for veterinary use in 1990. K. Smith WDT: p. 16, lines 12-16

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803. There is strong evidence from independent studies in numerous countries throughout the world that fluoroquinolone use in veterinary medicine (not in human medicine) is the primary force behind the increase in fluoroquinolone-resistance in *Campylobacter* infections of humans. K. Smith WDT: p. 16, lines 18-22

804. Studies from Spain demonstrate a temporal relationship between the use of fluoroquinolones in veterinary medicine and an increase in fluoroquinolone-resistant *Campylobacter* isolates in humans. K. Smith WDT: p. 16, lines 34-36

805. In a study from Spain, Perez-Trallero found that from 1990 to 1991 fluoroquinolone resistance in human *Campylobacter* isolates rose from 6.8% to 29%. K. Smith WDT: p. 16, lines 42; G-734

806. Human quinolone therapy does not explain the high prevalence of fluoroquinolone-resistant *Campylobacter* infections found by Perez-Trallero. K. Smith WDT: p. 16, lines 43-44; G-734

807. Perez-Trallero reports that in 1992, 42.5% of *Campylobacter* isolated from humans were resistant to nalidixic acid, and that percentage rose to 50% during the period of January through June 1993. K. Smith WDT: p. 17, lines 2-4; G-491

808. In a study from Spain, Reina found resistance to nalidixic acid (MIC ≥ 32 µg/ml) in 27.2% of 1220 strains of *Campylobacter* from pediatric patients from 1987-1993. K. Smith WDT: p. 17, lines 7-11; G-532

809. Reina found cross-resistance between nalidixic acid and ciprofloxacin in 89.1% of the *Campylobacter* strains isolated from pediatric patients during 1987-1993 tested. K. Smith WDT: p. 17, lines 13-15; G-532

810. In 1987, none of the *Campylobacter* isolated from pediatric patients by Reina were resistance to either nalidixic acid or ciprofloxacin. K. Smith WDT: p. 17, lines 16-17; G-532

811. In 1988 and 1989, Reina found both nalidixic acid and ciprofloxacin resistance to 2.3% and 3.4% of *Campylobacter* isolates from pediatric patients tested, respectively. K. Smith WDT: p. 17, lines 17-19; G-532

812. In 1990, Reina found 19% of the *Campylobacter* isolates from pediatric patients tested were resistant to nalidixic acid and 13% were resistant to ciprofloxacin. K. Smith WDT: p. 17, lines 19-20; G-532

813. In 1991, Reina found 31.8% of the *Campylobacter* isolates from pediatric patients tested were resistant to nalidixic acid and 30.5% were resistant to ciprofloxacin. K. Smith WDT: p. 17, lines 20-21; G-532

814.   In 1992, Reina found 36.7% of the *Campylobacter* isolates from pediatric patietns tested were resistant to nalidixic acid and 32.9% were resistant to ciprofloxacin. K. Smith WDT: p. 17, lines 22-23; G-532

815.   In 1993, Reina found 55.2% of the *Campylobacter* isolates from pediatric patients tested were resistant to nalidixic acid and 48.8% were resistant to ciprofloxacin. K. Smith WDT: p. 17, lines 23-24; G-532

816.   Reina found the rate of nalidixic acid resistance in *Campylobacter jejuni* isolated from humans rose from 0% in 1987 to 2.3% in 1988, to 3.4% in 1989, to 13% in 1990, to 30% in 1991 in Spain. K. Smith WDT: p. 17, lines 27-30; G-529

817.   In a study from Spain, Ruiz found 47.5% of human *Campylobacter jejuni* isolates tested were resistant to both nalidixic acid and ciprofloxacin in 1991; 63.5% of human *Campylobacter jejuni* isolates studied were resistant to both nalidixic acid and ciprofloxacin in 1992; 73% of human *Campylobacter jejuni* isolates studied were resistant to both nalidixic acid and ciprofloxacin in 1993; and 88% of *Campylobacter jejuni* isolates studied were resistant to both nalidixic acid and ciprofloxacin in 1994. K. Smith WDT: p. 17, lines 32-39; G-544

818.   In a study from Spain, Sanchez found 0% of human *Campylobacter* isolates studied were resistant to ciprofloxacin and ofloxacin and 2.6% were resistant to norfloxacin and nalidixic acid in 1988. K. Smith WDT: p. 17, line 46 – p. 18, line 1; G-557

819.   In a study from Spain, Sanchez found, 6.1% of human *Campylobacter* isolates tested were resistant to ciprofloxacin and ofloxacin, 8.1% were resistant to norfloxacin, and 20.4% were resistant to nalidixic acid in 1989. K. Smith WDT: p. 18, lines 2-3; G-557

820.   In a study from Spain, Sanchez found 8.6% of human *Campylobacter* isolates tested were resistant to ciprofloxacin, 8.7% were resistant to ofloxacin, 10.8% were resistant to norfloxacin, and 17.4% were resistant to nalidixic acid in 1990. K. Smith WDT: p. 18, lines 3-5; G-557

821.   In a study from Spain, Sanchez found 50.7% of human *Campylobacter* isolates tested were resistant to ciprofloxacin, 47.6% were resistant to ofloxacin, 52.3% were resistant to norfloxacin, and 58.7% were resistant to nalidixic acid in 1991. K. Smith WDT: p. 18, lines 5-7; G-557

822.   In a study in Spain, Sanchez found 49.5% of human *Campylobacter* isolates tested were resistant to ciprofloxacin, 45.6% were resistant to ofloxacin, 55.5% were resistant to norfloxacin, and 56.8% were resistant to nalidixic acid in 1992. K. Smith WDT: p. 18, lines 7-9; G-557

823.   In a study in Spain, Saenz found that 81% of broilers tested carried *Campylobacter* in 1997-1998. K. Smith WDT: p. 18, line 15; G-549

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824.   Testing of 5,800 isolates of *Campylobacter* isolated from humans in 1996 and 1997 in the United Kingdom revealed that 12% were resistant to ciprofloxacin (at a level of >8 μg/ml). K. Smith WDT: p. 18, lines 31-33; G-632

825.   Studies from Mexico demonstrate a temporal relationship between fluoroquinolone use in veterinary medicine and an increase in fluoroquinolone-resistant *Campylobacter* isolates in humans. K. Smith WDT: p. 18, lines 37-39

826.   Mexico produces a substantial amount of poultry meat; production increased from 1.7 x 10^9 lbs. in 1990 to 3.2 x 10^9 lbs. in 1997. K. Smith WDT: p. 19, lines 7-8

827.   Sales of quinolones for use in poultry, including ciprofloxacin, enrofloxacin, and danofloxacin, increased dramatically in Mexico, from 86 x 10^6 medicated liters in 1993 to 326 x 10^6 medicated liters in 1997. K. Smith WDT: p. 19, lines 8-11

828.   In a study by Chung-Chen in Taiwan, 92% of 12 *C. jejuni* and 91% of 23 *C. coli* isolates from retail chicken products were resistant to ciprofloxacin. K. Smith WDT: p. 19, lines 28-30; G-376

829.   Large amounts of fluoroquinolones are used in poultry in Taiwan. K. Smith WDT: p. 19, lines 30-31

830.   Fluoroquinolone-resistant *Campylobacter* has been isolated in retail chicken and in humans in the United States, the Netherlands, Spain, the United Kingdom, and Taiwan. K. Smith WDT: p. 19, lines 33-36

831.   Fluoroquinolone use in poultry and livestock is widespread in most regions of the world, including Europe, the United States, Asia, Latin America, and South Africa. K. Smith WDT: p. 19, lines 42-44

832.   The use of fluoroquinolones in poultry in foreign countries is an important contributor to infections with resistant *C. jejuni* among travelers to those countries. K. Smith WDT: p. 19, line 46 – p. 20, line 2

833.   Domestically acquired quinolone-resistant *C. jejuni* infections in Minnesota residents increased significantly after fluoroquinolones were licensed for use in poultry in the United States. K. Smith WDT: p. 20, lines 13-15

834.   The temporal relationship between the use of fluoroquinolones in veterinary medicine and the subsequent increase in fluoroquinolone resistance in human *Campylobacter* isolates has been born out again and again in different countries around the world. K. Smith WDT: p. 20, lines 17-21

835.   The presence of fluoroquinolone-resistant *Campylobacter* on retail chicken products has been documented in numerous countries around the world. K. Smith WDT: p. 20, lines 21-23

836. In K. Smith's study of retail chicken purchased in Minnesota in 1997, there is a statistically significant link between resistant *C. jejuni* isolates from retail chicken products and domestically acquired resistant *C. jejuni* in humans. K. Smith WDT: p. 20, lines 24-26; G-589

837. Retail poultry is a primary source of fluoroquinolone-resistant *Campylobacter* for humans in the Unites States and elsewhere in the world. K. Smith WDT: p. 20, lines 29-31

838. Treatment of bacterial gastroenteritis with fluoroquinolones shortens the duration of illness, if the infecting bacteria is susceptible to fluoroquinolones. K. Smith WDT: p. 20, lines 32-35

839. When the infecting *Campylobacter* strain is resistant to fluoroquinolones, and fluoroquinolones are used to treat these infections, the result (on a population level) is treatment failure and a longer duration of illness. K. Smith WDT: p. 20, lines 35-38

840. *Campylobacter* infections can become invasive and life threatening, particularly in the elderly and those immunocompromised for other reasons. K. Smith WDT: p. 20, lines 39-41

841. It often takes 2 days until stool culture and sensitivity results come back. K. Smith WDT: p. 21, lines 4-5

Robert Tauxe (G-1475)

842. Dr. Tauxe is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

843. Foodborne illness in the United States affects 1 in 4 Americans every year. Tauxe WDT: p. 2, lines 6-7

844. CDC has estimated there are 76 million cases of foodborne illnesses that occur each year from all different causes of foodborne infections. Tauxe WDT: p. 2, lines 7-8; G-410

845. Many people who become ill with a diarrheal illness do not visit a physician, either because the symptoms are relatively mild, or because they lack access to affordable care. Tauxe WDT: p. 2, lines 19-21

846. Many people who do not visit a physician with a diarrheal illness are not asked to provide a specimen for culture. Tauxe WDT: p. 2, lines 22-23; G-1790

847. Many specimens that are cultured are not reported, because the infection is not a reportable illness in many states. Tauxe WDT: p. 2, lines 24-25

848. *Campylobacter jejuni* is the most common type of *Campylobacter* that causes illness in humans. Tauxe WDT: p. 2, lines 40-42; G-1475

849. Between 1982 and 1986, among 37,713 human isolates of *Campylobacter* reported through the national *Campylobacter* surveillance system with species data, 37,556, or 99.6% were *Campylobacter jejuni* or *Campylobacter coli,* and of those that were either of the two 99.8% were *Campylobacter jejuni.* Tauxe WDT: p. 3, lines 2-5; G-617

850. Among human *Campylobacter* isolates reported to FoodNet between 1996 and 2000, 97% of *Campylobacter* infections for which a species was reported were either *Campylobacter jejuni* or *Campylobacter coli,* and of those that were either of those two, 95% were *Campylobacter jejuni; Campylobacter jejuni* represented 93% of all reported *Campylobacter* infections for which the species was reported. Tauxe WDT: p. 3, lines 7-11

851. People who become infected with *Campylobacter jejuni* typically become ill after 2-4 days. Tauxe WDT: p. 3, lines 15-16

852. *Campylobacter jejuni* illnesses cause crampy abdominal pain and diarrhea, often with fever and some nausea. The diarrhea often becomes bloody and the cramps severe. Tauxe WDT: p. 3, lines 16-18

853. *Campylobacter jejuni* illnesses usually last 5-7 days. Tauxe WDT: p. 3, lines 18-19 and p. 13, lines 7-8; G-580

854. In 1991, a ten-site collaborative survey of persons with diarrheal illness showed that among those from whom *Campylobacter* was isolated, including both inpatients and outpatients, 97% reported diarrhea, 80% reported abdominal cramps, 59% reported fever, 37% reported bloody diarrhea, 34% reported vomiting, and 21% were hospitalized. These symptoms are caused by the direct effect of the *Campylobacter* on the intestines. Tauxe WDT: p. 3, lines 21-28

855. *Campylobacter* infections can be complicated if the *Campylobacter* moves out of the intestinal tract into the person's bloodstream, causing severe septic illness and sometimes reaching other organs of the body and causing localized infections there. Tauxe WDT: p. 3, lines 30-33

856. In approximately 1 per 1000 infections, the person who is recovering from *Campylobacter* infection develops a severe paralysis that affects the major muscles of the limbs and trunk, starting with the feet, and ascending to affect more and more of the body until the person may be completely paralyzed, including the muscles they need to breathe. Tauxe WDT: p. 3, lines 39-43; G-444

857. The Neal study found that 13% of persons with documented *Campylobacter* infection developed symptoms of reactive arthritis, a very high rate that was specific to *Campylobacter*, more so than other enteric infections. Tauxe WDT: p. 4, lines 19-22; G-1475, p. 50-51

858. In Neal's study, the arthritis symptoms were correlated with the duration of illness. Persons that had a reactive arthritis were 2.7 times more likely to have had an acute illness

lasting more than 15 days than were persons without joint symptoms, a difference that was statistically significant. Neal's study suggests that antibiotic treatment which shortens the duration of illness and thus decreases the stimulation of the immune system could help prevent this complication, though this would need to be evaluated with a formal clinical treatment trial. Tauxe WDT: p. 4, lines 23-34

859. People become infected with *Campylobacter* by swallowing them. This usually occurs because Campylobacter was present on food or in water or other drinks that they consumed. It may also occur if *Campylobacter* is on their hands after contact with something that was contaminated and they put their hands on food or directly in their mouth. Tauxe WDT: p. 4, lines 43-46 and p. 5, line 1

860. In a volunteer feeding trial conducted at the University of Maryland, 800 organisms, the smallest number tested, were sufficient to cause disease in some of the volunteers. Tauxe WDT: p. 5, lines 3-6; G-67

861. *Campylobacter* are microscopic organisms, far smaller than can be seen with the human eye, and millions would fit on the heads of a pin. Tauxe WDT: p. 5, lines 7-8

862. A very small amount of contamination in food may contain enough organisms to cause illness. Tauxe WDT: p. 5, lines 8-9

863. *Campylobacter* does not spread easily from person-to-person. Tauxe WDT: p. 5, lines 36-37

864. Most *Campylobacter* infections are related to consuming food or water that is contaminated with animal feces. Tauxe WDT: p. 6, lines 1-3

865. *Campylobacter* infection has been associated with direct contact with infected animals that may or may not be ill. Tauxe WDT: p. 6, lines 3-4

866. The vast majority of *Campylobacter* infections occur sporadically, not as part of an outbreak. Tauxe WDT: p. 6, lines 14-16

867. A typical epidemiological study would involve interviewing patients with Campylobacter infection about things they had to eat or drink or other exposures they had in the week before they became ill, and comparing the frequency of those exposures with those of another group of people, who lived in the same area and were otherwise similar, but did not have Campylobacter infections. This technique, known as the case-control study, had been used to identify specific risk factors and specific exposures for a number of different infections. Tauxe WDT: p. 7, lines 25-31

868. The case-control study is a standard epidemiologic approach for defining those exposures that precede illness and are likely to be associated with getting the illness. Tauxe WDT: p. 7, lines 31-34

869. Case-controlled studies of sporadic *Campylobacter* infections in the United States and other countries typically identify exposure to undercooked poultry as a source of *Campylobacter* infections. Tauxe WDT: p. 8, lines 5-8

870. In the Washington State study, illness was associated with eating chicken, turkey and Cornish hens, particularly as undercooked. Tauxe WDT: p. 8, lines 10-12; G-268

871. In the Deming study conducted among University of Georgia students, most illness was associated with eating undercooked and even raw chicken. Tauxe WDT: p. 8, lines 13-15

872. *Campylobacter* on chickens, turkeys or other meats is easily transferable in the kitchen to other foods. This can happen via unwashed hands of food handlers, by the use of utensils, first on raw poultry and then on other foods such as fresh fruits or vegetables that might not be eaten before cooking, or because raw poultry drips onto another food. Tauxe WDT: p. 9, lines 29-34

873. In Hopkins's study in Colorado, *Campylobacter* infection was associated with handling raw chicken, as opposed to eating undercooked chicken, and it is likely that the persons became infected as a result of handling the raw chicken in the kitchen, even before it was cooked. Tauxe WDT: p. 9, lines 34-37; B-412

874. In Great Britain, an outbreak was reported at a school for chefs after a training exercise in how to pluck and slaughter a whole chicken. Tauxe WDT: p. 9, lines 38-40; G-1704

875. In Harris's case-control study in Seattle, infection among those eating chicken was strongly associated with not washing the kitchen cutting board and other indicators of cutting board hygiene, suggesting that practices in the kitchen can easily transfer the organisms to other foods. Tauxe WDT: p. 9, lines 41-45

876. Most poultry meat is contaminated with *Campylobacter* by transferring chicken feces to the carcass during the slaughter process. Tauxe WDT: p.10, lines 30-31

877. In one study in the United Kingdom, the number of *Campylobacter* organisms on the surface of a fresh chicken carcass, was estimated at 1,000-1,000,000 organisms per chicken. Tauxe WDT: p. 10, lines 37-39; G-1656

878. A drop of raw chicken juice would often include an infectious dose of 500 organisms. Tauxe WDT: p. 10, lines 40-41

879. The optimal temperature for growth of *Campylobacter jejuni* is the body temperature of a bird. Tauxe WDT: p. 11, lines 2-5

880. In a study from Israel, the most common serogroup isolated from humans was also the most common serogroup isolated from chicken meat. Tauxe WDT: p. 11, lines 35-36; G-1713

881.   In the Netherlands, comparison of biotypes and serotypes of human and animal isolates of *C. jejuni* showed that five of the six most common types present in human isolates were also common in chicken isolates, while there was little overlap with the types found in swine; testing the strains by hippurate hydrolysis, a laboratory test used to separate *C. jejuni* from *C. coli* also showed that poultry strains resembled human strains, while strains from pigs did not. This was particularly noteworthy as the Dutch were reported to eat four times as much pork as chicken. Tauxe WDT: p. 11, line 44-p.12, lines 6; G-1698

882.   In New Zealand, whole cell DNA restriction digest patterns were used to compare *Campylobacter* from a variety of sources, and it was reported that 50% of human isolates had patterns that were indistinguishable from those isolated from poultry. Tauxe WDT: p. 12, lines 9-12; G-1666

883.   *Campylobacter* infections typically last approximately 5-7 days. Tauxe WDT: p. 13, lines 7-8

884.   For persons with defective immune systems, including people with congenital defects in their immune system and people with human immunodeficiency virus infections, *Campylobacter* bacteremia can be a severe, debilitating febrile illness requiring multiple and prolonged courses of antibiotic treatment. Tauxe WDT: p. 13, lines 23-29

885.   Practice guidelines issued by the Infectious Diseases Society of America and CDC recommend considering empiric treatment of diarrheal illness with antibiotics if the diarrhea is visibly bloody, or is associated with fever, while waiting for the results of stool culture. Tauxe WDT: p. 14, lines 8-12

886.   In a survey of the physicians in FoodNet about when they ordered stool cultures, they reported that they ordered a stool culture from 79% of patients with bloody stools, and 40% of those without bloody stools. Tauxe WDT: p. 14, lines 17-19

887.   CDC reported that among persons with diarrhea who consulted a physician, 40% were treated with an antimicrobial agent. Tauxe WDT: p. 14, lines 20-22

888.   The principal textbook of infectious disease used in the U.S. states that treatment with antibiotics seems prudent in those patients with high fever, bloody diarrhea, or more than eight stools per day; in patients whose symptoms have not lessened or are worsening at the time the diagnosis is made; or in those in whom symptoms have persisted for more than 1 week. Tauxe WDT: p.14, lines 42-45, and p. 15, line 1; B-205

889.   The persons at greatest risk for invasive bloodstream infection with *Campylobacter* are the elderly and the immunocompromised. Tauxe WDT: p. 15, lines 4-5

890.   In the laboratory-based surveillance for *Campylobacter* from 1982-1986, 102/29468 or 0.03% of the infections were diagnosed by blood culture. Infection in the bloodstream was lowest, 0.2%, among persons aged 0-39, somewhat higher, 0.3%, among persons 40-69 years

of age, and highest, 1.2%, among persons 70 years old or older. Tauxe WDT: p. 15, lines 5-11

Fred Tenover (G-1476)

891. Dr. Tenover is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

892. PFGE uses the fragments produced by restriction enzyme digestion of the chromosomal DNA in the bacterial cell as means of strain identification. Tenover WDT: p. 2, lines 27-28

893. PFGE involves embedding organisms in an agarose gel matrix, lysing (breaking open) the organisms within the gel, and cleaving the chromosomal DNA into 15-20 fragments using enzymes called restriction endonucleases, then inserting slices of agarose containing the chromosomal DNA fragments into the wells of an agarose gel slab, and separating the DNA restriction fragments into a pattern of discrete bands by applying an electric current to the agarose gel. Tenover WDT: p. 2, lines 28-38

894. In PFGE the DNA restriction patterns of the isolates are compared with one another to determine the genetic relatedness of the bacterial isolates. Tenover WDT: p. 2, lines 38-41

895. Genotype is the genetic makeup of an organism encoded in its DNA. Tenover WDT: p. 3, line 14

896. Phenotype is the external manifestations of an organism's genetic makeup, i.e., how the organism's genes are expressed. Tenover WDT: p. 3, lines 16-17

897. Isolate is a general term for a pure culture of bacteria presumed to be derived from a single organism, for which no information is available aside from its genus and species. Tenover WDT: p. 3, lines 19-21

898. Genetically related isolates (clones) are isolates that are indistinguishable from each other by a variety of genetic typing tests (e.g., PFGE, multilocus enzyme electrophoresis, or ribotyping) or that are so similar that they are presumed to be derived from a common parent. Tenover WDT: p. 3, lines 28-31

899. A strain is an isolate or group of isolates that can be distinguished from other isolates of the same genus and species by biochemical characteristics or genetic characteristics or both. Tenover WDT: p. 3, lines 39-41

900. A strain is a descriptive subdivision of a species. Tenover WDT: p. 3, line 41

901. Genotyping techniques are essential for epidemiologic investigations of the sources of infection and routes of transmission in human and animal illnesses associated with nosocomial and foodborne bacterial pathogens. Tenover WDT: p. 10, lines 4-6

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902. PFGE is a good method for strain delineation of many common bacterial pathogens, and is one of several techniques that have been validated for *Campylobacter* species. Tenover WDT: p. 10, lines 6-8

903. The concentration of an antimicrobial agent (usually in µg/ml) that is required to inhibit the growth of the bacteria in the laboratory test is given is known as the minimal inhibitory concentration, or MIC, of the antimicrobial agent. Tenover WDT: p. 13, lines 9-11

904. In the United States, the National Committee for Clinical Laboratory Standards, or NCCLS, establishes the criteria that are used to interpret the results of antimicrobial susceptibility testing. Tenover WDT: p. 13, lines 13-15

905. The interpretive criteria developed by NCCLS are known as breakpoints. Tenover WDT: p. 13, lines 23-24

906. Breakpoints are used by microbiology laboratories to report the results of their antimicrobial susceptibility tests to physicians. Tenover WDT: p. 13, lines 23-25

907. PK/PD parameters include measurements such as protein binding, the peak serum concentration of the antimicrobial agent in the body (C_{max}) and the total concentration of the drug achievable in the serum over a given time period (as measured by the area under the serum concentration curve, or AUC). Tenover WDT: p. 15, lines 12-16

908. The peak MIC ratio and the 24-h AUC/MIC ratio are major determinants of the activity of fluoroquinolones (e.g., ciprofloxacin). Tenover WDT: p. 16, lines 3-5

909. Peak/MIC ratios should exceed 8, and 24-h AUC/MIC values should be >100, to successfully treat gram-negative infections and to prevent the emergence of resistant organisms during therapy with fluoroquinolones. Tenover WDT: p. 16, lines 5-7

Nathan M. Thielman (G-1477)

910. Dr. Thielman is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

911. Patients with campylobacteriosis characteristically present for medical care with an acute diarrheal illness that is clinically indistinguishable from that caused by *Salmonella, Shigella*, and some *E. coli* bacteria. Thielman WDT: p. 2, ¶ 3

912. In addition to diarrhea, patients with campylobacteriosis frequently complain of abdominal pain, fever, and headaches. Thielman WDT: p. 2, ¶ 3

913. Less frequent symptoms of campylobacteriosis are muscle aches, vomiting and bloody stools. Thielman WDT: p. 2, ¶ 3

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914. Untreated, the usual duration of campylobacteriosis is less than five days, but in several outbreaks the duration of illness was considerably longer. Thielman WDT: p. 2, ¶ 3

915. Campylobacteriosis lasting longer than one week has been documented in around 10-20% of patients seeking medical attention, and relapse occurs in about 5-10% of those who do not receive treatment. Thielman WDT: p. 2, ¶ 3

916. Complications from campylobacteriosis, such as associated blood stream infections occur more frequently in the elderly, the very young, or those who are immunocompromised by hypogammaglobulinemia or AIDS. Thielman WDT: p. 2, ¶ 3

917. Short-term complications of campylobacteriosis include colitis, sometimes complicated by toxic megacolon, septic abortion, cholecystitis, pancreatitis, and septic arthritis. Thielman WDT: p. 2, ¶ 3

918. Late complications from campylobacteriosis include rare cases of reactive arthritis and Guillain-Barré syndrome, a serious neurological condition resulting in ascending paralysis and sensory nerve changes. Thielman WDT: p. 2, ¶ 3

919. There are no clear-cut clinical features of *Campylobacter*-associated diarrhea that distinguish it from other inflammatory diarrheal illnesses. Thielman WDT: p. 2, ¶ 4

920. When a given patient presents with an inflammatory diarrheal illness, the microbiologic cause of the patient's illness is unknowable without appropriate culture test results. Thielman WDT: p. 2, ¶ 4

921. Rather than awaiting culture results, most practicing physicians will initiate empirical antibiotics in an effort to mitigate symptoms promptly and decrease associated complications. Thielman WDT: p. 2, ¶ 4

922. Empiric treatment means prescribing a drug that is effective for any number of possible causative pathogens. Thielman WDT: p. 2, ¶ 4

923. Since early initiation of therapy may have greater impact on resolution of symptoms than delayed treatment, it is often important to start a patient on therapy prior to getting a culture result. Thielman WDT: p. 2-3, ¶ 4

924. The Sanford Guide to Antimicrobial Therapy specifically recommends either ciprofloxacin 500 mg (a fluoroquinolone) by mouth twice daily or azithromycin 500 mg by mouth daily for three days for diarrhea associated with *Campylobacter jejuni*. Thielman WDT: p.3, ¶ 5

925. The Pocket Book of Infectious Disease Therapy lists erythromycin and fluoroquinolones as the preferred agents for diarrheal illnesses associated with *Campylobacter jejuni*. Thielman WDT: p 3, ¶ 6

926. The Infectious Diseases Society of America "Practice Guidelines for the Management of Infectious Diarrhea" recommends fluoroquinolones for adults with diarrheal illnesses. Thielman WDT: p 3, ¶ 6; G-261

927. In a study supported by Bayer Corporation and designed to evaluate the safety and efficacy of ciprofloxacin as empirical treatment for children with acute inflammatory diarrhea, the authors concluded that ciprofloxacin was as safe as intramuscular ceftriaxone. Thielman WDT: p 3, ¶ 6

928. Fluoroquinolones are generally well tolerated and easily prescribed on an outpatient basis. Thielman WDT: p.4, ¶ 7

929. Fluoroquinolone therapy typically consists of ciprofloxacin 500 mg administered twice daily for three to five days for diarrheal illnesses. Thielman WDT: p.4, ¶ 7

930. Because it takes up to 3 days to identify *Campylobacter* in a stool culture and would take several additional days to document resistance (particularly since resistance-testing is not routinely performed), practicing clinicians have no way of knowing whether the *Campylobacter* associated with a particular illness is fluoroquinolone-resistant for approximately one week - an interval during which the illness will either resolve on its own, persist or progress with complications, particularly in immunocompromised patients. Thielman WDT: p. 4, ¶ 7

931. While fluoroquinolones are not approved for the treatment of gastroenteritis in children in the U.S., physicians sometimes use drugs, including fluoroquinolones, in an off-label manner. Thielman WDT: p. 4, ¶ 8

932. Macrolides, such as erythromycin and azithromycin, can produce undesirable side effects including gastrointestinal distress. Thielman WDT: p. 4, ¶ 9

933. Although *Campylobacter*-associated diarrhea can be treated with a macrolide antibiotic such as erythromycin, fluoroquinolones are commonly, and appropriately, prescribed as first-line therapy for patients suffering with this illness especially since most gastroenteritis is treated empirically and fluoroquinolones are a broad spectrum antimicrobial that can be effective against most pathogenic gastrointestinal bacteria. Thielman WDT: p.4, ¶ 10

934. Practice guidelines and reference books, recognize that illness associated with *Campylobacter* are indistinguishable clinically from illness caused by other pathogens which are unresponsive to macrolides but easily treated with fluoroquinolones. Thielman WDT: p.4, ¶ 10

935. Fluoroquinolone antibiotics remain a critical first line therapy for *Campylobacter*-associated diarrhea. Thielman WDT: p. 5, ¶ 11

Linda Tollefson (G- 1478)

936.   Dr. Tollefson is qualified as an expert to testify as to the matters set forth in her written direct testimony submitted on December 9, 2002.

937.   The National Antimicrobial Resistance Monitoring System (NARMS) for zoonotic enteric pathogens in animals and humans became operational in January 1996 but planning began in 1995. Tollefson WDT: page 2, lines 16-19

938.   NARMS is an antimicrobial resistance monitoring system that helps ensure the continued safety and effectiveness of antimicrobial drugs for use in both animals and humans. Tollefson WDT: page 2, lines 19-22

939.   Development of antimicrobial resistant bacteria is a hazard associated with drug use in both human and veterinary medicine. The selection of antimicrobial resistant bacterial populations is a consequence of exposure to antimicrobial drugs and can occur from human, animal, and agricultural uses. Tollefson WDT: page 2, lines 27-32

940.   The use of antimicrobial drugs in food-producing animals is sometimes necessary to treat illnesses caused by bacteria. Unfortunately, food-producing animals can become reservoirs of bacteria capable of being transferred on food. Tollefson WDT: page 2, lines 32-36

941.   Resistant food borne pathogens that develop in response to antimicrobial drug use in food animals can be transmitted to humans through consumption of the contaminated food, among other routes. Tollefson WDT: page 2, lines 36-39

942.   If the resistant bacteria cause an illness in a consumer who needs treatment, medical therapy may be delayed, compromised or ineffective if the pathogenic bacteria are resistant to the drug used for treatment. Tollefson WDT: page 2, lines 40-43

943.   Several bacteria species are known to carry multidrug resistance genes that may confer resistance to a number of antimicrobials. Tollefson WDT: page 3, lines 1-3

944.   For foodborne pathogens, especially for those such as *Salmonella* and *Campylobacter* that are rarely transferred from person to person in developed countries, the most likely source of antibiotic resistance is use of antimicrobials in food-producing animals. Tollefson WDT: page 3, lines 8-12

945.   Antimicrobial agents can promote the emergence of resistant bacteria among both target pathogens and normal bacterial flora. Tollefson WDT: page 3, lines 12-14

946.   The normal bacterial flora in many species of animals include foodborne pathogens such as *Campylobacter*. *Campylobacter* can cause severe foodborne illness in humans even though they are non-pathogenic in animals such as poultry. Scientific evidence supporting these statements comes from a number of sources, including outbreak investigations,

laboratory surveillance, molecular subtyping, and studies on infectious dose and carriage rates. Tollefson WDT: page 3, lines 17-30; Exhibit G-285; Exhibit G-702; Exhibit B-252

947. *Campylobacter* has been cited as the most common known cause of foodborne illness in the United States. Tollefson WDT: page 3, lines 36-37; G-615

948. Foodborne diseases have a major public health impact in the United States. A recent reliable publication estimates that foodborne infections cause 5,000 deaths and 76 million foodborne illnesses annually in the United States. Tollefson WDT: page 3, lines 32-35;G-410

949. Development of resistance in foodborne pathogens complicates the medical and public health concern surrounding foodborne disease as important treatment options are compromised or lost. Tollefson WDT: page 3, lines 38-42; G-28; B-252

950. In 1995, the FDA approved sarafloxacin for control of mortality caused by *E. coli* in chickens and turkeys. In 1996, enrofloxacin was also approved for these indications and for control of turkey mortality associated with *Pasteurella multocida* (fowl cholera) infections. Tollefson WDT: page 3, line 46 through page 4, line 3

951. Sarafloxacin and enrofloxacin are fluoroquinolone antimicrobials that are administered in the drinking water for birds. Other fluoroquinolones, including Bayer Corporation's ciprofloxacin, are important in the treatment of several serious diseases in humans. Tollefson WDT: page 3, line 46 through page 4, line 7

952. The approval of the fluoroquinolones for use in animals intended as food raised serious public health concerns because of the potential risk of transfer of resistant bacteria from animals to humans. Tollefson WDT: page 4, lines 7-12

953. Cross-resistance occurs throughout the drug class of fluoroquinolones; thus, resistance to one fluoroquinolone compromises the effectiveness of all fluoroquinolone drugs whether used in animals or humans. Tollefson WDT: page 4, lines 12-16

954. FDA's joint Veterinary Medicine and Anti-Infective Drugs Advisory Committee was united in advising the agency that, if the products were to be approved, several restrictions should be placed on the use of the drugs in order to attempt to minimize the public health risks related to the development of resistant bacteria in animals. These restrictions included approval of fluoroquinolones only for therapeutic use by veterinary prescription, prohibition of extra-label use, and establishment of a nationally representative surveillance system to monitor resistance trends among both human and animal enteric bacteria. Tollefson WDT: page 4, lines 18-36

955. Shortly before FDA approved sarafloxacin in August 1995, Dr. Tollefson was asked to develop an antimicrobial resistance monitoring system consistent with the advisory committee's recommendation. Dr. Frederick Angulo of CDC, Dr. Paula Fedorka-Cray of USDA, and Dr. Tollefson were the primary scientists involved in designing, developing and

implementing an appropriate monitoring system. That system became operational in January 1996 and is known as the National Antimicrobial Resistance Monitoring System or NARMS. Tollefson WDT: page 4, lines 38-47

956.   The goals and objectives of NARMS are to: provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in enteric organisms from the human and animal populations; provide information to veterinarians, physicians and public health authorities so that timely action can be taken to protect public health; prolong the life span of approved drugs by promoting the prudent use of antimicrobials; identify areas for more detailed investigation; and guide research on antimicrobial resistance. Tollefson WDT: page 5, lines 29-38

957.   NARMS consists of three testing sites, or arms: 1) human (DHHS/CDC), 2) animal (USDA Agricultural Research Service, Food Safety Inspection Service, and Animal Plant Health Inspection Service) and 3) retail meats (DHHS/FDA/CVM). Tollefson WDT: page 6, lines 18 to 24

958.   *Campylobacter* isolates from poultry were not added to the animal arm of NARMS until 1998. Tollefson WDT: page 9, lines 4-5

959.   The animal NARMS surveillance program reported the following levels of resistance to fluoroquinolones in the chicken carcass isolates of *Campylobacter jejuni*: 9.4% for 1998; 9.3 % for 1999; 10.4% for 2000; and, for 2001, 17.6% by the "conventional" method and 20.3% by the "optimized" method. Tollefson WDT: page 12, lines 2-7; G-119; G-205; G-206; G-207; G-760; G-1363

960.   The animal arm of NARMS received only a subset of all the *Campylobacter* isolates from poultry, which resulted in an underestimate of fluoroquinolone resistance because some of the isolates that were perceived as not *Campylobacter jejuni/coli* because they were not susceptible to nalidixic acid were in fact resistant *Campylobacter jejuni/coli*, meaning they were resistant to both nalidixic acid and fluoroquinolones. Tollefson WDT: page 9, lines 31-46

961.   In 2001, retail meat testing was added to NARMS. Tollefson WDT: page 12, line 9

962.   Retail food represents the point of exposure that is closest to the consumer and, when combined with data from slaughter plant samples, provides a more representative picture of the prevalence of resistant pathogens in products derived from food-producing animals. Tollefson WDT: page 12, lines 9-14

963.   The poultry fluoroquinolone drugs were approved only for therapeutic use, by veterinary prescription. After approval, one of the first actions the Center took to minimize the public health risks for these drugs was to prohibit all extra-label uses of fluoroquinolones in food producing animals. This order, which became effective in August 1997 (21 CFR 530.4), also provided the Center with the authority necessary to enforce the prohibition. Violation of the prohibition could result in seizure of the drug and, in the case of repetitive or egregious

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situations, injunction or prosecution against the persons performing the prohibited act. Tollefson WDT: page 14, lines 13-24

964.   FDA established the National Antimicrobial Resistance Monitoring System to track changes in susceptibilities among enteric pathogens in both animals and humans. NARMS was specifically designed as an on-going monitoring system in both animal and human populations for the purpose of examining the impact of drug use in food-producing animals on human health. Evaluating the consequences of fluoroquinolone use in poultry was one of the specific purposes for which NARMS was created. Tollefson WDT: page 14, lines 26-35

965.   After approval of sarafloxacin in 1995 and enrofloxacin for poultry use in 1996, NARMS enabled CVM to detect fluoroquinolone resistance not only among the target pathogen, *E. coli*, but also among the human foodborne pathogen, *Campylobacter*. Tollefson WDT: page 14, lines 45-47 and page 15, lines 1-2

966.   Information from other CVM compliance and surveillance programs, such as monitoring the extra-label use prohibition and the tissue residue program, provided evidence that the fluoroquinolones were not being widely misused in food-producing animals. Tollefson WDT: page 15, lines 2-7

967.   By late 1999 – early 2000, all evidence available led CVM to conclude that the *E. coli* and *Campylobacter* organisms developed resistance to fluoroquinolones from the use of the fluoroquinolone drugs in chickens and turkeys even under the approved, labeled conditions of use. Tollefson WDT: page 15, lines 7-12

968.   The reason that there were few community-acquired fluoroquinolone-resistant *Campylobacter* infections in humans until 1996 is that there is little human-to-human transmission of these infections in the United States because generally the numbers of organisms present are low and fecal-oral transmission is required. Tollefson WDT: page 15, lines 24-32

969.   The agency contracted with a quantitative risk assessment expert to develop a quantitative risk assessment model to assess the human health impact of infections caused by fluoroquinolone-resistant *Campylobacter* organisms transmitted to humans through contaminated poultry. A mathematical model was derived to determine the relationship between the prevalence of fluoroquinolone-resistant *Campylobacter* infections in humans associated with the consumption of chicken to the prevalence of fluoroquinolone-resistant *Campylobacter* in chickens. Tollefson WDT: page 15, line 34 through page 16, line 25

970.   The potential hazard to humans from the use of fluoroquinolones in poultry is not limited to infections caused by consumption of, or contact with, chickens contaminated with resistant *Campylobacter* but extends to cross-contaminated food that is generally eaten raw, such as vegetables, that are contaminated in the consumer's kitchen by chickens contaminated with resistant *Campylobacter*. Tollefson WDT: page 16, lines 10-25

971. What the CVM *Campylobacter* risk assessment shows is that not only was there a quantifiable impact on human health from fluoroquinolone-resistant *Campylobacter* infections in humans acquired from chicken, but that the risk was substantial. Tollefson WDT: page 16, lines 30-34

972. To put the CVM *Campylobacter* risk assessment in perspective, the risk assessment calculated risks relative to various decreasing subsets of the U.S. population, beginning with all citizens, and then all citizens with campylobacteriosis, and so on. Those people who actually had campylobacteriosis, were ill enough to see a physician, and considered ill enough by the physician to be prescribed an antibiotic represent the people who are most seriously at risk from the failure of fluoroquinolone therapy. Tollefson WDT: page 16, lines 34-43

973. The CVM *Campylobacter* risk assessment showed that for 1999, the estimated mean number of people in the United States infected with fluoroquinolone-resistant Campylobacter from consuming or handling chicken, who saw a physician for their illness, and who subsequently received a fluoroquinolone as therapy is approximately 10,000 per year. This impact is a mean estimate, a value near the center of all feasible values for the expected number of people impacted. The 95th percentile estimate is just over 15,000 people impacted per year. Those 10,000 to 15,000 people were likely to have received ineffective or less effective therapy for their infections, resulting in adverse health effects. Tollefson WDT: page 15, line 34 through page 17, line 9; G-953

974. The CVM *Campylobacter* risk assessment modeled the general U.S. population, but it is likely that the impact of a fluoroquinolone-resistant *Campylobacter* infection is greater to some segments of the U.S. populations. Several population groups have increased susceptibility to foodborne infections, such as persons with lowered immunity due to HIV/AIDS and those on medications for cancer treatment or for organ transplantation, as well as pregnant women and their fetuses, young children, and the elderly. Tollefson WDT: page 17, lines 14-31

975. In concert with its mission to protect the safety of the food supply, it is FDA's responsibility to initiate regulatory activity long before an imminent threat to human health is evident. With respect to the hazard presented by antimicrobial-resistant foodborne pathogens, it is especially important to take action early because the nature of the problem is dynamic and cumulative. Tollefson WDT: page 19, lines 4-16

976. Unlike a static situation such as that which exists with residues of antimicrobial drugs in the tissues of food-producing animals, the development of resistant pathogens is the result of selective pressure from antimicrobial use and thus can be expected to increase over time rather than remain stable. Tollefson WDT: page 19, lines 10-16

977. CVM remains concerned that the harm of fluoroquinolone-resistant *Campylobacter* will continue to increase as more people will be unable to be effectively treated with fluoroquinolones when those drugs are needed for foodborne illness. Tollefson WDT: page 19, lines 18-22

978. CVM considers the fluoroquinolone resistance among *Campylobacter* found on chicken and turkey carcasses from the animal arm of NARMS to be underestimated until 2001 because of the methods employed in isolating the organisms, which selected only nalidixic acid-susceptible organisms. Tollefson WDT: page 19, lines 22-28

979. The retail meat studies undertaken by CVM show a greater prevalence of fluoroquinolone resistance among *Campylobacter* isolates, using methods that did not restrict the bacterial populations to nalidixic acid-susceptible strains; this prevalence is similar to that found among the *Campylobacter jejuni* isolated from chicken carcasses in the animal arm of NARMS during 2001. Tollefson WDT: page 19, lines 28-35

980. As a public health official, veterinarian and epidemiologist, Dr. Tollefson has examined the data demonstrating the selection of resistant strains of bacteria by fluoroquinolones, the inability to devise additional practicable and effective usage limitations for the poultry uses of fluoroquinolones, the international experience and the data from our own NARMS efforts. Taken as a whole, the evidence requires the Center for Veterinary Medicine to act to stop the poultry use of fluoroquinolones. This action will reduce the selection of fluoroquinolone-resistant *Campylobacter* and will thereby reduce the number of human cases rendered untreatable with the fluoroquinolones approved for human use. Tollefson WDT: p. 19, line 19 - p. 20, line 5

Curtis Travis (G- 1479)

981. Dr. Travis is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

982. Risk analysis is the process of answering a specific question regarding the risk of an existing or hypothetical hazard. There is no single process for conducting a risk assessment. The specific process followed, and the kinds of information needed, depend on the question being asked. Travis WDT: p. 2, lines 8-11

983. Risk analysis is best defined as the process of gathering and analyzing information to answer a specific question regarding the risk of an existing or hypothetical hazard. Travis WDT: p. 2, lines 24-25

984. The level of complexity needed to answer a question about risk depends, among other things, on the precision needed in the answer. In risk analysis, we tend to use the simplest approach possible consistent with the level of precision needed. Travis WDT: p. 2, lines 36-39

985. The International Society of Risk Analysis defines risk analysis as "a detailed examination ...performed to understand the nature of unwanted, negative consequences to human life, health, property, or the environment; an analytical process to provide information regarding undesirable events; the process of quantification of the probabilities and expected consequences for identified risks." Travis WDT: p. 2, lines 45-46 and p. 3, lines 1-4

986.   The National Research Council of the National Academy of Sciences defines risk assessment as "the characterization of the potential adverse health effects of human exposure to environmental hazards." Travis WDT: p. 3, lines 7-8, and lines 11-12

987.   The National Research Council of the National Academy of Sciences defined risk assessment as the "evaluation of information on the hazardous properties of substances, on the extent of human exposure to them, and on the characterization of the resulting risk." They note "Risk assessment is not a single, fixed method of analysis. Rather, it is a systematic approach to organizing and analyzing scientific knowledge and information of potentially hazardous activities or for substances that might pose risks under specified conditions." Travis WDT: p. 3, line 14, and lines 16-22

988.   The National Research Council of the National Academy of Sciences outlined a four-step process for human health risk assessment, but noted that not every risk assessment need contain all four steps. The four steps are:
- Hazard Identification: The determination of whether a particular hazard is causally related to a particular effect.
- Dose-response assessment: The determination of the relationship between magnitude of exposure and probability of effect.
- Exposure Assessment: Determination of the extent of exposure to the hazard.
- Risk Characterization: The integration of the first three steps to develop qualitative and quantitative estimates of nature and the magnitude of human risk. Travis WDT: p. 3, line 24, and lines 27-44, and p. 4, lines 1-22

989.   Guidance from the Office International des Epizooties (OIE) identified four components in antimicrobial resistance risk assessment:
- Release assessment
- Exposure assessment
- Consequence assessment
- Risk estimation. Travis WDT: p. 4, lines 4, 5, 16, 17, 19, 24, 29, and 37

990.   All of these methodological approaches to risk analysis can be placed into a more general framework that has three major components: (1) conceptualization of the problem (conceptual component); (2) analytical tools used in the analysis of the risk (analytical component); and (3) input parameters needed to apply to analytical tools (parameter component). Each component is described below.
- Conceptual component. The conceptual model is a conceptualization (blueprint) connecting the source of a risk with the impact being analyzed.
- Analytical Component. The analytical component specifies the computational tools (computer models, spreadsheet calculations, hand calculations) to be employed in the risk assessment and the general risk assessment methodology (deterministic, probabilistic, Bayesian) to be followed.
- Parameter Component. Estimates of risk are dependent of many input parameters. ... Parameter estimates also greatly influence the outcome of the risk assessments and can be another area of disagreement. Travis WDT: p. 4, lines 43 to 45, and page 5, lines 1 to 5, lines 15 to 18, lines 29 and 30, and lines 35 and 36

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991.	The decision on whether to use a "farm-to-fork" approach or "epidemiological" approach is a part of the conceptual component of a risk assessment. Travis WDT: p. 6, lines 5-6

992.	A "farm-to-fork" approach to antimicrobial resistance risk assessment is one in which the size of the pathogen population is modeled over all the steps in the production process: from farm, through transportation, to processor, to retailer, and finally to the consumer. It must account for all factors that increase or decrease microbial populations at each step. Travis WDT: p. 6, lines 8 to 12

993.	Risk analysis is the process of gathering and organizing information to answer a specific question. The type of information needed depends on the question asked. Travis WDT: p. 6, lines 38 and 39

994.	A model is a conceptualization of how something works. Travis WDT: p. 6, line 43

995.	In the case of the *Campylobacter* risk assessment, CVM was interested in developing a regulatory tool (a model) for predicting how the level of resistant foodborne bacterial infections in humans would change as a function of changes in the level of resistant bacteria in the food animal source. The particular case of interest was that of predicting how the level of fluoroquinolone-resistant *Campylobacter* infections in humans would change if the levels of fluoroquinolone-resistant *Campylobacter* in poultry were reduced. Risk analysis uses models to make such predictions. The output of the CVM *Campylobacter* risk assessment is an estimate (along with its attendant uncertainty) of the proportion of the U.S. population with fluoroquinolone-resistant *Campylobacter* attributable to the use of fluoroquinolones in poultry, and who are likely to be treated with a fluoroquinolone. Estimates of this quantity were provided for 1998 and for 1999. Travis WDT: p. 7, lines 7 to 17

996.	Another way to look at a model is that it is simply a relationship between a set of input variables and one or more output variables. Travis WDT: p. 7, lines 27 and 28

997.	All models are approximations of nature. Travis WDT: p. 7, line 40

998.	A predictive model is one that can be used to predict the behavior of a system under conditions different than those used to conceptualize the model. In risk analysis, we are usually concerned with two types of predictions: prediction of equilibrium behavior between known data points (interpolation) or prediction of future behavior starting from known data points (extrapolation). Travis WDT: p. 8, lines 9 to 13

999.	The CVM model of *Campylobacter* is a predictive model. It can be used to predict the level of fluoroquinolone-resistant *Campylobacter* in humans given the level of fluoroquinolone-resistance *Campylobacter* in poultry. Travis WDT: p. 8, lines 20 to 22

1000.	Models cannot be used to predict conditions that they were not designed to predict. The CVM Campylobacter model is not a "farm-to-fork" model. Thus, it cannot be used to predict how improvements in hygiene practices during the farm-to-fork trip will reduce microbial

loads on chicken at the point of consumption. This does not mean that the CVM Campylobacter model is not predictive. The CVM model was not designed to predict microbial loads on chicken at the point of consumption. The CVM model is predictive of the conditions it was designed to predict: how changes in levels of fluoroquinolone-resistance Campylobacter in chicken result in changes in fluoroquinolone-resistance levels of Campylobacter in humans. Travis WDT: p. 8, lines 24-32

1001. Models can be deterministic or probabilistic (stochastic is a term used interchangeably with probabilistic). In the deterministic case, one assumes that model input parameters are known exactly and when these parameters are inserted into the model, a single, exact estimate of the output parameter is obtained. In the probabilistic case, one assumes that the input parameters are not known exactly, but instead one knows a probability distribution around the input value. Travis WDT: p. 8, lines 36 and 37, lines 38 to 41, and lines 42 to 44

1002. If one propagates the probability distribution for x through the model, one obtains a probability distribution the output variable y. Travis WDT: p. 9, lines 1 to 3

1003. The CVM used a probabilistic approach to estimate the risk of fluoroquinolone-resistant Campylobacter infections in humans. Travis WDT: p. 9, lines 13 and 14

1004. One commonly accepted way of propagating a probability distribution through a model is called Monte Carlo simulation. CVM used a Monte Carlo simulation approach to propagate parameter variability through their model for fluoroquinolone-resistant Campylobacter in humans. Travis WDT: p. 9, lines 24 to 27

1005. A probabilistic output distribution indicates the most likely output value along with the range of other possible output values, thereby illustrating the degree of uncertainty in the final result. Travis WDT: p. 9, lines 38 to 40

1006. There are two views on calculating the probability associated with the occurrence of an event in nature. The frequency view holds that a unique probability can be associated with any event. According to this view, absolute estimates of probabilities can be determined by repeated sampling of nature. The probability of an event is the limiting relative frequency of the occurrence of an event in an infinite sequence of identical independent trials. Travis WDT: p. 9, lines 44 to 46, and p. 10, lines 1 to 3

1007. The subjective view holds that there is no absolute probability associated with an event. Bayes' Theorem provides a way to update our views as new information arrives, so that we retain a consistent view of the world. Travis WDT: p. 10, lines 5 and 6, and lines 8 to 10

1008. A Bayesian approach starts with the probability distribution, called the *prior* distribution, for the parameter of interest based on information available prior to collection of data specific to the situation. Travis WDT: p. 10, lines 28 to 30

1009. Bayes' Rule is applied to combine the prior distribution and the likelihood distribution to obtain a *posterior* distribution for parameter and situation of interest. Travis WDT: p. 10, lines 36 and 37

1010. The principle reason models are used in risk analysis is that risk analysis is usually concerned with predicting consequences of events that have not occurred. Travis WDT: page 11, lines 17 and 18

1011. Simply put, models are needed in risk assessment in situations where direct measurements are not available. Travis WDT: p. 11, lines 20 and 21.

1012. Risk analysis is usually concerned with events that have not occurred. Travis WDT: p. 11, lines 28 and 29

1013. Scenario Uncertainty is the incorrect conceptualization of past, present, or future conditions. Travis WDT: p. 11, lines 38 and 39

1014. Model Uncertainty is the inability of mathematical models to completely describe complex situations. Travis WDT: p. 11, lines 41 and 42

1015. Parameter Uncertainty. The parameters used in a risk assessment can be grouped into two categories. The first set consists of non-case specific parameters, which include chemical properties like solubility or toxicity, and exposure parameters like food ingestion rates. The second category includes parameters that are case specific. Travis WDT: p. 12, lines 4 to 8

1016. In the case of the CVM *Campylobacter* risk assessment, the major uncertainties come from parameter uncertainties. Different modelers may use different parameter values, yielding different results. For example, Cox and CVM use different parameter values for the fraction of *Campylobacter* cases attributable to chicken consumption and the proportion of *Campylobacter* infections from chicken that are resistant to fluoroquinolones. Travis WDT: p. 12, lines 10-15

1017. A model is a representation of how something works. A model is valid if it gives a correct representation of the system (under a specified set of conditions). That is, a model is valid if it can correctly predict the behavior of the system under different conditions. The process of determining if a model is valid is called model validation. This usually involving comparing model simulations (predictions) with actual measurements indicating how the system should perform. In practice, model validation is rarely done, primarily because insufficient data (measurements) exist with which to validate the model. Travis WDT: p. 12, lines 19 to 25

1018. Risk assessors usually rely on the logical structure of the model to convince them that it is a reasonable representation of a system. Travis WDT: p. 12, lines 28 and 29

1019. To evaluate the human health impact of antimicrobial use in animals, CVM developed a quantitative risk assessment model. The risk assessment was intended to estimate the risk to human health from antibiotic resistant food borne pathogens associated with the domestic use of antimicrobials in food producing animals. Specifically, a mathematical model was derived to relate the prevalence of fluoroquinolone-resistant *Campylobacter* infections in humans associated with the consumption of chicken to the prevalence of fluoroquinolone-resistant *Campylobacter* in chickens. Travis WDT: p. 12, lines 35-42

1020. The CVM *Campylobacter* Risk Assessment takes an epidemiologic approach to estimating the risk of fluoroquinolone-resistant illnesses in humans. It starts with the human epidemiologic data on the rate of campylobacteriosis in humans and then works backwards to estimate the fraction of this total illness burden that is fluoroquinolone-resistant and due to chicken consumption. Travis WDT: p. 12, lines 44 to 46, and p. 13, lines 1 to 4

1021. The incremental human health impact of resistant food borne disease can be determined without assessing all the factors influencing the cause of the food borne illness itself. Travis WDT: page 13, lines 27 to 29.

1022. Cox developed a risk assessment model of microbial hazards in food, using *Campylobacter jejuni* as an example. Travis WDT: p. 13, lines 33 and 34

1023. Cox's model is based on a discrete-event simulation (DES) model of the microbial load reaching consumers via ingested chicken. Microbial load is quantified in terms of colony-forming units (CFUs). The DES model simulates the probabilistic amplification and reduction on microbial load at successive states from farm to table. Ingested microbial load enters a non-linear dose-response model that predicts resulting probabilities of infection and illness. Travis WDT: p. 13, lines 39 to 44

1024. Cox takes a "farm-to-fork" approach. A "farm-to-fork" microbial risk assessment tracks the level of bacterial contamination in food products at each step in the process from the farm to the table. These steps include slaughtering, processing, transportation, storage, retail and food handling prior to consumption. Such an assessment attempts to predict (model) the actual number of colony-forming units of *Campylobacter* at each step in the trip from farm-to-fork. Such an assessment must consider sources of contamination at each step and subsequent microbial growth and reduction events between stages. Travis WDT: p. 14, lines 1 to 7

1025. The major components of the Cox model are:
- A "farm-to-fork" microbial loading model that produces a probability distribution of the load of *Campylobacter jejuni* (measured as the number of colony-forming units, CFU, of *Campylobacter jejuni*) on individual chickens at the point of consumption.
- A nonlinear dose-response model that predicts the probability of illness resulting from consumption of chicken contaminated with a given number of CFU of *Campylobacter jejuni*. This component of the model has been extended to predict probability of infection and illness for different age groups. Travis WDT: p. 14, lines 14 to 24

1026. Cox describes the microbial loading component of his model as being able to predict how different processes within the farm-to-table supply-chain affect the microbial load. The model considers the effects of:
- The initial microbial load of chickens leaving the farm (by season),
- The effects of transport from farm to processing plant,
- The effects of the processing plant itself, including cross-contamination, and
- The effects of storage and preparation practices prior to consumption. Travis WDT: p. 14, lines 26 to 33

1027. To be able to predict the impact of fluoroquinolone-resistant *Campylobacter* in poultry meat on human health using Cox's model, it is necessary to know the dose-response function relating *Campylobacter* consumption with the probability of illness as an intermediary step. Cox assumes a Beta Poisson model fit to the Black et al. data to define the dose-response function. In assuming a Beta Poisson with the specified parameters Cox assumes that an ingested dose of less the 500 colony forming units (CFU) has a zero probability of producing illness in humans. Travis WDT: p. 14, lines 35-41

1028. Cox takes a farm-to-fork approach to predicting the prevalence of campylobacteriosis in the human population. However, he does not estimate (predict) the fraction of fluoroquinolone-resistant *Campylobacter* at each step on the trip from farm to human exposure to illness. Travis WDT: p. 14, lines 45 and 46 and p. 15, lines 1 and 2

1029. To estimate the prevalence of fluoroquinolone-resistant *Campylobacter* in humans, Cox assumes that the fraction of *Campylobacter* cases in humans that is fluoroquinolone-resistant is 0.6809 times the fraction of *Campylobacter* isolates in chicken that is fluoroquinolone-resistant. Travis WDT: p. 15, lines 9 to 12

1030. Cox uses a dose-response model to predict the probability that a person will develop a *Campylobacter jejuni* infection or illness following consumption of chicken contaminated with a fixed number of *Campylobacter jejuni* CFU. The human dose-response model used by Cox was developed by Teunis et a., based on empirical testing data from Black et al. Travis WDT: p. 15, lines 16 to 20

1031. At the lowest doses used in the Black et al study (800 CFU), 50% of subjects had positive stool cultures and 10% developed diarrhea or fever. The strain used, A3249, was the weaker of the two strains used in this study, thus other strains of *Campylobacter* may be more virulent at low doses. Travis WDT: p. 15, lines 30-34

1032. Black et al concluded that even low does of *C. jejuni* may produce infection and illness in humans. Travis WDT: p. 16, lines 29 and 30

1033. Teunis et al. found that the Beta Poisson model appears to be well suited to describe the majority of known results from human feeding studies. They also concluded that this does not mean that no better models could be constructed. Travis WDT: p. 15, line 38, and lines 43 to 45

1034. There is a great deal of uncertainty in dose-response for *Campylobacter* at low doses. Travis WDT: p. 16, lines 11 and 12

1035. To overcome the problem of not knowing the correct parameter values to use in the "farm-to-fork" model, Cox calibrates the model. Travis WDT: p. 16, lines 17 and 18

1036. Calibration consists of finding values for the model parameters so that if the model starts with the assumed initial level of *Campylobacter* infection (CFUs) on chickens at the farm, it is able to predict the assumed current prevalence of campylobacteriosis. Travis WDT: p. 16, lines 25 to 27

1037. It is difficult, if not impossible, to identify all the various factors that can influence the presence and growth of bacterial during the trip from farm-to-fork. Travis WDT: p. 17, lines 8 and 9

1038. Thus, it is highly likely that in developing a "farm-to-fork" model, important factors will be overlooked and omitted from the model. Travis WDT: p. 17, lines 16 and 17

1039. There currently does not exist sufficient knowledge of parameter values to parameterize the model used by Cox. Travis WDT: p. 17, lines 23 to 25

1040. The difficulty in applying the Cox model is that Cox does not know all the parameter values that should be used in each component of the microbial loading model. Travis WDT: p. 17, lines 28-30

1041. It is generally agreed that currently there does not exist sufficient data to develop a predictive "farm-to-fork" model for any one of the common food borne pathogens and that the current use of such a model is limited to identifying intervention points in the farm-to-fork process where action might help reduce the overall level of bacterial contamination in food products at the consumer consumption level. Travis WDT: p. 17, lines 32 to 36

1042. The Cox implementation of the Teunis et al. model makes two assumptions:
- An ingested dose of *Campylobacter jejuni* less than 500 CFU has a zero probability of producing illness in humans.
- A Beta Poisson model provides an adequate fit to the Black et al. data. Travis WDT: p. 17, lines 40 to 45

1043. In his model, Cox states, that the minimum infective dose for *Campylobacter jejuni* in the Black et al. study was 800 CFU. Other research has shown that the minimum dosage may be as low as 500 CFU. The Cox statement is somewhat misleading and may be misunderstood. It could be mistaken to mean that doses below 800 CFU were ineffective in the Black et al study. In fact, 800 CFU was the lowest dose used in the Black et al study and this dose level produced a 50% infection rate and a 10% illness rate. Thus, the 800 CFU dose level in the Black et al. study was not a dose level below which there is a zero probability of producing an illness. The Black et al. study actually predicts that one CFU of

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Campylobacter jejuni has a positive probability of causing infection and illness. Travis WDT: p. 18, lines 3-14, G-284

1044. Holcomb et al. compared six microbial dose-response models, including exponential and Beta Poisson models considered in the Teunis et al study, for their ability to fit four microbial dose-response data sets from human feeding studies. Travis WDT: p. 18, lines 22 to 25

1045. Holcomb et al. concluded that when applied to the Black et al. *Campylobacter jejuni* data set, the models predicted nine orders of magnitude (billion-fold) difference in the dose estimated to infect one percent of the subjects (ID_{01}). However, three of the predicted ID doses were less than 1.0 CFU, meaning that a dose of 1.0 CFU infects more than one percent of the population. Travis WDT: p. 18, lines 29 to 33

1046. The basic limitations of incorporating a dose-response function into Cox's *Campylobacter* risk assessment are:
- There is uncertainty in the appropriate shape of the dose-response function for *Campylobacter* infection
- The shape of the dose-response for *Campylobacter* infection may not be the same as the shape of the dose-response for *Campylobacter* illness
- It is not clear, and it is not likely, that an ingested dose of *Campylobacter jejuni* less than 500 CFU has a zero probability of producing illness in humans. Travis WDT: p. 18, lines 35 to 45

1047. CVM estimates 153,580 cases of fluoroquinolone-resistant *Campylobacter* cases resulting from chicken consumption, while Cox estimates the number to be 38,419 cases. The difference between these two estimates can be traced to differences in the following parameter choices:
- Fraction of *Campylobacter* cases Attributable to Chicken Consumption
    - CVM uses 57.4%, based on two case-control studies.
    - Cox uses 60%, based on the same two case-control studies.
- Proportion *of Campylobacter* Infections From Chicken That are Resistant to Fluoroquinolone
    - CVM uses 19.6% in 1999, based on subtracting the proportion of fluoroquinolone-resistant *Campylobacter* isolates from NARMs for which the resistance might have been derived through foreign travel or through having received a fluoroquinolone prior to stool culture. The proportion to be subtracted was determined from the CDC's *Campylobacter* Case Control study.
    - Cox uses 6.4%, based on two CDC FoodNet *Campylobacter* Case Control Studies.
- Children Under the Age of One (27.5% of cases)
    - CVM removed children from its calculations by multiplying the number expected to receive an antibiotic by the fraction expected to receive a fluoroquinolone since children are not expected to be prescribed this class of drugs.
    - Cox removed children from its calculations by removing the number of U.S. citizens below the age of 2 from the starting U.S. population size in his model. Travis WDT: p. 19, lines 4-35

1048. The difference between the Cox and CVM estimate of the number of fluoroquinolone-resistant *Campylobacter* cases attributable to chicken consumption does not result from Cox's use of a more complex model. Travis WDT: p. 19, lines 39 to 41

1049. Both Cox and CVM use the same method for estimating the number of fluoroquinolone-resistant *Campylobacter* cases attributable to chicken consumption. Travis WDT: p. 20, lines 1 to 3

1050. CVM estimates 153,580 cases of fluoroquinolone-resistant *Campylobacter* cases resulting from chicken consumption while Cox estimates the number to be 38,419 cases. Travis WDT: p 19, lines 7 and 8

1051. For the fraction of cases attributable to chicken consumption, CVM uses 57.4%, based on two case-control studies. Cox uses 60%, based on the two case-control studies. Travis WDT: p. 19, lines 13 and 14

1052. For the proportion of Campylobacter infections from chickens that are resistant to fluoroquinolones, CVM uses 19.6% in 1999, Cox uses 6.4%, based on two CDC FoodNet *Campylobacter* Case Control Studies. Travis WDT: p. 19, line 18 and lines 25 and 26

1053. The Cox and CVM estimates differ solely because of differences in the parameter values. Travis WDT: p. 20, lines 4 and 5

David Vose (G-1480)

1054. Dr. Vose is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

1055. Risk assessment is a decision-support tool. Vose WDT: p. 2, line 20

1056. Risk analysis applies methods of analysis to matters of risk. Its aim is to increase understanding of the substantive qualities, seriousness, likelihood, and conditions of a hazard or risk and of the options for managing it. Vose WDT: p. 2, lines 32-35

1057. Risk analysis uses observations about what we know to make predictions about what we don't know. Risk analysis is a fundamentally science-based process that strives to reflect the realities of Nature in order to provide useful information for decisions about management risks. Risk analysis seeks to inform, not to dictate, the complex and difficult choices among possible measures to mitigate risks. Risk analysis enriches fair and transparent deliberative decision-making processes in a democratic society. Vose WDT: p. 2, lines 37-41

1058. Risk analysis seeks to integrate knowledge about the fundamental physical, biological, social, cultural, and economic processes that determine human, environmental, and technological responses to a diverse set of circumstances. Because decisions about risks are usually needed when knowledge is incomplete, risk analysts rely on informed judgment and

on models reflecting plausible interpretations of the realities of Nature. Vose WDT: p. 2, lines 42-46

1059. If a risk characterization is to fulfill its purpose, it must (1) be decision driven, (2) recognize all significant concerns, (3) reflect both analysis and deliberation, with appropriate input from the interested and affected parties, and (4) be appropriate to the decision. Vose WDT: p. 3, lines 19-22

1060. Risk characterization is a synthesis and summary of information about a potentially hazardous situation that addresses the needs and interests of decision makers and of interested and affected parties. Risk characterization is a prelude to decision making and depends on a iterative, analytic-deliberative process. Vose WDT: p. 3, lines 28-31

1061. Both the Society for Risk Analysis and the National Research Council conclude that risk assessments need to address decision questions, and that the form of that risk assessment will be driven by the decision-makers needs. The risk assessment produced by CVM does exactly that. Vose WDT: p. 3, lines 33-35

1062. The Georgetown University risk assessment discussed in Anderson et al (2001) was not a farm-to-fork model, but began its risk assessment with retail meat, and only modeled consumer handling and a dose-response relationship, rather than looking at farm, slaughter and processing practices where the key food safety controls are. Vose WDT: p. 4, lines 4-7

1063. The food safety risk assessment community is beginning to recognize that farm-to-fork risk assessments have not produced what was hoped, and that more decision-question focused assessments are necessary, using more efficient alternative modeling approaches. Vose WDT: p. 4, lines 37-40

1064. An antimicrobial risk assessment is similar in principle to a microbial risk assessment, the principle difference being the hazard (a risk analysis term defined by CODEX in food safety as influenced by foodborne agents as 'a biological, chemical or physical agent in or property of food that may have an adverse health effect'): in a microbial food safety risk assessment, the hazard is a bacterium or other human pathogen, whilst in an antimicrobial food safety risk assessment it is a resistant determinant. Vose WDT: p. 4, lines 45-48, and p. 5, lines 1-2

1065. The risk management question asked by CVM was whether the use of antimicrobials under their authority is introducing a significant human health burden and, if so, whether any action they could taken would significantly reduce that burden. Vose WDT: p. 5, lines 41-43

1066. The CVM conducted a draft risk assessment to determine the human health impact of the use of an antimicrobial drug in food producing animals. The CVM also produced versions of the risk assessment model that could be run and modified by anyone with access to Microsoft Excel, a commonly used spreadsheet program. The CVM risk assessment was released and made available on the CVM Internet homepage, including the downloadable versions of the risk assessment model. There followed a public comment period and even a conference dedicated to the assessment, including a food safety and risk assessment expert panel from

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around the world who provided independent comments on the strengths and weaknesses of the draft assessment. Vose WDT: p. 6, lines 25-33

1067. CVM chose a predictive model approach that would provide it with meaningful and useful input in a timely fashion to help determine whether to allow continued use of fluoroquinolone in poultry. The approach was supported by reliable data, and was designed to help enable industry to manage its use of fluoroquinolone so that the human health impact did not become unacceptable. CVM also made exceptional efforts to incorporate stakeholders' views and data. Vose WDT: p. 6, lines 38-43

1068. The CVM risk assessment estimates the amount of domestically-produced poultry meat after slaughtering that contains fluoroquinolone-resistant *Campylobacter*. It also estimates the number of people who get ill from consuming that fluoroquinolone-resistant *Campylobacter* contaminated meat. Both estimates are based almost entirely on U.S. federally collected data, the Centers for Disease Control (CDC) for the human data and the United States Department of Agriculture (USDA) for the poultry meat data. Vose WDT: p. 6, lines 47-48 and p. 7, lines 1-4

1069. In the CVM *Campylobacter* risk assessment model, K is the aggregate probability of all possible pathways via which people get exposed, combined with the conditional probability distribution of how many bacteria would be received in the exposure, and the dose-response probability function added up over the entire population. Vose WDT: p. 7, lines 25-28

1070. The draft Danish farm-to-fork risk assessment "Risk Assessment on *Campylobacter jejuni* in Chicken Products" demonstrates exactly the same behavior as we have assumed, i.e. if the prevalence of contaminated product at the end of the slaughter plants increases by some factor, the incidence of human illness will, on average, also increase by this factor. Vose WDT: p. 7, lines 34-35, and lines 37-40

1071. The CVM modeling approach is mathematically simple, with few assumptions, but which nevertheless addressed the decision question and provided the ability to predict future levels of human health impact resulting from changes in the level of consumption, prevalence of contaminated meats, etc. Vose WDT: p. 8, lines 7-10

1072. CVM did not use a farm to fork model. A farm-to-fork risk assessment tracks the bacterial on the food-producing animal, usually from the time it leaves the farm, through to the final food product being consumed and the effect of the consumption of the bacteria. A farm-to-fork model requires modeling an almost infinitely complex system, regarding all important components of the farm-to-fork continuum with respect to the attenuation, growth, redistribution and cross-contamination of the bacteria in question. Vose WDT: p. 8, lines 14-20

1073. An expert group of highly regarded risk assessors working on the Joint FAO/WHO Activities on Risk Assessment of Microbiological Hazards in Food, produced the report 'Hazard identification, hazard characterization and exposure assessment of *Campylobacter*

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spp. in broiler chickens' ... This report discusses at length the data available to quantify a dose-response relationship and noted:

1074. There is insufficient information in the epidemiological literature, that we have been able to review, to allow a dose-response relationship to be derived using this type of data. There is one human feeding trial study that has been conducted Black et al. (1988). This study used healthy young adult volunteers from the Baltimore community. The challenge dose was administered in milk, and the volunteers fasted for 90 minutes before and after ingesting the organism. This study involved the use of two strains of C. jejuni (A3249 and 81-176). Vose WDT: p. 9, lines 14-25

1075. Later the expert group working of the Joint FAO/WHO report state: 'The human feeding trial data does not indicate a clear dose-response relationship for the conditional probability of illness following infection." Vose WDT: p. 9, lines 29-30

1076. CVM did not use a farm to fork risk analysis because:
- A farm-to-fork analysis would require significant assumptions that could not be supported by data. The much simpler analysis performed by the CVM nonetheless had to deal with data gaps and thus required making a number of assumptions described in the report. A farm-to-fork analysis would have suffered far more from gaps in data;
- A farm-to-fork would be costly and difficult to maintain and update because any changes in husbandry, transportation, processing, and human behavior could require new studies and data gathering activity;
- A farm-to-fork requires a dose-response (D-R) model, for which available data on *Campylobacter* were poor, based on feeding trials (known to be poor predictions of real world risk) and for which there is no generally agreed upon analysis;
In the CVM modeling approach chosen, yearly updated K and p values can track change, as they relate to this risk question, of the consumer risk/lb contaminated meat. In other words, if industry found methods to reduce the human health impact during processing, storage, consumer handling, retail, etc. this could have been taken into account without the need of large data collection studies. Vose WDT: p. 10, lines 24-40

1077. The WHO expert panel concluded, in their efforts to produce a *Campylobacter* spp. in broiler chickens farm-to-fork risk assessment:
"Given the value of knowing the relative importance of consumer behaviour variables in food safety (cooking temperature profiles and cross-contamination processes), the ultimate risk characterization (in both the absolute and comparative senses) will be highly dependent upon several 'ungrounded' assumptions. It is possible that a carefully directed research effort could elucidate some of these issues. In the near term, however, risk characterization as it relates to consumer risk factors of cooking and cross-contamination will continue to be largely a matter of mathematical combinations of unvalidated assumptions. These may be useful for conceptualizing the risk factors in support of food safety decisions, but are not likely to be sufficient to stand-along as providing reliable numerical estimates of risk." Vose WDT: p. 10, lines 42-49, and p. 11, lines 1-3

1078. The CVM investigated alternative approaches to modeling the risk issue, but concluded that the paucity and ambiguity of available data made taking other modeling approaches impractical. Their conclusions have subsequently been borne out by the WHO expert panel. Vose WDT: p. 11, lines 5-8

1079. The risk assessment implicitly includes pathogen load in the factor K. Vose WDT: p. 11, lines 16-17

1080. K is effectively the probability that a pound of contaminated meat at slaughter plant will produce a case of campylobacteriosis in humans. If one were to break up that probability into smaller steps, one could do so as follows:
-      The probability the contaminated meat contains X bacteria;
-      The probability that after processing, storage, etc the bacteria contains Y bacteria given it started off with X bacteria;
-      The probability that exposure to the Y bacteria then causes an illness.
Integrating these probabilities for all values of X, all values of Y, and all dose-response relationships for the individuals in the U.S., one arrives at K. The integration over all X in the inclusion of the bacterial load at the slaughter plant. The integration to Y is taking into account the distribution of bacteria at the point of consumption. Vose WDT: p. 11, lines 19-31

1081. One possible method of correction for year-to-year differences in the bacterial contamination load was discussed in Section 1 of the CVM *Campylobacter* risk assessment report under the heading: *Accounting for changes in the bacterial load of contaminated carcasses*. Vose WDT: p. 11, lines 44-46

1082. Simple corrections to the model to account for future changes in medical practice, patient behavior, and resistant *Campylobacter* prevalence in poultry, and for changes in the number of U.S. citizens were also discussed in the same section, highlighting the adaptive nature of the CVM model. Vose WDT: p. 11, lines 45-49 and p. 12, lines 1-2.

1083. The method proposed in the report to correct for bacterial load was to make an approximation that the number of bacteria on a contaminated carcass was log-exponentially distributed (i.e. the log of the number of bacteria is exponentially distributed). Then a fractional reduction of bacterial load would be mathematically equivalent to a reduction in the prevalence of contaminated carcasses, with the remaining carcasses having the same load distribution as the original. The method is fairly crude but by continuous updating of the parameter K only very sudden changes from one year to the next of the bacterial load distribution (for example, with the introduction of irradiation of carcasses) would need to be addressed in this manner. Other correction methods could, of course, also be explored. Vose WDT: p. 12, lines 4-12

1084. Although CVM's model does not attempt to explicitly incorporate modeling of pathogen load, the effect of pathogen load is nonetheless implicitly incorporated into the model. Vose WDT: p. 12, lines 35-37

1085. The model assumes that the distribution of pathogen load remains fairly constant between successive years, but possible corrections are available if this were to change dramatically. Vose WDT: p. 12, lines 40-42

1086. An *explicit* dose-response step need only be included in a microbial or antimicrobial risk assessment if its inclusion materially improves the quality of the decision that would be made from it. Vose WDT: p. 13, lines 2-4

1087. It was unnecessary for the purpose of the CVM risk assessment to explicitly include a dose-response component. In fact, given the very poor current understanding of what that dose-response relationship might be between *Campylobacter* and the human illness, it was of considerable value in improving the robustness of the analysis to be able to find an alternative risk assessment approach that did not oblige defining this dose-response relationship. Vose WDT: p. 13, lines 47-48 and p. 14, lines 1-5

1088. A key assumption in the CVM model is that fluoroquinolone use in poultry results in reduced susceptibility of *Campylobacter* in the poultry to fluoroquinolones, and that humans are exposed to these bacteria and become ill. Vose WDT: p. 14, lines 8-10

1089. There are a number of guidelines for attempting to validate causal relationships. The most important are 1) we can postulate why one variable (in this case, prevalence of resistance in U.S. *Campylobacter* is domestically-reared poultry, call it X) influences the size of another variable (in this case, the number of domestically-acquired fluoroquinolone-resistant case of human campylobacteriosis, call it Y); and 2) we can observe a lagged correlation between these values (in this case, and increase in X produced a corresponding increase in Y some time later). Vose WDT: p. 14, lines 20-27

1090. Anderson et al (2001), in their AHI-sponsored risk assessment, discuss the significant evidence internationally for correlation between the introduction of fluoroquinolone for use in food-producing animals and the increase in fluoroquinolone-resistant cases of campylobacteriosis. Vose WDT: p. 14, lines 40-43

1091. The incidence of cases of campylobacteriosis is very seasonal, due in part to travelling (which we excluded from our analysis), in part probably to changes in cooking practices, food handling and eating practices, and also probably in part due to the weather allowing greater survivability of these thermophilic bacteria. ... the model does not require estimating seasonal variations because it estimates an average for the year. This is valid mathematically because a special feature of the Poisson mathematics that is used in the CVM model is that the expected number of cases for each season can be added together." Vose WDT: p. 15, lines 4-7 and lines 9-12

1092. All food safety models contain important assumptions. Vose WDT: p. 15, line 16

1093. CVM called for data in the Federal Register, making available the draft report and model on the Web, sponsored a public conference to discuss the draft assessment, sponsored experts from around the world to discuss the assessment in an open forum in that conference, and

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evaluated and responded to comments received. CVM has taken great care to collect, evaluate and list sources of data used in its risk assessment. Despite the simplicity of the modeling, the report uses 125 references, obviously not including material that was read and found irrelevant. CVM even provided data, advice and personnel time to Dr. Cox to help in his efforts to produce an alternative model. Vose WDT: p. 15, lines 27-37

1094. In conclusion, all CVM assumptions have been thoroughly investigated, explained and debated with the risk management staff. The structure of CVM's model means that there are a minimal number of these assumptions which increases confidence in using the results. Vose WDT: p. 16, lines 34-36

1095. The Society for Risk Analysis and the National Research Council agree that the form that a risk assessment takes should be driven by the decision-makers needs. The CVM model did this, whereas a farm-to-fork would not have. Vose WDT: p. 16, lines 40-42

1096. Antimicrobial risk assessment needs to address more complicated issues than microbial risk assessment, and requires a flexible modeling approach. Vose WDT: p. 16, lines 43-44

1097. The CVM model was predictive and provided CVM management with meaningful and useful decision-support in a timely fashion. Vose WDT: p. 16, lines 45-46

1098. The CVM model was designed to help industry manage their use of fluoroquinolones in the least restrictive way possible to protect the human health. Vose WDT: p. 16, lines 47-48

1099. The CVM model requires the minimum of assumptions, and was supported by largely federally collected data. Vose WDT: p. 17, lines 1-2

1100. The CVM investigated alternative approaches, and cooperated with Dr. Cox to help develop his models, but concluded that the CVM approach provided the greatest decision-support. Vose WDT: p. 17, lines 3-5

1101. The CVM went to great lengths to invite debate about their approach and collect useful information. Vose WDT: p. 17, lines 6-7

1102. The CVM modeling approach was able to avoid direct modeling of bacterial load and dose-response relationships for which data are sparse and theories are currently tenuous. This was consistent with NRC/NAS guidelines. Vose WDT: p. 17, lines 8-12

Robert D. Walker (G-1481)

1103. Dr. Walker is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

1104. In vitro antimicrobial susceptibility testing is performed by exposing a known concentration of a pure bacterial culture, in the appropriate growth phase, to increasing concentrations of antimicrobial agents. Walker WDT: p. 3, lines 1-3

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1105. The results of in vitro antimicrobial susceptibility testing may be reported qualitatively (*susceptible, intermediate or resistant*) or quantitatively, via a numerical value representing the minimum concentration of the drug that is required to inhibit the growth of the pathogen. Walker WDT: p.3, lines 3-6

1106. The quantitative value of in vitro antimicrobial susceptibility testing is referred to as the minimal inhibitory concentration (MIC). Walker WDT: p. 3, lines 6-7

1107. The MIC values are expressed in micrograms per milliliter or milligrams per liter. Walker WDT: p. 3, lines 7-8

1108. MIC refers to the lowest concentration of an antimicrobial agent that it takes to inhibit the growth of a bacterium. Walker WDT: p. 3, lines 8-9

1109. Standardized susceptibility testing methods use one of three methods. These are agar dilution, the "Gold Standard" of susceptibility testing, broth dilution, and agar diffusion. Walker WDT: p. 3, lines 15-17

1110. Agar dilution tests are performed by incorporating the antimicrobial agent to be tested into the appropriate agar medium using serial two-fold dilution and applying the bacterial inoculum to the surface of the agar plate, usually by using a multi-pin replicating apparatus. Walker WDT: p. 3, lines 17-21

1111. The endpoint for agar dilution tests, the MIC, is determined by the unaided visual inspection of the agar surface. Walker WDT: p. 3, lines 21-22

1112. The results of agar dilution tests may be reported qualitatively, if appropriate interpretive criteria has been determined for the bacterium/drug combination that was tested and/or quantitatively. Walker WDT: p. 3, lines 22-24

1113. *Broth dilution* may be performed using macro (volumes greater than one mL) or micro (usually volumes of 100 µL or less). Walker WDT: p. 3, lines 26-27

1114. With *broth dilution*, a standardized suspension of bacteria is tested against two-fold serial dilutions of an antimicrobial agent in a standardized liquid medium. Walker WDT: p. 3, lines 27-29

1115. The endpoint for the broth dilution is also the MIC. Walker WDT: p. 3, lines 29-30

1116. Broth dilution results may be reported as qualitative and/or quantitative. Walker WDT: p. 3, lines 32-33

1117. The *disk diffusion* testing method generates an endpoint based on the diffusion of an antimicrobial agent from a solid carrier *(e.g., paper disk)* into a solid culture medium that has had the surface seeded with a known bacterial inoculum. Walker WDT: p. 3, lines 35-38

1118. The diffusion of the antimicrobial agent into the culture medium produces an antimicrobial gradient. Walker WDT: p. 3, lines 38-39

1119. In the disk diffusion method, when the concentration of the antimicrobial is sufficient to inhibit the growth of the bacterium growing on the surface of the agar, a zone of inhibition is formed. Walker WDT: p. 3, lines 39-41

1120. In the disk diffusion method, the boundary of this zone of inhibition correlates with the MIC for that particular bacterium/antimicrobial combination. Walker WDT: p. 3, lines 41-43

1121. In the disk diffusion method, the larger the zone of inhibition, the smaller the concentration of antimicrobial required to inhibit the organism's growth. Walker WDT: p. 3, lines 43-44

1122. The Etest represents a diffusion testing method that can generate quantitative results. Walker WDT: p. 4, lines 1-2

1123. With Etest, a predefined concentration gradient of an antimicrobial drug is impregnated into one side of a plastic strip which is approximately 5 mm wide and 60 mm long. The gradient covers a continuous concentration range which corresponds to 15 two-fold dilutions in a conventional MIC method. This plastic strip is placed on a seeded agar surface similar to the method in which the disks are placed for the disk diffusion test. Walker WDT: p. 4, lines 2-7

1124. When an Etest strip is applied to an inoculated agar plate, there is an immediate release of the antimicrobial from the plastic carrier surface into the agar matrix. A continuous and exponential gradient of antimicrobial concentrations is created directly underneath the Etest strip. After incubation, a symmetrical inhibition ellipse centered along the strip is observed on the agar plate. Walker WDT: p. 4, lines 7-12

1125. In the Etest method, the MIC value for the bacterium/drug combination is read from the scale on the strip in terms of µg/mL where the ellipse edge intersects the strip. Walker WDT: p. 4, lines 12-14

1126. One of the advantages of the Etest is that because it comprises a continuous gradient, MIC values between two-fold dilutions can be obtained. Walker WDT: p. 4, lines 14-16

1127. Broth dilution and agar dilution testing methods may generate MICs such as 2, 4 or 8 µg/mL (doubling dilutions), where the Etest can generate these same MIC values plus 3, 5, 6 and 12 µg/mL. Walker WDT: p. 4, lines 16-19

1128. Regardless of the testing method, susceptibility results are generated as numerical values. These values may be expressed as the MIC of a bacterium/drug combination (e.g. 0.5 µg/mL) or the size of the zone of inhibition (e.g., 33 mm). Walker WDT: p. 4, lines 23-25

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1129. The interpretative criteria used for bacterium/antimicrobial agent interactions are susceptible, intermediate and resistant. Walker WDT: p. 4, lines 31-33

1130. "Susceptible" implies that there is a high likelihood of a favorable clinical outcome when the drug is administered at its label dose. Walker WDT: p.4, lines 33-35

1131. "Intermediate" is a "buffer zone" to minimize the impact of small, uncontrolled technical factors. Walker WDT: p. 4, lines 35-36

1132. Intermediate is also used for antimicrobial agents that can inhibit bacterial pathogens causing disease in body sites where the drug may be concentrated. Walker WDT: p.4, lines 36-38

1133. "Resistant" implies that the concentration of the drug required to inhibit the growth of the pathogen is such that there would not be a favorable clinical outcome. Walker WDT: p.4, lines 38-40

1134. The MIC, or zone diameter size, used to determine if a bacterium is susceptible, intermediate or resistant is called the breakpoint. In other words, the breakpoint is a dividing point at which a distinction is made within a population. Walker WDT: p. 4, lines 40-43

1135. In reference to bacteria and antimicrobial agents, the breakpoint is the concentration, expressed as a MIC or the size of the zone of inhibition, that distinguishes between a susceptible, intermediate or resistant bacteria. Walker WDT: page 5, lines 1-4

1136. NCCLS has published two documents, the M23-A2 and the M37-A2, which describe guidelines that a drug sponsor needs to follow to establish interpretive criteria acceptable to the NCCLS. Walker WDT: page 5, lines 21-24; G-1795 and G-1797

1137. The pharmacology of antimicrobial chemotherapy can be divided into two principal components; pharmacokinetics (PK) and pharmacodynamics (PD). Walker WDT: page 6, lines 3-4

1138. Pharmacokinetics refers to the absorption, distribution and elimination of drugs in the body. Walker WDT: page 6, lines 5-6

1139. Pharmacodynamics includes the relationship between antimicrobial concentration, either in serum or at the site of infection or both, and the pharmacological and toxicological effects of the antimicrobial drug. Walker WDT: page 6, lines 11-14

1140. With respect to antimicrobial drugs, the essential factor is the relationship between concentration of the antimicrobial drug and the antimicrobial effect against the bacterium. Walker WDT: page 6, lines 14-16

1141. The maximum concentration of drug attained in serum after a dose is referred to as the Cmax. Walker WDT: page 6, lines 16-17

1142. The product of detectable drug concentration relative to time is described by the area under the curve (AUC). In other words, AUC is the area under the graphed serum-drug-concentration vs. time curve following administration of an antimicrobial agent. Walker WDT: page 6, lines 17-20

1143. Regarding the PK-PD parameters for fluoroquinolones, the AUC:MIC ratio generally has the strongest correlation with successful outcome in animal and human infections. Walker WDT: p. 6, lines 22-30

1144. There are no NCCLS interpretive criteria for any antimicrobial agent for the in vitro antimicrobial susceptibility testing of *Campylobacter*. Walker WDT: p. 6, lines 34-35

1145. There is a NCCLS approved standardized testing method and quality control ranges for five antimicrobial agents (Ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem). Walker WDT: p. 6, lines 36-38

1146. The resistance breakpoints put forth by the British Society for Antimicrobial Chemotherapy (BSAC) for *Campylobacter* are ≥4 µg/mL for ciprofloxacin and ≥2 µg/mL for erythromycin. Walker WDT: p. 6, lines 42-44

1147. The proposed resistance breakpoints for *Campylobacter* by the Comite de L'Antibiogramme de la Societe Francaise de Microbiologie are >2 µg/mL for ciprofloxacin and >4 µg/mL for erythromycin. Walker WDT: p. 6, line 44 and p. 7 lines 1-2

1148. The Danish surveillance system lowered its resistance breakpoint for ciprofloxacin to ≥1 µg/mL in 2001, based on the distribution of MIC values in the population of *Campylobacter* analyzed in their surveillance laboratories. Walker WDT: p. 7, lines 3-6

1149. Many scientific reports use the NCCLS interpretive criteria generated for *Enterobacteriaceae* to determine susceptibility and resistance to ciprofloxacin for *Campylobacter* (≥4 µg/mL). Walker WDT: p. 7, lines 8-10

1150. The clinical efficacy of the fluoroquinolones is dependent on achieving high peak serum concentrations to MIC ratios (8 to 12) or high AUC/MIC ratios (≥ 125 or more with ratios of 100 or less more likely to select for resistance). Walker WDT: p. 7, lines 17-20

1151. For ciprofloxacin and *Campylobacter,* a susceptible breakpoint of ≤ 0.25 µg/mL would result in serum Cmax/MIC ratios of 12 and AUC/MIC ratios of 100. These ratios have been shown to correlate well with clinical efficacy. Walker WDT: p. 7, lines 32-35

1152. The overall agreement of MICs between the Etest and agar dilution methods was 61.9%. Walker WDT: p. 9, lines 7-8

1153. Ciprofloxacin MIC agreement between the Etest and agar dilution methods was 85.2%. Walker WDT: p. 9, lines 9-10

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1154. Using a ciprofloxacin resistant breakpoint of either 1.0 or 4.0 μg/mL, Etest under-reported the number of ciprofloxacin-resistant strains. Walker WDT: p. 9, lines 10-11

1155. Surveillance programs or diagnostic laboratories using the Etest for measuring ciprofloxacin susceptibility in *Campylobacter* are likely to underestimate the true prevalence of fluoroquinolone-resistant strains. Walker WDT: p. 9, lines 14-16

1156. MICs obtained using Etest were generally lower than those by agar dilution regardless of the antimicrobial tested. Walker WDT: p. 9, lines 18-19

1157. The College of American Pathologists (CAP), the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and the United States Food and Drug Administration (FDA) have all accepted the NCCLS as the standards setting body for antimicrobial susceptibility testing. Walker WDT: p. 9, lines 31-35

1158. Based on the absorption, distribution and elimination pattern of ciprofloxacin in humans, a bacterial pathogen (including *Campylobacter*) with an MIC >1.0 μg/mL to ciprofloxacin would most likely not respond to therapy and thus should be considered resistant. Walker WDT: p. 10, lines 11-14

Nicholas Weber (G-1482)

1159. Dr. Weber is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

1160. A residue means any compound present in edible tissues of the target animal which results from the use of the sponsored compound, including the sponsored compound, its metabolites, and any other substances formed in or on food because of the sponsored compound's use. Weber WDT: p. 1, line 48 – p. 2, line 1

1161. A withdrawal period is the interval between the time of the latest administration of the new animal drug and the time the animal can be safely slaughtered for food. Weber WDT: p. 2, lines 16-18

1162. If proper withdrawal times are followed, the use of drugs other than Baytril in poultry (e.g., sulfonamides) will not result in unsafe drug residues. Weber WDT: p. 6, lines 10-11

1163. The basic ring structure of nalidixic acid is very closely related to that of a quinolone. Weber WDT: p. 7, lines 7-8

1164. Enrofloxacin is a fluoroquinolone antibiotic. Weber WDT: p. 7, line 16

1165. Ciprofloxacin is a fluoroquinolone that has bactericidal properties similar to other members of the fluoroquinolone family including enrofloxacin. Weber WDT: p. 7, lines 27-28

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1166. Ciprofloxacin is a drug widely used in human medicine. Weber WDT: p. 7, line 28 – p. 8, line 1

1167. Ciprofloxacin is a metabolite of enrofloxacin. Weber WDT: p. 8, line 1

1168. Nalidixic acid, enrofloxacin, and ciprofloxacin all have very similar core structures. Weber WDT: p. 8, lines 5-6

1169. Nalidixic acid, enrofloxacin, and ciprofloxacin often inhibit a specific receptor molecule called a topoisomerase. Weber WDT: p. 8, lines 5-7

1170. When bacteria develop resistance by a slight chemical alteration in their topoisomerase genes, the bacteria often show cross-resistance to compounds of very similar chemical structure. Weber WDT: p. 8, lines 9-11

1171. Bacteria resistant to enrofloxacin are also often resistant to ciprofloxacin and nalidixic acid as well as other fluoroquinolones. Weber WDT: p. 8, lines 12-13

Henrik Wegener (G-1483)

1172. Dr. Wegener is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

1173. Thermophilic *Campylobacter*, notably *Campylobacter jejuni* and *Campylobacter coli*, are normal inhabitants of the gastrointestinal tract of most warm-blooded animals including the major food-animals cattle, swine and poultry. Wegener WDT: page 2, lines 43-46

1174. *Campylobacter* does not usually cause disease in the food animals. *Campylobacter* bacteria colonize the animals' intestines together with hundreds of other species of harmless bacteria. Wegener WDT: page 4, lines 2-4

1175. While poultry and cattle predominantly are colonized by *Campylobacter jejuni*, swine are predominantly colonized by *Campylobacter coli*. *Campylobacter jejuni* causes the majority of human infections in all countries investigated. Wegener WDT: page 4, lines 8-10

1176. Nearly all animals, wild and domesticated, harbor *Campylobacter* as a normal inhabitant of the gastrointestinal tract. Food animals reared in continuous production systems such as cattle and swine probably acquire the infection from the parent animals by faecal-oral transmission. Broiler chicken and other poultry, where there is no contact between the parent bird and the progeny, acquire the infection from the environment. Wegener WDT: page 4, lines 14-18

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1177. The animal gastrointestinal tract is probably the only significant place where *Campylobacter* grow and multiply in the farm to fork chain. Wegener WDT: page 4, lines 18-20

1178. Faeces can, and will, contaminate the animal carcass during slaughter and, consequently, *Campylobacter* is smeared onto the surface of the meat during processing of the fresh meat products. Wegener WDT: page 4, lines 25-27

1179. During the process called evisceration, where the intestines and other internal organs are removed from the killed animal, some degree of faecal contamination is inevitable no mater how stringent hygiene measures are applied. Although, from the moment the animal is slaughtered and the intestines removed, the *Campylobacter* present on that carcass do not multiply further, they may be passed onto other food products by cross-contamination. Wegener WDT: page 5, lines 1-6

1180. One process during the slaughter of broilers that is considered a potential "hot spot" for *Campylobacter* contamination is the defeathering process, where feathers are removed from the killed bird by rotating rubber fingers rubbing the surface of the carcass. Wegener WDT: page 5, lines 11-14

1181. Prior to defeathering, the birds are immersed in hot water (50-52 degrees C. soft scald; 56-58 degrees C. hard scald) to loosen the feathers. Varying proportions of the *Campylobacter* present on the surface of the bird may be killed during scalding depending primarily on the water temperature and time submersed. Wegener WDT: page 5, lines 14-18

1182. Poultry evisceration is carried out by a mechanical claw, a process that inevitably causes some degree of faecal contamination. Wegener WDT: page 5, lines 19-20

1183. Broiler carcasses destined for freezing are usually chilled in a cold-water bath (a "spin-chiller"). During this process, *Campylobacter* transmits from contaminated to non-contaminated carcasses ("cross contamination"). Wegener WDT: page 5, lines 22-24

1184. Several anatomical features in broiler chickens can serve as insulating "pockets" for *Campylobacter*, supporting enhanced survival during freezing and thawing. Wegener WDT: page 5, lines 27-28

1185. Water is not a natural reservoir for *Campylobacter*. Wegener WDT: page 9, lines 1-7

1186. In poultry, notably chicken, several anatomical features support the survival of *Campylobacter* during cooking. Wegener WDT: page 9, lines 29-30

1187. Contaminated meat products can serve as sources of contamination of other food products anywhere along the line of processing and distribution. Wegener WDT: page 9, lines 38-39

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1188. Complete avoidance of cross-contamination, both in the professional processing plant and in the private kitchen, is virtually impossible. Wegener WDT: page 10, lines 3-9

1189. Cross-contamination to products that are consumed without heating, such as ready to eat meat products, vegetables and salads, probably takes place frequently in the consumers' kitchen, as indicated by studies tracing the spread of drip fluid from chicken to the kitchen environment, kitchen utensils, and food products during preparation of a meal. Wegener WDT: page 10, lines 15-19

1190. Chicken products contain *Campylobacter* much more frequently and in much higher numbers that products of beef and pork because the processing of chicken favors the survival of *Campylobacter* whereas the processing of beef and pork includes steps that effectively reduce the *Campylobacter* load in the products. Wegener WDT: page 10, lines 23-26

1191. While optimal kitchen hygiene and cooking probably can reduce the risk of *Campylobacter* infection from food, this risk cannot be eliminated. Investigations of professional food handlers as well as ordinary consumers ' practices in the kitchen document that the majority are either unable or unwilling to adhere to the necessary strict hygiene practices. Wegener WDT: page 10, lines 32-36

1192. The putative sources of human *Campylobacter* infections are direct animal contacts, food, water, environment, and human contacts. While all sources undoubtedly contribute to the total number of human infections, a single source probably predominates over all others in industrialized countries. Poultry, notably broiler chicken, is the most important source of foodborne campylobacteriosis in the industrialized world. Wegener WDT: page 10, line 38 through page 11, line 21; page 13, line 6 through page 15, line 7 and lines 34-36

1193. A number of independent and methodologically different studies from multiple different countries support the conclusion that, poultry, notably broiler chicken, is the most important source of foodborne campylobacteriosis in the industrialized world. While each study in itself may have limitations, the sum of studies only leaves one conclusion possible that broiler chicken is the single most important reservoir of human *Campylobacter* infections, and that broiler products are the single most important sources of human campylobacteriosis in the industrialized world. Wegener WDT: page 10, line 38 through page 11, line 21; page 13, line 6 through page 15, line 7 and lines 34-36; G-1777; G-185; G-602; G-1686; G-182; G-307; G-1718; G-334; G-474; G-299; B-412

1194. The detection of *Campylobacter* in broilers and broiler products shows that broilers are a potential source of infection; indeed there are no examples where human pathogenic bacteria can be consistently detected in a fresh meat product and where this particular product cannot be linked to human disease. Wegener WDT: page 11, lines 10-13

1195. There exists a close correlation between the prevalence of *Campylobacter* in broiler chicken and chicken products and human disease incidence, both when comparing absolute prevalence between otherwise comparable countries such as Denmark and Norway and when evaluating seasonal variations within a given country. Figure 4, "Seasonality of

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Campylobacter in humans and broilers in Denmark", 1998-2001, inserted in Dr. Wegener's testimony shows the trend in human campylobacteriosis and the trend in *Campylobacter* in broiler flocks in Denmark. Wegener WDT: page 11, lines 14-21; G-1777

1196. The seasonal variation observed in broiler poultry flocks can also be found in the derived food products. Furthermore, the incidence in domestically acquired infections displays the same pattern as the poultry curve. This finding is common to a number of nations irrespective of which hemisphere. Wegener WDT: page 12, lines 1-4

1197. There are few examples of documented *Campylobacter* outbreaks. Wegener WDT: page 12, line 6

1198. If *Campylobacter* occurred frequently in water or milk, which is shared by many persons at the same time, outbreaks would occur much more frequently. Wegener WDT: page 12, lines 9-11

1199. By far the majority of human *Campylobacter* infections are registered as sporadic infections; that is, they cannot be linked to any other patient by a common source of the infection and appear to be isolated events with no common source of the infection. Wegener WDT: page 12, lines 18-20

1200. In a questionnaire-based investigation, it is extremely difficult to identify risk factors if they are very common. For example, most people eat chicken at least once a week. Therefore, asking cases as well as controls if they ate chicken in the week before the disease onset (or the week before receipt of the questionnaire, for the controls) is likely to lead to a similar result, i.e., both groups had chicken at least once. This outcome would fail to identify chicken as a risk factor even if it was one. Wegener WDT: page 12, lines 21-39

1201. The findings of twelve independent studies from three different continents and nine different countries strongly indicate that chicken is a source of human *Campylobacter* infection and indeed a frequent source in most industrialized countries. Table 4 inserted in Dr. Wegener's testimony is a survey of 16 published case-control studies. Wegener WDT: page 14 (Table 4 "Risk Factors for Campylobacter infections identified in case control studies from 1979-1998"); page 15, lines 4-7

1202. Epidemiological studies provide strong scientific support that poultry, notably chicken, is an important risk factor for human campylobacteriosis. Wegener WDT: page 15, lines 31-33

1203. Multiple epidemiological studies, which have compared patients to healthy controls by means of interviewing, have shown that consumption of poultry, notably chicken, is a risk factor for *Campylobacter* infection. Wegener WDT: page 15, lines 34-36

1204. Effler's case-control study found that eating chicken prepared by a commercial food establishment was a statistically independent predictor of illness caused by *Campylobacter* (adjusted OR, 1.8; p=0.03). Effler's study was conducted in Hawaii during a five-month

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period in 1998 and enrolled 211 cases and 211 controls matched on age and telephone exchange. Wegener WDT: page 14; G-185

1205. Studahl's case-control study found a statistically significant association between eating chicken and having a *Campylobacter* infection (OR 2.29, 95% CI: 1.29, 4.23). Studahl's study was conducted in Sweden during the twelve-month period in 1995 and enrolled 101 cases and 198 controls matched on age, sex, and district of residence. Wegener WDT: page 14; G-602

1206. Neal's case-control study found that eating chicken was independently associated with *Campylobacter* gastroenteritis and that this association was statistically significant (OR 1.4, 95% CI: 1.1, 1.8). Neal's study was conducted in the United Kingdom during a 14-month period between 1994-1995 and enrolled 313 cases and 512 controls matched on sex and age group. Wegener WDT: page 14; G-1686

1207. Eberhart-Phillips' case-control study found that risk of campylobacteriosis was strongly associated with recent consumption of raw or undercooked chicken (matched OR 4.52, 95% CI: 2.88, 7.10) and that there was also an increased risk associated with chicken eaten in restaurants (matched OR 3.84, 95% CI: 2.52, 5.88). Eberhart-Phillips' study was conducted in New Zealand during a nine-month period between 1994-1995 and enrolled 621 cases and 621 controls matched on sex, age group, and home telephone prefix. Wegener WDT: page 14; G-182

1208. Ikram's case-control study found that eating poultry at a friend's house (OR 3.18; CI: 1.0, 10.73; p=0.03), eating poultry at a barbecue (OR 3.00; CI: 0.99, 9.34; p.=0.03), or eating undercooked chicken (OR 4.94; CI: 1.03, 23.62; p=0.05) were risk factors for acquiring a *Campylobacter* infection. Ikram's study was conducted in New Zealand during a two-month period in 1992-1993 and enrolled 100 cases and 100 controls matched on sex and age. Wegener WDT: page 14; G-182; G-307

1209. In Schorr's case-control study, consumption of poultry liver was shown to be an independent risk factor for *Campylobacter* enteritis (adjusted matched OR 5.7, 95% CI: 1.4, 22.8). Schorr's study was conducted in Switzerland during an eleven-month period in 1991 and enrolled 167 cases and 282 controls matched on sex. Wegener WDT: page 14; G-1718

1210. Kapperud's case-control study found that eating poultry that was brought into the house raw (frozen or refrigerated) was independently associated with *Campylobacter* illness (OR 3.20, p=0.024). Kapperud's study was conducted in Norway during an 18-month period between 1989-1990 and enrolled 52 cases and 103 controls matched by sex, age, and geographic location. Wegener WDT: page 14; G-334

1211. Oosterom's case-control study found that significantly more index patients with a *Campylobacter jejuni* infection had eaten chicken meat (47 v. 29, p=0.0002) particularly at barbecues (14 v. 2, p=0.0015) compared with controls. Oosterom's study was conducted in the Netherlands during four-month period in 1982 and enrolled 54 cases and 54 controls. Wegener WDT: page 14; G-474

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1212. Among chicken-eaters in the Hopkins/Olmstead case-control study, eating undercooked chicken was identified as a risk factor for sporadic *Campylobacter jejuni* infection (unmatched OR 2.77, 95% CI 1.01, 12.7; matched OR 6.27, 95% CI 0.90, 43.84). The Hopkins/Olmstead study was conducted in Colorado during a 2.5-month period in 1981 and enrolled 40 cases and 71 controls matched on age and sex. Wegener WDT: page 14; G-299

1213. The Hopkins/Scott case-control study determined that handling raw chicken or preparing chicken was significantly associated with *Campylobacter jejuni* illness. This study was conducted in Colorado during a one-month period in 1982 and enrolled 10 cases and 15 controls. Wegener WDT: page 14; B-412

1214. Different methods are used for typing of *Campylobacter*. The most informative methods currently used for typing of *Campylobacter* are pulsed-field gel-electrophoresis (PFGE), amplified fragment-length polymorphisms (AFLP) and, more recently, Multilocus Sequence Typing (MLST). Wegener WDT: page 16, line 27 and lines 30-32

1215. Investigation of strains of *Campylobacter* from animals food and human patients by genetic fingerprinting and other sensitive methods for tracing sources of human infection has provided scientific support for the hypothesis that poultry, notably chicken, is a source of human fluoroquinolone-resistant *Campylobacter* infections. Furthermore, the route of transmission from the farm to the patient has been supported. Wegener WDT: page 17, line 41 through page 18, line 3

1216. In Belgium, a food scare caused by detection of a harmful toxin "dioxin" in animal feed led the authorities to require withdrawal of Belgian poultry and eggs from the market. Imported poultry and other meat products remained available to the consumers. The incidence of human *Campylobacter* infections declined by 40% in the period that Belgian poultry and eggs were withdrawn from the shops. When Belgian poultry and eggs were readmitted in the market, incidence of human *Campylobacter* infections returned to the "normal" level. The Belgian investigators concluded that the decline in the number of *Campylobacter* infections in Belgium by 40% was due to the withdrawal of Belgian poultry from the market. Wegener WDT: page 18, lines 13-21; G-672

1217. In Iceland, a sharp increase in human *Campylobacter* infections occurred in the period from 1997 to 1999. This increase coincided with the marketing of fresh chicken products where, in the past, most chicken products have been frozen products. Chicken marketed in Iceland is almost exclusively of domestic origin. A control program was implemented by which flocks are tested for *Campylobacter* a week prior to slaughter; *Campylobacter*-positive flocks are slaughtered independently of *Campylobacter*-negative flocks, and chicken meat from positive flocks is frozen before it is marketed. Following the introduction of this control program, the incidence of domestically acquired campylobacteriosis has been reduced by approximately 70%. Wegener WDT: page 18, line 25 through page 19, line 13

1218. In Norway, a *Campylobacter* action plan was initiated in 2002 based on the same principles as the plan of Iceland. A nearly 50% reduction in domestically acquired human

campylobacteriosis has been observed in the first 39 weeks of 2002 compared to the same time period in 2001. Wegener WDT: page 19, lines 14-18

1219. The three independent intervention studies in Belgium, Iceland, and Norway document, beyond reasonable scientific doubt, that poultry, notably chicken, constitutes a major source of human campylobacteriosis in these countries. Interventions primarily or exclusively aimed at poultry have reduced the human incidence of campylobacteriosis by 40-70% in the respective countries. Wegener WDT: page 20, lines 4-8

1220. When resistance emerges in *Campylobacter* in animals, resistant *Campylobacter* transmits to humans. Wegener WDT: page 20, lines 24-25

1221. The increase in *Campylobacter* resistant to quinolones in broiler chicken in Denmark was paralleled by an increase in human infections with *Campylobacter* resistant to quinolones. This is consistent with the pattern observed in many other countries. In Denmark this increase has occurred later than in many other European countries, but, as in other countries, the onset of the increase has occurred shortly after the licensing of fluoroquinolones for use in food animals including poultry. The different times of the onsets of the increase in levels of resistance in different countries, and the common association with the licensing of quinolones for food animals in all countries, strongly support that veterinary use of quinolones and not the medical use of quinolones is the driving factor behind the increase in animals as well as in humans. Wegener WDT: page 23, lines 5-15

1222. *Campylobacter* is among the most common causes of travelers' diarrhea in industrialized countries. Wegener WDT: page 23, lines 17-20

1223. On *Campylobacter*, the WHO consensus statement reads: "Following the introduction of fluoroquinolones for use in poultry there has been a dramatic rise in the prevalence of fluoroquinolone-resistant *Campylobacter jejuni* isolated in live poultry, poultry meat and from infected humans. Moreover, prior to any use in poultry, no resistant strains were reported in individuals with no previous exposure to quinolones. Fluoroquinolone-resistant *Campylobacter* has been associated with treatment failures." (World Health Organization 1997 Meeting). Wegener WDT: page 25, lines 1-6

1224. In 1998, the World Health Organization convened another meeting addressing in particular the use of quinolones in food animals and potential impact on human health. It was a meeting of experts and stakeholder including pharmaceutical industries, and a consensus report was produced from the meeting. Dr. Wegener participated in the meeting as an invited expert and speaker. The consensus statements were agreed upon by these experts and stakeholders, including pharmaceutical industry representation by Bayer Corporation. Wegener WDT: page 25, lines 10-17

1225. A WHO consensus statement reads: "*Campylobacter jejuni* is a frequent commensal in poultry and cattle, and *C. coli* is a frequent commensal in swine and poultry. There is a temporal association between the introduction of fluoroquinolones for use in poultry and a substantial rise in the prevalence of quinolone-resistant *Campylobacter jejuni* isolated in live

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poultry, poultry meat and from infected humans. Moreover, prior to any use in poultry, no resistant strains were reported in individuals with no previous exposure to quinolones." (World Health Organization 1998 Meeting). Wegener WDT: page 25, lines 18-23

1226. A WHO consensus statement reads: *"Campylobacter* species are the commonest cause of bacterial gastroenteritis in developed countries. Sporadic cases of campylobacteriosis, which comprise the largest number of reported cases, are predominantly associated with consumption of contaminated food, primarily poultry, in most developed countries." (World Health Organization 1998 Meeting). Wegener WDT: page 25, lines 24-27

1227. Neimann investigated the duration of illness in patients and found a tendency that patients infected with a quinolone-resistant *Campylobacter* and treated with a fluoroquinolone had a longer duration of illness (average excess duration of 5 days) than the duration of illness in patients with a quinolone-sensitive illness and treated with a fluoroquinolone. Wegener WDT: page 26, lines 1-6; B-561

1228. In 2000, the World Health Organization convened a consultation of experts on the increasing incidence of human campylobacteriosis. Twenty-nine internationally recognized scientific experts in *Campylobacter* and campylobacteriosis participated in the consultation. Dr. Wegener participated in the consultation as an expert, speaker, co-chair, and local secretariat. There were agreed-upon conclusions and recommendations from the consultation. Wegener WDT: page 26, lines 7-13

1229. A WHO agreed-upon conclusion reads: *"Campylobacter* is the leading cause of zoonotic enteric infections in developed and developing countries." (World Health Organization 2000 Meeting). Wegener WDT: page 26, lines 14-15

1230. A WHO agreed-upon conclusion reads: "The reported incidence of campylobacteriosis in most developed countries has risen substantially during the past 20 years, and especially since 1990. In developing countries campylobacteriosis is widespread and causes significant morbidity, and even mortality in infants and children. Additional concern is raised by the increasing number of newly described *Campylobacter* species, as well as the increasing number of antibiotic-resistant strains of the common species, *C. jejuni.* Recently, too, it has been recognized that the paralytic condition, Guillan-Barré Syndrome (GBS), is a serious complication of *Campylobacter* infection. (World Health Organization 2000 Meeting). Wegener WDT: page 26, lines 16-23

1231. A WHO agreed-upon conclusion reads: "In developed countries, for example, handling and consumption of poultry meat are primary sources of infection and are likely to account for much of the increased incidence of campylobacteriosis." (World Health Organization 2000 Meeting). Wegener WDT: page 26, lines 24-26

1232. A WHO agreed-upon conclusion reads: "...the most alarming increase in resistance is to the fluoroquinolone group of antimicrobials. This is because adult patients suffering from severe gastrointestinal disease are likely to be treated with a fluoroquinolone prior to confirmation of the diagnosis, and if the strain of *Campylobacter* is fluoroquinolone-

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resistant, the duration of the illness may be prolonged. One of the major reasons for the increase in fluoroquinolone-resistant strains in human disease is the use of these antibiotics in poultry." (World Health Organization 2000 Meeting). Wegener WDT: page 26, lines 27-32

1233. Three different World Health Organization meetings with participation from different disciplines and sectors have all reached essentially the same conclusions supporting that poultry is a major source of human *Campylobacter* infections, including quinolone-resistant *Campylobacter* infections: (1) "Following the introduction of fluoroquinolones for use in poultry there has been a dramatic rise in the prevalence of fluoroquinolone-resistant *Campylobacter jejuni* isolated in live poultry, poultry meat and from infected humans. Moreover, prior to any use in poultry, no resistant strains were reported in individuals with no previous exposure to quinolones."; (2) "*Campylobacter* species are the commonest cause of bacterial gastroenteritis in developed countries. Sporadic cases of campylobacteriosis, which comprise the largest number of reported cases, are predominantly associated with consumption of contaminated food, primarily poultry, in most developed countries."; and (3) "In developed countries, for example, handling and consumption of poultry meat are primary sources of infection and are likely to account for much of the increased incidence of campylobacteriosis." Wegener WDT: page 26, line 33 through page 27, line 9

1234. The available scientific evidence supports, beyond reasonable scientific doubt, that poultry products, notably chicken meat, are the major source of human campylobacteriosis infections in industrialized countries. Wegener WDT: page 27, lines 12-27

David White (G-1484)

1235. Dr. White is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

1236. Most foodborne illness goes undiagnosed and unreported. White WDT: p. 2, line 27

1237. Nearly 2.4 million cases of foodborne illness are caused by *Campylobacter* each year in the United States. White WDT: p. 2, lines 29-30; G-410; G-1373

1238. *Campylobacter* is recognized as one of the leading causes of foodborne gastroenteritis in the United States. White WDT: p. 2, lines 38-40

1239. *Campylobacter* is one of the most frequent causes of acute bacterial enteritis worldwide. White WDT: p. 2, lines 38-40

1240. In food producing animals such as cattle, poultry and swine, fecal *C. jejuni/C. coli* is regarded as a commensal organism (i.e., does not cause disease). White WDT: p. 2, lines 42-43

1241. Raw poultry meats are commonly contaminated with *Campylobacter*, with prevalence rates reported up to as high as 100%. White WDT: p. 2, lines 46 - p. 3 line 2

1242. Zhao's study found that 70.7% of raw chicken sampled (n=184) between June, 1999 to July, 2000 in the Greater Washington, DC area were contaminated with *Campylobacter*. White WDT: p. 3, line 7-13; G-727

1243. Zhao's study found that approximately 14% of raw turkey samples (n=172) between June, 1999 to July, 2000 in the Greater Washington, DC area yielded *Campylobacter*. White WDT: p. 3, lines 7-13; G-727

1244. Ge's results from antimicrobial susceptibility testing of 135 *Campylobacter* isolates recovered from retail meat (81 *Campylobacter jejuni* isolates: from chickens, 75 from 32 samples; 4 from a beef sample; and, 2 from a pork sample; 39 *Campylobacter coli* isolates: 39 from 14 chickens; 7 from 3 turkeys; and, 8 from 3 pork samples) in the Greater Washington, DC area during the summer and autumn of 1999 show 21.5% resistant to nalidixic acid and 20.7% resistant to ciprofloxacin. White WDT: p. 3, lines 26-35; G-763

1245. 33.3% of *Campylobacter coli* identified in Ge's study were resistant to ciprofloxacin and 31.5% were resistant to nalidixic acid. White WDT: p. 3, lines 26-37; G-763

1246. 12.3% of *Campylobacter jejuni* identified in Ge's study were resistant to ciprofloxacin and 14.8% were resistant to nalidixic acid. White WDT: p. 3, lines 26-37; G-763

1247. *Campylobacter jejuni* and *Campylobacter coli* isolates resistant to antimicrobials used for treating campylobacteriosis are common in retail meats. White WDT: p. 3, lines 41-43; G-763

1248. In 2002, NARMS expanded into surveillance of retail meats to determine the presence of antimicrobial resistance among certain bacteria including *Campylobacter*. White WDT: p. 3, lines 45-47

1249. Preliminary data as of November 2002 for the retail meat arm of NARMS show that 58% of 356 chicken breast samples analyzed and 8% of 372 ground turkey samples were positive for *Campylobacter*. White WDT: p. 4, lines 12-15

1250. In a 1986 study conducted by the Washington State Department of Health, 57% of poultry processing plant samples and 23% of retail chickens carried *C. jejuni*. White WDT: p. 4, lines 24-26; B-387

1251. In K. Smith's study of retail chicken from September to November 1997, 88% of 91 retail chicken products surveyed in Minnesota by Smith had *Campylobacter*. White WDT: p. 4, lines 29-32; G-589

1252. There is an association between molecular subtypes of resistant *C. jejuni* strains that are acquired domestically in humans and those found in retail chicken products. White WDT: p. 4, lines 45-47

1253. The 1994-1995 FSIS baseline study in chickens estimated that the prevalence of *Campylobacter* contaminated chicken broiler carcasses sampled from July, 1994 through June, 1995 was 88%. White WDT: p. 5, lines 4-11; G-652

1254. The 1996-1997 FSIS baseline study in turkeys estimated that the prevalence of *Campylobacter* contaminated young turkey carcasses sampled from August, 1996 to July, 1997 was 90%. White WDT: p. 5, lines 14-17; G-651

1255. In 1996, FSIS estimated the national prevalence of *Campylobacter jejuni/coli* on raw ground chicken to be 59.8% in 1996. White WDT: p. 5, lines 25-27;G-653

1256. In 1996, FSIS estimated the national prevalence of *Campylobacter jejuni/coli* on raw ground turkey to be 25.4% in 1996. White WDT: p. 5, lines 29-32; G-654

1257. The January-June 1999 FoodNet survey of retail chickens found that 44% of retail chicken meat samples were contaminated with *Campylobacter*. White WDT: p. 5, lines 35-47; G-541; G-1528

1258. In the January-June 1999 FoodNet survey or retail chickens, overall 11% of retail chicken samples yielded ciprofloxacin resistant *Campylobacter*; 24% of the *Campylobacter jejuni* contaminated chicken samples were resistant to ciprofloxacin. White WDT: p. 5, lines 35; p. 6, line 2; G-541; G-1528

1259. Studies have shown that some human clinical *C. jejuni* isolates display PFGE patterns indistinguishable from PFGE patterns observed in *C. jejuni* strains recovered from tested poultry carcasses. White WDT: p. 6, lines 9-12; G-1785

1260. 69.4% of 360 broilers carcasses purchased at a supermarket from January 1994 through December 1994 and tested by Willis were positive for *Campylobacter jejuni*. White WDT: p. 6, lines 15-19; G-701

1261. Stern found approximately 65% of 2075 poultry carcass rinses contaminated with *Campylobacter*. White WDT: p. 6, lines 25-27; G-791

1262. Agar dilution involves the incorporation of an antimicrobial agent into an agar medium in a geometrical progression of concentrations followed by the application of a defined bacterial inoculum to the agar surface of the plate. White WDT: p. 7, lines 14-17

1263. The standardized agar dilution method for testing *Campylobacter* was developed by scientists at CVM. White WDT: p. 7, lines 26-27

1264. In the Iowa retail meat study, 20% of 654 retail meats purchased from March, 2001 to March, 2002, were positive for *Campylobacter*. White WDT: p. 6, lines 38-40 and 45-46, and p.7, lines 29-30; G-746; G-1352

1265. In the Iowa retail meat study, chicken accounted for most (73%) of the *Campylobacter*-positive test results. White WDT: p. 7, lines 29-31; G-746; G-1352

1266. In the Iowa retail meat study, 20% of retail meats were positive for *Campylobacter;* only 1% of retail pork samples and none of retail ground beef samples yielded *Campylobacter.* White WDT: p. 7, lines 31-33; G-746; G-1352

1267. 27% of *C. jejuni* isolates recovered from either retail chicken or turkey exhibited ciprofloxacin resistance (MIC $\geq$ 4 μg/ml) and 27% of *C. coli* isolated from retail chicken, turkey and pork exhibited ciprofloxacin resistance in the Iowa retail meat study. White WDT: p. 7, lines 34-37; G-746; G-1352

1268. *Campylobacter* contamination of retail meats is not limited to poultry raised and slaughtered in the United States. White WDT: p. 8, lines 4-5

1269. The prevalence of *Campylobacter* and fluoroquinolone-resistant *Campylobacter* on retail poultry in other countries is relevant because foreign travel to certain countries has been implicated as a risk factor for acquiring *Campylobacter* and fluoroquinolone-resistant *Campylobacter.* White WDT: p. 8, lines 5-9

1270. In the summer of 1994, 1853 fresh chicken breasts of German, Dutch and French origin were purchased at local markets and analyzed for the presence of bacteria. *Campylobacter* was isolated from 28% of the fresh chicken meat. White WDT: p. 8, lines 12-15; A-169

1271. Researchers from the United Kingdom looked at a total of 300 raw samples of chicken purchased in New South Wales, U.K. *Campylobacter* was isolated from 68% of the samples and in 34% of the meat packaging. White WDT: p. 8, lines 20-24; G-270

1272. In Spain, a group of scientists looked at 198 samples of retail chicken from retail outlets and supermarkets during February 1999 to November 1999 and found that the prevalence of *Campylobacter* in retail chicken was 49.5%. White WDT: p. 8, lines 26-28; G-730

1273. The reported rate of *Campylobacter* contamination on pork products is 1.3% in the U.S. and 2% in Belgium. White WDT: p. 8, lines 31-32

1274. The prevalence of *Campylobacter* in beef is generally low. White WDT: p. 8, lines 33-34

1275. The majority of studies indicate that poultry meat products are most often contaminated with *Campylobacter* compared with other retail meat commodities. White WDT: p. 8, lines 35-37

1276. Poultry, in particular chicken, represent a major reservoir for human *Campylobacter* infections, including fluoroquinolone-resistant variants, to which humans are routinely exposed through the food supply. White WDT: p. 8, lines 40-42

1277. Humans are routinely exposed to *Campylobacter* and fluoroquinolone-resistant *Campylobacter* through the food supply. White WDT: p. 8, lines 41-42

1278. Poultry constitutes the most important reservoir for human *Campylobacter* infections. White WDT: p. 8, lines 44-45

1279. The use of fluoroquinolones in poultry has played a principal role in increasing resistance to fluoroquinolone among *Campylobacter* isolates recovered from human illness. White WDT: p. 8, lines 5-47

Christopher Ohl (G-1485)

1280. Dr. Ohl is qualified as an expert to testify as to the matters set forth in his written direct testimony submitted on December 9, 2002.

1281. Bacterial enteritis is a very common cause of traveler's diarrhea. Ohl WDT: p. 4, lines 20 to 22

1282. Gastroenteritis is the medical term for inflammation of the stomach and intestine. Ohl WDT: p. 4, lines 6-7

1283. Gastritis refers to such disorders of the stomach and is predominately a vomiting illness, while enteritis involves the small or large intestine and manifests with diarrhea. Ohl WDT: p. 4, lines 7-9

1284. Gastroenteritis is an acute illness afflicting both the stomach and intestines. Ohl WDT: p. 4, lines 9-10

1285. Gastroenteritis is a common disease with up to 1 billion cases per year worldwide causing 3.3 million deaths, mostly due to severe dehydration. Ohl WDT: p. 4, lines 15-16

1286. In the United States there are an estimated 100 to 375 million gastroenteritis episodes, ~3000 attributable deaths, and depending on the patient's age, 1.5 to 5 episodes per person per year. Ohl WDT: p. 4, lines 18-20

1287. Gastroenteritis is the third most common syndrome seen in general practice. Ohl WDT: p. 4, lines 24-25

1288. In 1985, 8.2 million Americans visited a physician and 250,000 persons required hospitalization because of diarrhea. More recent data show 28 million health care provider visits, 45 million physician phone calls and 1.8 million hospitalizations that are associated with 116 million antidiarrheal and 19 million antibiotic medication prescriptions. Ohl WDT: p. 4, lines 25-30; reference 4

1289. Military populations are particularly at risk for diarrheal illness. Ohl WDT: p. 4, line 32

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1290. Diarrheal illness is one of the most common nontraumatic reasons for military population hospitalizations in peace as well as wartime. Ohl WDT: p. 4, lines 32-34

1291. *Campylobacter jejuni* is the most common cause of bacterial enteritis in the United States. Ohl WDT: p. 4, lines 41-42

1292. Bacterial gastroenteritis is acquired through swallowing viable bacteria and subsequent passage of the bacteria from the stomach to the intestines. Ohl WDT: p. 5, lines 1-2

1293. Person to person spread with *Campylobacter* is unusual. Ohl WDT: p. 5, lines 7-8

1294. The predominate symptom of bacterial gastroenteritis or enteritis is diarrhea accompanied by cramping abdominal pain. Ohl WDT: p. 5, lines 12-13

1295. Persons afflicted with bacterial gastroenteritis may have between 3 and 15 loose or watery stools per day. Ohl WDT: p. 5, line 14

1296. Additional symptoms of bacterial gastroenteritis may include fever, headache, muscle aches, rectal pain with defecation, weakness, fatigue, malaise, and occasionally vomiting. Ohl WDT: p. 5, lines 15-17

1297. In most cases people are not able to function normally or be productive until their illness begins to resolve. Ohl WDT: p. 5, lines 32-33

1298. Patients who seek medical care for bacterial enteritis due so in order to obtain treatment to relieve their symptoms and hasten the resolution of their illness. Ohl WDT: p. 5, lines 33-35

1299. It is estimated that there are probably more than 2 million *Campylobacter jejuni* infections in the U.S. annually. Ohl WDT: p. 6, lines 7-9

1300. *Campylobacter jejuni* is the most common organism that is grown from stool specimens of patients with inflammatory diarrhea. Ohl WDT: p. 6, lines 9-11

1301. Most patients acquire *Campylobacter* infection from contaminated food, milk, or water. Ohl WDT: p. 6, lines 12-13

1302. The majority of patients become infected with *Campylobacter* through the ingestion of undercooked or post-cooking contaminated poultry including chicken, and turkey. Ohl WDT: p. 6, lines 13-15

1303. *Campylobacter* is a common cause of traveler's diarrhea, particularly for visitors to southeast Asia. Ohl WDT: p. 6, lines 17 and 18

1304. *Campylobacter jejuni* causes moderate to severe inflammation of the small and large intestine resulting in the secretion of large amounts of fluid. Ohl WDT: p. 6, lines 20-21

1305. Patients with campylobacteriosis are usually dehydrated and in the elderly and young infants this dehydration can be profound and cause death if not treated with fluids. Ohl WDT: p. 6, lines 28-30

1306. In 10 percent of cases, particularly those not treated with antibiotics, *Campylobacter* intestinal infection can relapse and cause recurrent diarrheal illness, with or without associated systemic symptoms. Ohl WDT: p. 6, lines 38-41

1307. Cases in which *Campylobacter* invades the bloodstream always require antibiotic treatment. Ohl WDT: p. 7, lines 2-4

1308. Children less than one year of age, the elderly or patients with cancer, HIV infection, or low levels of antibodies are always treated for *Campylobacter* infection if confirmed or suspected in order to alleviate symptoms, reduce the rate of reoccurrence and prevent complications. Ohl WDT: p. 7, lines 11-15

1309. Inflammatory, bacterial enteritis is usually suspected in patients who have more diarrhea than vomiting and accompanying fever and systemic symptoms. Ohl WDT: p. 8, lines 2-4

1310. Physical examination of the patient is usually not that helpful in differentiating a bacterial cause from viral or parasitic cause of illness. Ohl WDT: p. 8, lines 5-7

1311. A culture of stool for the specific types of bacteria that cause diarrhea is required to confirm the diagnosis and identify the exact bacterium responsible for the illness. Ohl WDT: p. 8, lines 14-16

1312. Many offices and some hospitals currently send out laboratory specimens to a large centralized lab which delays the plating of the culture and decreases the chances of it growing. Ohl WDT: p. 8, lines 25-27

1313. *Campylobacter,* in particular, is difficult to culture from stool as many hospital and clinical laboratories do not have the filter apparatus and special incubation jars that have been shown to yield optimal culture results. Ohl WDT: p. 8, lines 28-30

1314. The perception of many clinicians is that stool cultures are not cost effective. Ohl WDT: p. 8, lines 30-32

1315. Many health-care providers will treat infectious diarrhea without the use of stool cultures. Ohl WDT: p. 8, lines 33-34

1316. Studies of the efficiency of stool cultures have confirmed their expense and a low yield between approximately 1 and 15%. Ohl WDT: p. 8, lines 37-38

1317. Most physicians treating adults or older adolescents do not wait for the results of an *E. coli* 0157:H7 culture or toxin test before prescribing an antibiotic unless the patient has a known risk for this specific illness in the setting of an epidemic. Ohl WDT: p. 9, lines 8-11

1318. *E. coli* 0157:H7 is a rare illness and outside of children the risk of HUS is very small. Ohl WDT: p. 9, lines 12-13

1319. Most cases of HUS occur in children, and fluoroquinolone antibiotics (including ciprofloxacin), the most commonly used antibiotic for inflammatory diarrhea, are contraindicated for this age group. Ohl WDT: p. 9, lines 17-19

1320. For moderate to severe cases of inflammatory diarrhea, most medical care providers will start treatment with an antibiotic before stool culture results are available. Ohl WDT: p. 10, lines 28-30

1321. Moderate to severe inflammatory diarrhea associated with fever, and systemic symptoms with or without blood in the stool, should be treated with antibiotics. Ohl WDT: p. 10, lines 43-45

1322. Patients benefit symptomatically from antibiotic therapy, recover from illness more quickly and are able to return to work earlier. Ohl WDT: p. 10, line 46; - p. 11, line 2

1323. Antibiotic therapy hastens recovery from traveler's diarrhea. Ohl WDT: p. 11, lines 2-3

1324. Guidelines of the Infectious Diseases Society of America (ISDA) recommend that immunocompromised patients, including those with antibody deficiencies, cancer, organ transplants, and human immunodeficiency virus infection; infants; and patients who are pregnant, elderly, or ill with diabetes or chronic liver or intestinal disease, should be treated with antibiotics for inflammatory diarrhea presumed to be due to a bacterial infection. Ohl WDT: p. 11, lines 11-18; G-261

1325. Soldiers and sailors are likely to be treated for milder symptoms of gastroenteritis. Ohl WDT: p. 11, line 26

1326. Many times antibiotic therapy is started without specific knowledge of the cause of the bacteria because of the lack of submitted stool culture or the institution of therapy before culture results are available. Ohl WDT: p. 11, lines 31-34

1327. The most appropriate antibiotic for suspected bacterial enteritis of unknown etiology is one that has the appropriate spectrum to cover the usual bacteria that cause the syndrome. Ohl WDT: p. 11, lines 38-40

1328. The ISDA guidelines recommend the fluoroquinolone antibiotic ciprofloxacin as the preferred empiric treatment for bacterial enteritis and traveler's diarrhea in adults if therapy is required. Ohl WDT: p. 11, line 44-p. 12, line 2; G-261

1329. In addition to better efficacy, there are fewer side effects for patients who take ciprofloxacin than alternative drugs for treatment of bacterial enteritis. Ohl WDT: p.12, lines 10-12

1330. Studies of patients with moderate to severe diarrhea due to *Campylobacter* who received antibiotics early in their illness (~2 days) have shown that antibiotic treatment shortens the duration of illness, decreases severity of symptoms, and reduces the number of diarrheal stools in addition to decreasing the number of days of shedding of the bacterium. Ohl WDT: p. 12, line 43-p. 13, line 1; G-172; G-707; G-399

1331. Most authorities now recommend that moderate to severe symptomatic infectious diarrhea due to *Campylobacter* be treated with antibiotics, particularly if it is accompanied by bloody stools, fever, chills, and worsening or non-resolving symptoms. Ohl WDT: p. 13, lines 5-8

1332. Antibiotic treatment in campylobacteriosis cases decreases the duration of diarrhea symptoms by 2-3 days. Ohl WDT: p. 13, lines 2-3

1333. Antibiotic therapy of *Campylobacter* enteritis considerably reduces the chances of relapse of the illness. Ohl WDT: p. 13, lines 14-16

1334. For *Campylobacter jejuni* bacteria that are not resistant to antibiotics, erythromycin, ciprofloxacin, and azithromycin have been shown to be effective antibiotics for killing the bacterium in the test tube and improving the symptoms and duration of diarrheal illness. Ohl WDT: p. 13, lines 20-23

1335. Dr. Bartlett, in his guide *Therapy of Diarrhea, Community Acquired Acute*, recommends erythromycin or ciprofloxacin for *Campylobacter* enteritis. Ohl WDT: p. 13, lines 26-27

1336. The Sanford Antibiotic Guide recommends ciprofloxacin or azithromycin as the preferred therapy and erythromycin as alternative therapy. Ohl WDT: p. 13, lines 27-29

1337. Dr. Cunha recommends in his guide book either ciprofloxacin, doxycycline, or erythromycin and as alternative therapy azithromycin or clarithromycin. Ohl WDT: p. 13, lines 29-31

1338. Ciprofloxacin or azithromycin is tolerated better by adult patients, with less side effects than erythromycin, and requires fewer administered doses per day. Ohl WDT: p. 13, lines 32-33

1339. Most cases of bacterial enteritis are treated empirically without the identification of a causative bacteria, and that the majority of these cases will be undiagnosed *Campylobacter* enteritis. Ohl WDT: p. 13, lines 40-42

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1340. Ciprofloxacin, the antibiotic indicated for empiric therapy of bacterial enteritis due to an unknown bacterium, will be used for treatment of the majority of cases of *Campylobacter* enteritis. Ohl WDT: p. 13, lines 42-45

1341. Many patients with campylobacteriosis treated with ciprofloxacin will benefit with reduced symptom severity and duration and a faster return to a functional status. Ohl WDT: p. 13, lines 45-46

1342. The treatment of *Campylobacter* enteritis is becoming complicated by the development of antibiotic resistance of this bacterium to ciprofloxacin, erythromycin and azithromycin. Ohl WDT: p. 14, lines 4-6

1343. Patients with inflammatory bacterial enteritis due to antimicrobial resistant *Campylobacter* are less likely to realize the benefits of treatment with antibiotics than patients infected with an antibiotic sensitive *Campylobacter*. Ohl WDT: p. 14, lines 11-13

1344. Symptomatic relapse developing during or after treatment with ciprofloxacin has been described with *Campylobacter* infection due to ciprofloxacin resistance in this bacterium. Ohl WDT: p. 14, lines 18-20

1345. In Marano's multistate surveillance study, patients treated with a fluoroquinolone antibiotic for *Campylobacter* enteritis had diarrhea that lasted significantly longer amongst those infected by a fluoroquinolone-resistant strain than by a fluoroquinolone-sensitive strain (8 days vs 6 days, p=0.02). Ohl WDT: p. 14, lines 23-27; G-394

1346. In K. Smith's study, patients treated with a fluoroquinolone for *Campylobacter* enteritis caused by a fluoroquinolone-resistant strain showed a slower response to therapy by 3 days (p=0.03). Ohl WDT: p. 14, lines 27-30;G-589

1347. Data show that the morbidity of ciprofloxacin-resistant *Campylobacter* gastroenteritis is higher than that of ciprofloxacin-sensitive illness due to this bacterium. Ohl WDT: p. 14, lines 32-34

1348. A recent analysis of attributable morbidity and mortality due to antimicrobial resistance in *Campylobacter jejuni* infections in the United States calculated that each year 22,085 infections, hospitalizations and 1 death are attributable to fluoroquinolone-resistant *Campylobacter jejuni*. Ohl WDT: p. 14, lines 34-38

1349. The increasing rate of fluoroquinolone resistance in *Campylobacter jejuni* will make treatment more and more difficult for afflicted patients and deprive them of an opportunity for treatment to reduce the severity and duration of symptoms and an earlier return to work. Ohl WDT: p. 14, lines 38-42

1350. For patients who are at higher risk for complications of *Campylobacter species* enteritis, such as invasion of the blood stream or other organs, fluoroquinolone-resistance is potentially life-threatening. Ohl WDT: p. 14, lines 42-44

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1351. Most medical providers will initiate empiric therapy based on treatment guidelines or guide books in the hopes of reducing the severity and duration of diarrhea and its associated symptoms. Ohl WDT: p. 15, lines 19-22

1352. The empiric therapy of presumed bacterial enteritis is ciprofloxacin for adults. Ohl WDT: p. 15, lines 22-23

1353. If treatment with ciprofloxacin is initiated early in the course of illness, many patients will realize benefits in terms of lessened severity and duration of illness. Ohl WDT: p. 15, lines 23-25

1354. The development of ciprofloxacin-resistance in *Campylobacter* species is complicating the treatment of this illness and resulting in treatment failures. Ohl WDT: p. 15, lines 35-37

1355. Continued development of antimicrobial resistance in this *Campylobacter* will increase the morbidity and mortality of infections due to this bacterium. Ohl WDT: p. 15, lines 37-39

Gregory Burkhart (B-1900)

1356. The retail and slaughter data confirm that fluoroquinolone-resistant *Campylobacter* can contaminate poultry intended for human consumption. Burkhart WDT: p. 3, line 8-9.

1357. When used in poultry, enrofloxacin is administered in the drinking water to all birds in the same housing unit as those birds with a suspected *E. coli* or *Pasteurella* infection. Burkhart WDT: p. 6, line 11-12.

1358. Several publications have provided bacteriological sampling data from retail chicken products in the United States as well as from carcasses sampled in slaughter houses and these data confirm the presence of fluoroquinolone-resistant *Campylobacter* at the retail at surprisingly high rates given the fairly low rate of use of enrofloxacin in poultry production. Burkhart WDT: p. 9, 10-14.

1359. The most likely exposure to fluoroquinolone-resistant *Campylobacter*, given all current evidence, would be uncooked or undercooked food that contains fluoroquinolone-resistant *Campylobacter*. Burkhart WDT: p. 9, line 42-43.

1360. The 1996-1998 database from Minnesota (i.e., the Smith study) is a source of multi-year data in the United States on fluoroquinolone-resistant *Campylobacter* that is: (a) derived from a well-defined denominator; (b) not based upon non-random sampling of reported cases; and (c) captured data on foreign travel and prior fluoroquinolone use. Burkhart WDT: p. 16, line 40-44.

1361. The Minnesota data from 1996-1998 (i.e., the Smith study) are probably the most robust multiyear dataset in the United States, and perhaps the world, containing information on foreign travel and prior fluoroquinolone use. Burkhart WDT: p. 17, line 12-14.

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1362. If there is no increased virulence, there is no reason to believe that the total *Campylobacter* incidence would change at all if domestic fluoroquinolone-resistant cases could be eliminated; resistant cases would simply be replaced by non-resistant cases. Burkhart WDT: p. 33, line 7-11.

1363. Irrespective of foreign travel or prior fluoroquinolone use, resistant cases with no use of an antidiarrheal agent tended to have a longer duration of diarrhea by 1-2 days. Burkhart WDT: p. 37, line 6-8.

1364. The 1996-1998 Minnesota data (i.e., the Smith study) are likely to be the most valid data in the United States that are available to study the issue of increasing domestically acquired fluoroquinolone-resistant *Campylobacter*. Burkhart WDT: p. 45, line 14 through p. 46, line 2.

1365. There is good evidence to support the conclusion that enrofloxacin use in poultry can select for fluoroquinolone-resistant *Campylobacter*, and that such selection, leads to fluoroquinolone-resistant *Campylobacter* on poultry at slaughter and at the retail level. Burkhart WDT: p. 48, line 4-6.

Louis Anthony Cox, Jr. (B-1901)

1366. Cox determined his figure for population-attributable risk (PAR) for chicken consumption by treating the absence of a response to a yes-or-no survey question as a "no". This had the effect of reducing that calculation of that PAR from 11% to 3.1%. Later he concedes that the estimate of a 24% or 25% PAR for eating chicken in restaurants depends heavily on how such missing data are treated. Cox WDT: p. 56, first of three "Note" paragraphs; Cox WDT: p. 57, first full paragraph.

1367. After substituting some values pursuant to his testimony, and using his (Cox-Popken) model, Cox testified that the baseline version of his model predicts that 2,814 treatment failures per year would be averted by a "ban" [withdrawal of approval] of enrofloxacin. Cox WDT: p. 84, last three lines.

Roger Feldman (B-1902)

1368. The majority of *Campylobacter* cases are sporadic. Feldman WDT: p. 15, line 1

1369. In the Harris study, excluding travel to underdeveloped nations and consumption of raw milk did not alter the association of *Campylobacter* enteritis with unprocessed poultry or processed turkey consumption. Feldman WDT: p. 19, lines 18-20; G-268

1370. The Niemann case control study finds a major risk of campylobacteriosis with eating undercooked chicken. Feldman WDT: p. 20, lines 5-7; B-561

1371. The Oosterom study reports an increased risk of campylobacteriosis with eating chicken at home or in barbecue. Feldman WDT: p. 21, lines 4-7; G-474;

1372. The Eberhart-Philips study found an increased risk of *Campylobacter* infection with eating chicken. Feldman WDT: p 21, lines 8-10; G-182

1373. The Studahl study found an increased risk of campylobacteriosis from eating chicken and from contact with chickens. Feldman WDT: p 21, lines 16-19; G-602

1374. Case control studies are an acceptable way to investigate risk factors of sporadic disease in a population. Feldman. WDT: p 23, lines 7-8

John R. Glisson (B-1903)

1375. Baytril (enrofloxacin) is delivered to chickens and turkeys through the drinking system in a water soluble form. Glisson WDT: p. 3, lines 20-21

1376. Administration via water is typical of therapeutic antibiotic usage in the poultry industry. Glisson WDT: p. 3, lines 21-22

1377. For commercially grown broiler chickens and turkeys in the United States, it is neither feasible nor practical to administer enrofloxacin, or other therapeutic antibiotics, on an individual bird basis. Glisson WDT: p. 3, line 22 – p. 4, lines 1-2

1378. The label instructions for Baytril allow a dosage of 25 ppm - 50 ppm for 3-7 days. Glisson WDT: p. 5, lines 7-8

1379. Enrofloxacin usage is by prescription only and only under veterinary supervision. Glisson WDT: p. 5, lines 16-17

1380. Enrofloxacin is used for therapeutic purposes and is not used for growth promotion. Glisson WDT: p. 5, lines 17-18

Charles Haas (B-1904)

1381. Bayer Witness Haas concedes that the epidemiological studies used by Vose [in the CVM *Campylobacter* resistance risk assessment] to estimate the poultry related fraction of campylobacteriosis were peer reviewed in refereed journals. Haas WDT: p. 17, lines 11-12

1382. Manfred Kist (B-1906)

1383. *Campylobacter jejuni* is a common cause of bacterial diarrhea worldwide. Kist WDT, p. 2, line 15

1384. Most cases of campylobacteriosis are sporadic in nature. Kist WDT: p. 3, line 7

1385. Symptoms of campylobacteriosis includes headache, back pain, general malaise, fever, cramps and frequent bowel movements, loose or watery diarrhea and bloody diarrhea in about half the cases. Kist WDT: p. 3, lines 10-14

1386. People can die from *Campylobacter* infections. Kist WDT: p. 3, line 21 – p. 4, line 7

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1387. Campylobacteriosis establishes itself after an incubation period of 24 to 72 hrs. Kist WDT: p. 3, line 10

1388. Extraintestinal infections do occur as a complication of campylobacteriosis. Kist WDT: p. 4, lines 17-18

1389. *Campylobacter* infection may lead to symptomatic sequelae. Kist WDT: p 4, lines 20-21

1390. Complications of *Campylobacter* infection include bacteremia, cholecystitis, pancreatitis, appendicitis, meningitis, encephalopathy, septic abortion, hemolytic uremic syndrome, reactive arthritis, and Guillain-Barre syndrome. Kist WDT: p. 5, line 1 – p. 8, line 17

1391. Neonatal meningitis can be a life-threatening complication of *Campylobacter* bacteremia of the pregnant mother. Kist WDT: p. 6, lines 6-7

1392. In the United States, more than 99% of reported infections with *Campylobacter* are with *Campylobacter jejuni*. Kist WDT: p. 8, line 19

1393. Indications for antimicrobial treatment are high fever for more than 2 days, bloody stools, prolonged illness, pregnancy, infection with HIV, other immunocompromised states, and living in an institution. Kist WDT: p. 10, lines 2-4

1394. In Germany, the decrease of *C. coli* relative to *C. jejuni* is probably due to increased consumption of poultry in that period. Kist WDT: p. 9, lines 3-5

1395. In Sweden and Belgium, fluoroquinolones are not recommended to treat *Campylobacter* infections because of the high rates of *Campylobacter* resistance to fluoroquinolones. Kist WDT: p. 11, lines 3-5

1396. Some countries have already lost the use of fluoroquinolones to treat campylobacteriosis because of high resistance rates. Kist WDT: p. 11, lines 3-5

1397. Quinolones are prescribed as an empiric treatment to treat diarrhea without knowledge of its causative agents. Kist WDT: p. 11, lines 13-15

1398. In the US, quinolones are prescribed in approximately 5.2% of all food-borne bacterial diarrhea cases. Kist WDT: p. 11, lines 15-16

1399. At least some patients who need antibiotics are prescribed fluoroquinolones that are ineffective because they have fluoroquinolone-resistant *Campylobacter*. Kist WDT: p. 12, lines 1-11

1400. Ciprofloxacin is effective in shortening the duration of diarrhea in patients with *Campylobacter* infection whose pretreatment *Campylobacter* isolates are susceptible to ciprofloxacin. Kist WDT: p. 13, lines 16-18

1401. Erythromycin has a narrow spectrum of activity. Kist WDT: p. 12, lines 16-18

1402. There is a rising incidence of quinolone resistance in human *Campylobacter* infections. Kist WDT: p. 14, lines 5-6

1403. Patients with fluoroquinolone-resistant *Campylobacter* infections are more likely to be hospitalized than patients with fluoroquinolone-susceptible *Campylobacter* infections. Kist WDT: p. 15, lines 16-19

1404. In a study of *Campylobacter* isolates from humans in 4 states in the U.S., 20 of 164 were resistant to ciprofloxacin. Of 16 patients who were interviewed, 5 were hospitalized overnight, compared with only 1 of 31 patients with fluoroquinolone-sensitive *Campylobacter* infection. Kist WDT: p. 15, lines 16-19; B-1803

Diane Newell (B-1908)

1405. *Campylobacter jejuni* and the related organism *C. coli* are motile, thermophilic, microaerophilic, Gram negative bacteria, which can colonize the intestinal mucous of a range of hosts , including humans and poultry. Newell WDT: p. 3, lines 14-17

1406. Most published information is derived from studies in broiler chicken flocks for *Campylobacter* colonization. The prevalence of flock infection varies worldwide from 10% to over 90%. Flock colonization is seasonal and can vary between countries. Newell WDT: p. 3, lines 17-23

1407. Few studies have been undertaken in turkeys, or other poultry, and the general assumption has been that the ecology and physiology of campylobacters in all birds is the same. Newell WDT: p. 4, lines 1-3

1408. *C. jejuni* in particular appears to have evolved to preferentially colonize the avian gut as part of the normal gut flora. Newell WDT: p. 4, lines 23-24

1409. In chickens reared under intensive conditions, there are few natural restrictions to Campylobacter growth. Newell WDT: p. 5, lines 2-3

1410. Once the birds have become detectably infected at about 2-3 weeks of age, colonization can reach high level in such birds (about 10^8 per g caecal contents) and is chronic for the life of the bird (usually 6-7 weeks). Newell WDT: p. 5, lines 3-6

1411. Surveys undertaken with turkeys indicate that colonization occurs by 7 days of age and is persistent with between 80-100% of birds detectably colonized at slaughter. Newell WDT: p. 5, lines 10-12

1412. Longitudinal epidemiological investigations indicate that naturally-acquired *Campylobacter* infection in broilers is age-dependent. Most flocks become infected only 2-3

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weeks after placement into a house. Once the first positive birds are detected transmission is extremely rapid which may reflect enhanced colonization potential. Newell WDT: p. 5, lines 13-20

1413. Published epidemiological evidence indicates that chicks are Campylobacter-free on hatching. Newell WDT: p. 6, lines 8-9

1414. Potential horizontal sources of *Campylobacter* contamination for poultry include feed, water, air and an environment contaminated by previous flocks. Newell WDT: p. 7, lines 1-3

1415. Campylobacters can be isolated from environmental sources around broiler houses. Newell WDT: p. 8, line 24 and page 9, line 1

1416. Colonization in turkeys is also chronic and most birds are colonized at slaughter though shedding may be intermittent. Newell WDT: p. 10, lines 13-14

1417. As early as 1981 the development of Campylobacter resistance to antimicrobials, including erythromycin and nalidixic acid was being reported. In 1985 cross-resistance between nalidixic acid and enoxacin, a first generation fluoroquinolone, was reported. Newell WDT: p. 11, lines 4-8

1418. Resistance to the fluoroquinolones has only become of major interest since the 1995 International *Campylobacter* Workshop as the reports of increasing resistance have emerged. Newell WDT: p. 11, lines 8-11

1419. Bayer witness Newell uses in her testimony "resistance" for *Campylobacter* to mean a MIC of 4 μg/mL, measured *in vitro*. Newell WDT: p. 11, footnote 1

1420. In *C. jejuni/coli* it appears that the major molecular basis of fluoroquinolone resistance is by a single point mutation in the *gyr*A gene. Newell WDT: p. 12, lines 2-3

1421. *Campylobacter* resistance to fluoroquinolones occurs naturally as a point mutation in the *gyr*A gene and is selected by the presence of fluoroquinolones. Newell WDT: p. 12, lines 21-22

1422. Fluoroquinolone-resistant *Campylobacters* may be isolated from poultry as a direct result of either the fluoroquinolone treatment of *Campylobacter*-infected poultry or the acquisition by poultry of already fluoroquinolone-resistant organisms. Newell WDT: p. 13, lines 13-16

1423. There are no standardized methods for the measurement of fluoroquinolone resistance in *Campylobacters*. Newell WDT: p. 13, lines 17-18

1424. In the United Kingdom, the Public Health Laboratory Service has adopted a MIC of 1 μg/mL as resistant for *Campylobacter*. Newell WDT: p. 13, lines 21-22

1425. In Denmark 6% of *C. jejuni* but no *C.coli* strains were ciprofloxacin resistant during the 2001 abattoir survey. Diane G. Newell, Exhibit B-1908, page 14, lines 5-7

1426. The levels of fluoroquinolone resistance in poultry at slaughter varies; studies have found 32% were resistant in chicken strains in Japan; 19% in Germany, and 99% in Spain. Newell WDT: p. 14, lines 7-10.

1427. Emerging fluoroquinolone resistance in poultry campylobacters worldwide has been reported subsequent to the licensing of fluoroquinolones for use in poultry. Newell WDT: p. 14, lines 11-12

1428. NARMS data suggests that between 1997-2000 25% of 180 retail chickens carried fluoroquinolone-resistant campylobacters. Smith *et al.* found a similar level (19%) of resistance in 91 retail chicken products while Ge *et al* (2002) found 25% resistance in 155 isolates from chicken and turkey meat. Newell WDT: p. 14, lines 22-24, page 15, line 1

1429. Turkey *Campylobacters* may be more exposed to fluoroquinolones than strains from broilers. Newell WDT: p. 15, lines 22-23

1430. The treatment of broiler flocks, which are already colonized with *Campylobacter,* can result in the selection of resistant organisms, which will naturally occur during bacterial growth. Jacobs-Reitsma *et al* clearly demonstrated that resistant campylobacters are readily recovered from experimentally-infected chickens exposed to fluoroquinolones. Newell WDT: p. 16, lines 13-16

1431. Bayer witness Newell acknowledges that "treatment of broiler flocks with fluoroquinolones does result in the selection of resistant organisms." Newell WDT: p. 16, lines 23-24

1432. Newell concludes that "In poultry flocks reared under intensive conditions colonization with *Campylobacters* can reach extremely high levels and during processing such gut contents can contaminate poultry meat." Newell WDT: p. 17, lines 17-19

1433. The importance of thermophilic *Campylobacters* as a cause of acute human bacterial enteritis was first recognized in 1977 . Newell WDT: p. 19, lines 4-5

1434. Most human *Campylobacter* infections are due to *C. jejuni.* Newell WDT: p. 19, lines 7-8

1435. *Campylobacter jejuni* and *C.coli* colonize the intestinal mucous of a range of hosts, including humans and poultry. In susceptible humans, particularly in the industrialized world, colonization with *Campylobacter* is associated with disease. Newell WDT: p. 19, lines 18-21

1436. Surveillance has shown that campylobacteriosis is the major cause of acute bacterial intestinal infectious disease in many industrial countries. Newell WDT: p. 20, lines 3-5

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1437. In Great Britain campylobacteriosis is the most common cause of acute bacterial enteritis. Newell WDT: p. 20, lines 5-6

1438. CDC has estimated the incidence of campylobacteriosis infections in the United States at 2.4 million cases per year, including non-reported cases. Newell WDT: p. 20, lines 15-16

1439. Campylobacteriosis is generally considered to be foodborne. Newell WDT: p. 21, line 16

1440. Because of their fastidious growth requirements *Campylobacters* only naturally multiply within the intestinal tract of a suitable host such as poultry and humans. Newell WDT: p. 21, lines 21-23

1441. The ability of *Campylobacters* to survive and be disseminated around the domestic kitchen is acknowledged as a risk factor. Newell WDT: p. 22, lines 10-11

1442. One compelling argument for poultry as a source of infection is the contamination of retail poultry products with *Campylobacters*. The level of this contamination can be high; between $10^2 - 10^5$ CFU per chicken carcass. The levels of contamination on other retail meats are significantly lower. Newell WDT: p. 23, lines 10-14

1443. On average, the delay between exposure to *Campylobacter* and illness is 3-5 days and diagnosis by culture can take a further 3-5 days. Newell WDT: p. 25, lines 8-9

1444. Many studies have used genotyping techniques, like fla-typing, ribotyping or PFGE, to compare isolates from humans and poultry or livestock to determine the degree of population overlap. Newell, WDT: p. 30, lines 20-22

1445. From 35-80% of the *Campylobacter* strain types found in poultry are the same as the types of strains recovered from humans with disease. Newell WDT: p. 35, lines 1-2

1446. The population structure studies confirm that the lineage of many of the *Campylobacter* strains isolated from poultry is the same as strains isolated from humans with disease. Newell WDT: p. 35, lines 16-17.

1447. In Clow's study, the proportion of *Campylobacter* types in poultry not associated with disease in humans was estimated to be about 10%. Newell WDT: p. 36, lines 7-9

1448. A proportion of patients, probably ranging from 5-10% of those with *Campylobacter* infections may require hospitalization. Newell WDT: p. 37, lines 3-4

1449. In the past, the drug of choice for patients who may benefit from antimicrobial therapy for *Campylobacter* infections was Erythromycin but increasingly Ciprofloxacin and other fluoroquinolones are prescribed, as these have activity against most enteric bacterial pathogens. Newell WDT: p. 37, lines 8-14

1450. An observed increase in resistance of *Campylobacters* infecting humans to antimicrobials has recently caused concern. Skirrow and Blaser have stated that the use of fluoroquinolones for treatment of campylobacteriosis has been severely compromised by increasing resistance rates in some countries. These concerns are realistic. Newell WDT: p. 37, lines 15-21, G-580

1451. Because the mechanism of resistance is primarily a single point mutation and is not known to be horizontally transferred, fluoroquinolone-resistant *Campylobacters* may be isolated from humans as a direct result of either the fluoroquinolone treatment of *Campylobacter* infected humans or the acquisition by humans of already fluoroquinolone-resistant organisms from another treated host. Newell WDT: p. 37, lines 23-24, p. 38, lines 1-5

1452. Transmission of *Campylobacter* from human to human is rare. Newell WDT: p. 38, line 15

1453. Infection of susceptible humans with *C. jejuni* or *C. coli* can cause severe symptoms: in one study in England, published in 2000, all adults presenting to general practitioners with campylobacteriosis had diarrhea; severe in 65% of the cases, and bloody in 17% of them. Ninety-two percent of the patients had abdominal pain and 76% had fever. Their mean duration of illness was 6 days. Newell WDT: p. 42, line 21 – p. 43, line 2.

James Patterson (B-1910)

1454. Fluoroquinolone resistance among *Campylobacter* strains is worrisome with regard to the treatment of human *Campylobacter* infections. Patterson WDT: p. 6, lines 12-13

1455. Waste products associated with animal husbandry and meat products processing contain *Campylobacter*, including fluoroquinolone-resistant *Campylobacter*. Patterson WDT: p. 10, lines 15-16

1456. Farm drainage can transport *Campylobacter* into surface waters and ground waters. Patterson WDT: p. 10, lines 16-17

1457. Most *Campylobacter* infections in humans occur as sporadic isolated cases or as part of a small cluster of cases. Patterson WDT: p. 22, lines 17-18

1458. Most documented waterborne instances of campylobacteriosis are associated with outbreaks. Patterson WDT: p. 23, lines 15-16

Michael Robach (B-1911)

1459. Most human *Campylobacter* illness is caused by *Campylobacter jejuni*. Robach WDT: p. 5, lines 2-3

1460. The most common bacterial causes of food borne disease in the United States are *Salmonella* and *Campylobacter*. Robach WDT: p. 4, lines 5-6

1461. Most people who become ill from *Campylobacter* develop diarrhea 2-5 days after exposure to the organism. Robach WDT: p. 4, lines 10-12

1462. Campylobacteriosis usually occurs in single, sporadic cases. Robach WDT: p. 5, line 17

1463. Chickens colonized with *Campylobacter* usually show no signs of illness. Robach WDT: p. 5, line 20

1464. When an infected bird is slaughtered, *Campylobacter* can be transferred from the intestinal tract to the skin or to the meat. Robach WDT: p. 5, lines 22-23

1465. Many chicken flocks are infected with *Campylobacter* but show no signs of illness. Robach WDT: p. 5, line 20

1466. *Campylobacter* can easily spread from bird to bird through a common water source, or through contact with infected feces. Robach WDT: p. 5, lines 21-22

1467. When an infected bird is slaughtered, *Campylobacter* can be transferred from the intestinal tract to the skin or to the meat. Robach WDT: p. 5, lines 22-23

1468. Foods of animal origin are an important cause of human illness. Robach WDT: p. 7, lines 21-22

1469. During the slaughtering process, workers, equipment, and the processing environment can serve as sources of contamination for the finished product. Robach WDT: p. 8, lines 1-2

1470. *Campylobacter* are naturally occurring bacteria that reside in the gut of healthy birds. Robach WDT: p. 9, lines 9-10

1471. *Campylobacter* organisms are capable of causing foodborne illness in humans. Robach WDT: p. 9, line 10

1472. The two human enteropathogens most frequently associated with poultry are *Salmonella* and *Campylobacter jejuni*. Robach WDT: p. 10, lines 19-20

1473. The intestines of poultry are easily colonized with *Campylobacter*. Robach WDT: p. 10, lines 21-22

1474. *Campylobacter* grows best at 37 – 42 C. Robach WDT: p. 11, line 2

1475. *Campylobacter* grows best in a microaerophillic environment (5% O_2, 10% CO_2, and 85% N_2). Robach WDT: p. 11, lines 2-3

1476. *Campylobacter* is rarely found in chickens until after the third or fourth week of grow-out. Robach WDT: p. 12, lines 1-3

1477. Once *Campylobacter* is introduced in the poultry house, almost the entire house will be colonized in the span of a few days, and remain that way through slaughter. Robach WDT: p. 12, lines 1-3

1478. Evisceration can be a major source of fecal contamination, particularly if the intestines are cut. Robach WDT: p. 14, lines 7-8

1479. Adding chlorine to chill tank water does not kill all bacteria. Robach WDT: p. 14, lines 18-21

1480. High pre-chill contamination of poultry with bacteria results in high post-chill contamination of poultry with bacteria. Robach WDT: p. 14, lines 20-21

1481. Foods of animal origin are an important source of the enteric organisms that cause human illness. Robach p. 17, lines 5-7

Scott Russell (B-1912)

1482. Broilers (chickens) are typically raised on broiler farms comprised of two or three houses per farm with 20,000 to 25,000 broilers per house. Russell WDT: p. 3, lines 14-15

1483. Broilers are reared to 42-58 days. Russell WDT: p. 3 lines 15-16

1484. Scalders have water between 130 - 132°F. Russell WDT: p. 4, lines 13-14

1485. Fecal contamination of chickens at the evisceration stage is easily spread to adjacent birds due to the bird to bird contact on the hang line. Russell WDT: p. 5, lines 5-6

1486. Chill tank water is about 33 - 34°F. Russell WDT: p. 6, lines 3- 4

1487. Campylobacteriosis usually occurs in single, sporadic cases. Russell WDT: p. 10, lines 7-8 and p. 10, line 14

1488. *Campylobacter* has been associated with handling raw poultry. Russell WDT: p. 10, line 9

1489. *Campylobacter* has been associated with eating raw or undercooked poultry. Russell WDT: p. 10, lines 9-10

1490. A very small number of *Campylobacter* organisms can cause illness in humans. Russell WDT: p. 10, lines 10-11

1491. As few as 500 *Campylobacter* organisms can cause illness in humans. Russell WDT: p. 10, lines 10-11

1492. Campylobacteriosis is estimated to affect over 2 million persons every year. Russell WDT: p. 10, line 18

1493. People can die from campylobacteriosis. Russell WDT: p. 11, lines 1-2

1494. During viscera removal, opportunities exist for the digestive tract to be cut or torn, releasing fecal material onto the interior or exterior surfaces of the carcass. Russell WDT: p. 13, lines 4-5

1495. Line speeds at chicken slaughter plants are 70 broilers per minute or higher. Russell WDT: p. 15, lines 16-17

1496. Only a certain percentage of bacteria will be susceptible to chlorine in the chiller. Russell WDT: p. 15, line 22

1497. Some bacteria on the chicken is protected from the chiller by the chicken fat, location on the carcass, or because they are lodged in a feather follicle. Russell WDT: p. 16, lines 1-2

1498. Highly contaminated pre-chiller carcasses will result in highly contaminated post chiller carcasses. Russell WDT: p. 16, lines 3-4

1499. Chickens in a chiller are susceptible to cross-contamination. Russell WDT: p. 16, lines 6-7

1500. In the chiller, bacteria from carcasses contaminated with feces can spread to other carcasses. Russell WDT: p. 16, lines 7-8

1501. The chiller does not reduce high bacterial count numbers. Russell WDT: p. 19, line 14

1502. The chiller will reduce bacteria by a certain degree but the number of bacteria does not change significantly if the numbers are high or low going into the chiller. Russell WDT: p. 19, lines 14-16

1503. *Campylobacter jejuni* is a leading cause of diarrhea disease and foodborne gastroenteritis worldwide. Russell WDT: p. 24, lines 18-19

1504. Contaminated poultry is a common vehicle of transmission of *C. jejuni* in humans. Russell WDT: p. 24, lines 20-21

Terry TerHune (B-1915)

1505. The current label dose for enrofloxacin is 25 to 50 ppm for broiler chickens and turkeys. TerHune WDT: p. 5, line 16 – p. 6, line 1

Anthony E. van den Bogaard (B-1916)

1506. Selection and dissemination of resistance is an inevitable result of any antibiotic use. van den Bogaard WDT: p. 3, lines 5-6.

1507. In most cases, two mutations in the genome of a bacterium are required in order to cause clinically relevant resistance against fluoroquinolones but only one mutation is necessary to cause resistance in *Campylobacter*. van den Bogaard WDT: p. 3 lines 10-14

1508. Bayer witness Dr. van den Bogaard testified that data and information has demonstrated that fluoroquinolone use in poultry acts as a selection pressure resulting in the emergence of fluoroquinolone-resistant *Campylobacter* spp. in poultry that may be transferred to humans cause therapy failures in humans when those infections are treated with a fluoroquinolone. van den Bogaard WDT: p. 5, lines 2-11

1509. The emergence of fluoroquinolone-resistance of *Campylobacter spp.* in chickens was first suggested by Endtz et al as isolates from chicken meats were frequently found resistant to fluoroquinolones. van den Bogaard WDT: p. 5 line 29 – p. 6, line 1, G-755, G-190

1510. An experiment by Jacobs-Reitsma et al experimentally infected broiler chickens with quinolone-sensitive *C. jejuni*, and then treated the birds with enrofloxacin, which did not eradicate the *Campylobacter* colonization of the broilers, but caused the emergence of quinolone-resistant strains that persisted until slaughter. *Campylobacters* in chicks of a control group, not receiving enrofloxacin treatment, remained fluoroquinolone-sensitive. van den Bogaard WDT: p. 6, lines 4-11, B-432

1511. Recent studies confirm the conclusion that fluoroquinolone use in poultry does act as a selection pressure resulting in the emergence of fluoroquinolone-*resistant Campylobacter jejuni* in poultry. van den Bogaard WDT: p. 7, lines 17-21, B-868

1512. Endtz et al. documented the prevalence of ciprofloxacin resistance bracketing the introduction of fluoroquinolones into human medicine and enrofloxacin into veterinary medicine. van den Bogaard WDT: p. 8, lines 18-22

1513. Among human *Campylobacter* isolated examined by Endtz et al., no ciprofloxacin resistance was found during 1982 to 1983 or in 1985. The percentage of ciprofloxacin-resistant isolated increased to 8% during 1987 to 1988 and to 11% in 1989. In a follow-up study in humans, the prevalence of fluoroquinolone-resistant Campylobacter isolates amounted to approximately 25% in 1990. van den Bogaard WDT: p. 8, lines 24-28

1514. Among poultry isolates examined by Endtz et. al., no resistance was found in poultry isolates from 1982 to 1983; the percentage of resistant isolates increased to 8% during 1987 to 1988 and to 14% during 1989. van den Bogaard WDT: p.8 lines 18-28. G-755, B-22

1515. Fluoroquinolones are very important for human patients with life-threatening bacterial infections. van den Bogaard WDT: p. 14, lines 22-23

1516. Like with other foodborne pathogenic bacteria, the colonization of *Campylobacter* spp. in animals, the high rate of contamination of meat products and inappropriate handling at home as well as in commercial kitchens are the basic problems. van den Bogaard WDT: p.15, lines 16-18

Dennis P. Wages (B-1917)

1517. Toms are raised until 16 - 22 weeks of age and weight 32- 40 pounds; hens are raised from 14-20 weeks of age and weigh 14 - 20 pounds. Wages WDT: p. 3, lines 5-6

1518. The typical turkey grow-out complex has two brooder houses and four grow-out houses per farm. Wages WDT: p. 3, lines 20-21

1519. Turkey brooder and grow-out houses are usually 40-50 feet wide and 300-400 feet long. Wages WDT: p. 4, line 3

1520. Turkey brooder and grow-out houses are usually oriented east-west. Wages WDT: p. 4, lines 3-4

1521. Turkey brooder and grow-out houses are usually curtain sided, wood constructed with tin roofs. Wages WDT: p. 4, lines 3-4

1522. Inside the turkey house, there are usually two automatic feed lines in a brooder house and one automatic feed line in the grow-out houses. Wages WDT: p. 4, lines 5-6

1523. There are usually two rows of automatic, Plasson type (open) drinkers in turkey houses. Wages WDT: p. 4, lines 6-7

1524. There are typically 8,000 -12,000 turkeys raised in a brooder house. Wages WDT: p. 4, line 7

1525. There are normally 4,000 to 6,000 turkeys raised in the grow-out houses. Wages WDT: p. 4, lines 7-8

1526. All turkey feed in the U.S. industry is pelleted. Wages WDT: p. 4, line 11

1527. Baytril 3.23% concentrate oral solution was approved for control of turkey mortality associated with *E. coli* and *Pasteurella multocida* (fowl cholera) susceptible to enrofloxacin. Wages WDT: p. 8, lines 6-7

1528. Baytril is administered in the drinking water of the infected house. Wages WDT: p. 18, lines 2-3

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1529. In the United States, enrofloxacin is approved for use only by prescription and only under veterinary supervision. Wages WDT: p. 18, lines 7-8

1530. In the United States, enrofloxacin is approved for therapeutic use only and is not approved for growth promotion. Wages WDT: p. 18, lines 8-9

1531. In the United States, extra-label use of enrofloxacin is prohibited by law for food producing animals. Wages WDT: p. 18, lines 9-10

1532. Extra-label use of enrofloxacin is prohibited in the United States. Wages WDT: p. 18, line 11

1533. Labeled dosage of enrofloxacin for turkeys is 25 ppm - 50 ppm. Wages WDT: p. 18, line 12

1534. Typical dosage of enrofloxacin for turkeys is 50 ppm. Wages WDT: p. 18, line 12

Richard Carnevale (A-199)

1535. It is currently not possible, even under the most hygienic conditions, to produce raw poultry that is sterile, or at least completely free of any harmful pathogens. Carnevale WDT: p. 21, line 1-2.

1536. Millions of bacteria may be carried by birds upon entry to a slaughter establishment. Carnevale WDT: p. 20, line 17 through p. 21, line 3.

1537. *Campylobacter* contamination of raw poultry carcasses is considered unavoidable; this is true regardless of the antimicrobial susceptibility profile of the *Campylobacter*. Carnevale WDT: p. 21, line 10-14

Bradley DeGroot (A-200)

1538. Seasonal variation explains only a small proportion of the number of resistant isolates submitted by Minnesota to the human NARMS surveillance program, beyond what can be expected based on overall resistance measured for the state. DeGroot WDT: p. 24, line 1-7.

1539. If participating laboratories do not change culture and isolation methods and if the relative contribution of laboratories using various methods remain consistent through time, any differences in laboratory methods will have minimal effect on comparisons of resistant rate estimates over time. DeGroot WDT: p. 28, line 15-18.

1540. The intent of the slaughter sampling component of the animal NARMS surveillance program is sound. DeGroot WDT: p. 64, line 12-13.

Eric Gonder (A-201)

1541. The traditional turkey housing system includes a farm with a brooder house and 1-3 grow-out houses. Gonder WDT: p. 4, line 13

1542. Turkeys start in the brooder house, move to the grow-out houses at 5-7 weeks of age, and are replaced by a new group in the brooder house after several weeks. Gonder WDT: p. 4, lines 13-15

1543. Two ages of turkeys may be present on the farm at the same time. Gonder WDT: p. 4, lines 15-16

1544. Most turkey farms have 2 to 4 houses while the national range is 2 to 10 houses. Gonder WDT: p. 6, line 2

1545. Brooder houses, where the turkeys reside for the first several weeks of life, are 32-70 feet wide and 250-600 feet long. Gonder WDT: p. 6, lines 3-4

1546. Brooder houses will contain 10,000-20,000 turkey poults, although some very large houses used in a few locations may contain 70,000 turkey poults. Gonder WDT: p. 6, lines 10-11

1547. Finishing or grow-out houses usually contain one-third to one-half as many turkey as brooder houses due to the need to provide additional space for the birds to grow. Gonder WDT: p. 6, lines 11-13

1548. Typically, the number of turkeys in a grow-out house is on the order of 10,000 to 20,000 birds. Gonder WDT: p. 6, lines 13-14

1549. CDC reports that 95 - 99% of human *Campylobacter* infections are caused by C. *jejuni*. Gonder WDT: p. 13, lines 5-6

Chuck Hofacre (A-202)

1550. The US turkey (272 million) and broiler chicken (8.5 billion) industries are similar to each other. The integrated company purchases the parent breeders at 1 day of age or hatching eggs from a primary breeder or genetic selection company. These birds are raised on farms contracted by the company under specific company guidelines for antibiotic usage. The offspring (broiler chickens or commercial turkeys) of these breeders are hatched in company-owned hatcheries, and placed on a contract or company-owned farm, where the farmer must follow strict company guidelines for all aspects of raising the birds, including antibiotic usage. All feed that is fed to the breeders, broiler chickens or commercial turkeys is manufactured in a company-owned feedmill under specific guidelines of a company. The company nutritionist specifies the nutritional composition of the feed, and the veterinarian determines any antibiotic usage requirements. The birds are then slaughtered in a company-owned processing plant. Hofacre WDT: p. 32, lines 4-14

1551. The typical US broiler chicken farm typically has on the order of 100,000 chickens, divided equally into four houses. Hofacre WDT: p. 3, lines 15-16

1552. Day old broiler chicks are delivered to a contract broiler grower farm where they go into an environmentally controlled house that is on average 40 feet wide and 500 feet long with approximately 25,000 broilers per house. Hofacre WDT: p. 5, lines 20-22

1553. The label dose for Baytril is 25 to 50 ppm for 3 to 7 days for the treatment of *E. coli* infections in chickens and turkeys plus *Pasteurella multocida* infections of turkeys. Hofacre WDT: p. 22, lines 21-22

Ronald Prucha (A-203)

1554. About one-half of foodborne bacterial infections from the major bacterial pathogens are attributable to meat and poultry sources. Prucha WDT: p. 4, lines 3-5

1555. In 1993, 50% of *Campylobacter* cases were attributed to meat and poultry sources. Prucha WDT: p. 4, lines 5-6

1556. In 1995, 41% to 53% of *Campylobacter* cases were due to meat and poultry sources. Prucha WDT: p. 4, lines 6-7

1557. The number of *Campylobacter* cases attributed to meat and poultry are rising. Prucha p. 4, lines 5-7

1558. The skin and feathers of live birds are highly contaminated during their grow-out periods, and their intestinal tracts contain millions of bacteria, some of which may be pathogenic to humans. Prucha WDT: p. 7, lines 18-20

1559. The evisceration process can release heavy bacteria loads through fecal contamination to poultry carcasses. Prucha WDT: p. 8, lines 11-12

1560. Fecal contamination in chill tanks can cross-contaminate thousands of other carcasses. Prucha WDT: p. 8, lines 12-13

Robert Tompkin (A-204)

1561. A multistate survey between 1996-1997 found that only 1.5% of people drink raw milk. Tompkin WDT: p. 12 line 1

1562. *Campylobacter* is now recognized as the leading cause of zoonotic enteric infections in most developed and developing countries. Tompkin WDT: p. 14, lines 11-12

1563. Campylobacteriosis is usually caused by *C. jejuni* or to a lesser extent by *C. coli*. Tompkin WDT: p. 14, lines 12-13

1564. Most human *Campylobacter* infections are classified as sporadic single cases. Tompkin WDT: p. 14, line 13

1565. FoodNet is the most sensitive means employed by the public health community to document the extent of diarrheal disease in the U.S. Tompkin WDT: p. 15, lines 14-15

1566. Vertical transmission of *Campylobacter* is an unlikely source of infection. Tompkin WDT: p. 44, line 19

1567. Once a poultry flock has been exposed to colonization, water and feed play an important role in the dissemination of colonization throughout the flock. Tompkin WDT: p. 45, lines 5-6

1568. There is contamination of the exterior of a large proportion of birds in the transport vehicle. Tompkin WDT: p. 46, lines 15-16

1569. As birds enter the scald tank there may be involuntary defecation, leading to accumulation of fecal matter in the tank. Tompkin WDT: p. 49, lines 13-14

1570. Scalding may lead to external contamination if an uncontaminated poultry carcass is passed through contaminated scald water. Tompkin WDT: p. 49, lines 21-22

1571. Defeathering machines are major sites of potential cross-contamination in primary processing. Tompkin WDT: p. 50, lines 5-6

1572. The spinning action of the plucker heads during mechanical defeathering produces aerosols which spread contamination. Tompkin WDT: p. 50, lines 9-11

1573. The process of defeathering has been demonstrated to generally increase the number of carcasses contaminated with organisms. Tompkin WDT: p. 50, lines 11-12

1574. For birds colonized with *Campylobacters,* gross contamination may result if damage occurs to the viscera during evisceration. Tompkin WDT: p. 51, lines 3-4

1575. As the carcasses move through the operation, the major source of contamination can result during mechanical evisceration. Tompkin WDT: p. 51, lines 10-11

1576. The majority of campylobacteriosis in humans is due to *C. jejuni.* Tompkin WDT: p. 57, line 2

Joint Stipulations

1577. Fluoroquinolone use in chickens and turkeys can act as a selection pressure for fluoroquinolone-resistant bacteria in the chicken and turkey digestive tract. Revised Joint Stipulation No. 7

1578. The parties do not have any facts or data demonstrating horizontal gene transfer for fluoroquinolone resistance in *Campylobacter*. Revised Joint Stipulation No. 10

1579. The National Committee for Clinical Laboratory Standards (NCCLS) is a standards-developing organization that develops and disseminates standards, guidelines and best practices for medical testing in clinical laboratories. Revised Joint Stipulation No. 11

1580. NCCLS has established guidelines for susceptibility testing of certain bacteria to certain antimicrobial agents. Revised Joint Stipulation No. 12

1581. A NCCLS recognized breakpoint indicating loss of clinical effectiveness has not been established for fluoroquinolone drug use in *Campylobacter* infections in humans. Revised Joint Stipulation No. 14

1582. Many persons sick with gastroenteritis do not seek medical care. Revised Joint Stipulation No. 20

1583. For commercially grown broiler chickens and turkeys in the United States, it is neither feasible nor practical to administer enrofloxacin on an individual bird basis. Revised Joint Stipulation No. 36

1584. In the United States, a broiler grow-out house typically contains on the order of 20,000 to 25,000 broilers. Revised Joint Stipulation No. 37

1585. In the United States, a turkey grow-out house typically contains on the order of 10,000 to 20,000 turkeys. Revised Joint Stipulation No. 38

1586. Baytril 3.23% concentrate oral solution was approved in the United States on October 4, 1996 for control of chicken mortality associated with *Escherichia coli* susceptible to enrofloxacin and for control of turkey mortality associated with *E. coli* and *Pasteurella multocida* (fowl cholera) susceptible to enrofloxacin. Revised Joint Stipulation No. 39

1587. The horizontal transfer of genes conferring fluoroquinolone resistance in *Campylobacter* has not been demonstrated. Revised Joint Stipulation No. 40

1588. *Campylobacter jejuni* and *Campylobacter coli* can be human pathogens. Revised Joint Stipulation No. 41

1589. Common criteria for the antimicrobial treatment of human *Campylobacter* infection include: severe illness, severe systemic toxicity, high fever, severe symptoms of dysentery; prolonged illness; worsening and/or relapsing symptoms despite appropriate supportive therapy; underlying primary and acquired immunodeficiency states such as HIV, immunoglobulin deficiency states, allograft recipients; chronic illness; and the elderly. Revised Joint Stipulation No. 42

1590.   In 2001, there were 8.6 billion broilers (chickens) raised for slaughter in the United States. Revised Joint Stipulation No. 43

1591.   In 2000, there were 270 million turkeys raised for slaughter in the United States. Revised Joint Stipulation No. 44

1592.   The use of enrofloxacin in chickens and turkeys can exert a selection pressure that can lead to fluoroquinolone resistance. Revised Joint Stipulation No. 45

1593.   SaraFlox WSP was approved in the United States on August 18, 1995 for the control of mortality in growing turkeys and broiler chickens associated with *Escherichia coli* organisms susceptible to sarafloxacin. Revised Joint Stipulation No. 47

1594.   SaraFlox Injection was approved in the United States on October 12, 1995 for the control of early mortality in day old broiler chickens associated with *E. coli* organisms susceptible to sarafloxacin. Revised Joint Stipulation No. 48

1595.   Bayer's ciprofloxacin product was first registered in Austria on June 26, 1987; Bayer's enrofloxacin product for poultry was first registered in Austria on May 3, 1988. Revised Joint Stipulation No. 51

1596.   Bayer's ciprofloxacin product was first registered in Belgium on January 20, 1989; Bayer's enrofloxacin product for poultry was first registered in Belgium on January 19, 1988. Revised Joint Stipulation No. 52

1597.   Bayer's ciprofloxacin product was first registered in Denmark on March 23, 1988; Bayer's enrofloxacin product for poultry was first registered in Denmark on December 27, 1991. Revised Joint Stipulation No. 53

1598.   Bayer's ciprofloxacin product was first registered in Finland on July 8, 1987. Revised Joint Stipulation No. 54

1599.   Bayer's ciprofloxacin product was first registered in France on July 24, 1987; Bayer's enrofloxacin product for poultry was first registered in France on December 31, 1991. Revised Joint Stipulation No. 55

1600.   Bayer's ciprofloxacin product was first registered in Germany on January 30, 1987; Bayer's enrofloxacin product for poultry was first registered in Germany on January 17, 1990. Revised Joint Stipulation No. 56

1601.   Bayer's ciprofloxacin product was first registered in Greece on April 6, 1988; Bayer's enrofloxacin product for poultry was first registered in Greece on January 22, 1990. Revised Joint Stipulation No. 57

1602. Bayer's ciprofloxacin product was first registered in Ireland on December 20, 1988; Bayer's enrofloxacin product for poultry was first registered in Ireland on October 1, 1988. Revised Joint Stipulation No. 58

1603. Bayer's ciprofloxacin product was first registered in Italy on March 1, 1989; Bayer's enrofloxacin product for poultry was first registered in Italy on September 19, 1990. Revised Joint Stipulation No. 59

1604. Bayer's ciprofloxacin product was first registered in Luxembourg on June 16, 1987; Bayer's enrofloxacin product for poultry was first registered in Luxembourg on February 23, 1990. Revised Joint Stipulation No. 60

1605. Bayer's ciprofloxacin product was first registered in The Netherlands on August 15, 1988; Bayer's enrofloxacin product for poultry was first registered in The Netherlands on April 8, 1987. Revised Joint Stipulation No. 61

1606. Bayer's ciprofloxacin product was first registered in Portugal on August 23, 1988; Bayer's enrofloxacin product for poultry was first registered in Portugal on June 20, 1994. Revised Joint Stipulation No. 62

1607. Bayer's ciprofloxacin product was first registered in Spain on May 26, 1988; Bayer's enrofloxacin product for poultry was first registered in Spain on October 1, 1990. Revised Joint Stipulation No. 63

1608. Bayer's ciprofloxacin product was first registered in Sweden on February 4, 1988; Bayer's enrofloxacin product for poultry was first registered in Sweden on Septembers, 1989. Revised Joint Stipulation No. 64

1609. Bayer's ciprofloxacin product was first registered in the United Kingdom on February 2, 1987; Bayer's enrofloxacin product for poultry was first registered in the United Kingdom on November 11, 1993. Revised Joint Stipulation No. 65

1610. Bayer's ciprofloxacin product was first registered in Australia on December 18, 1987. Revised Joint Stipulation No. 66

1611. Bayer's ciprofloxacin product was first registered in New Zealand on February 4, 1988. Revised Joint Stipulation No. 67

1612. Bayer's ciprofloxacin product was first registered in Thailand on May 23, 1988; Bayer's enrofloxacin product for poultry was first registered in Thailand on November 30, 1988. Revised Joint Stipulation No. 68

1613. Bayer's ciprofloxacin product was first registered in Taiwan on July 3, 1990; Bayer's enrofloxacin product for poultry was first registered in Taiwan on November 1, 1990. Revised Joint Stipulation No. 69

1614. Bayer's ciprofloxacin product was first registered in Japan on March 29, 1988; Bayer's enrofloxacin product for poultry was first registered in Japan on November 15, 1991. Revised Joint Stipulation No. 70

1615. Bayer's ciprofloxacin product was first registered in Vietnam on July 16, 1994. Revised Joint Stipulation No. 71

1616. Bayer's ciprofloxacin product was first registered in Israel on September 1, 1988; Bayer's enrofloxacin product for poultry was first registered in Israel in July 1991. Revised Joint Stipulation No. 72

1617. Bayer's ciprofloxacin product was first registered in Turkey on March 16, 1989; Bayer's enrofloxacin product for poultry was first registered in Turkey on March 20, 1989. Revised Joint Stipulation No. 73

1618. Bayer's ciprofloxacin product was first registered in the Russian Federation on September 26, 1996; Bayer's enrofloxacin product for poultry was first registered in the Russian Federation on October 25, 1989. Revised Joint Stipulation No. 74

1619. Bayer's ciprofloxacin product was first registered in Norway on October 9, 1990. Revised Joint Stipulation No. 75

1620. Bayer's ciprofloxacin product was first registered in Canada on January 9, 1989; Bayer's enrofloxacin product for poultry was first registered as a turkey egg dip in Canada on December 5, 1988. Revised Joint Stipulation No. 76

1621. Bayer's ciprofloxacin product was first registered in Mexico on November 3, 1987; Bayer's enrofloxacin product for poultry was first registered in Mexico on September 18, 1992. Revised Joint Stipulation No. 77

1622. Bayer's ciprofloxacin product was first registered in Switzerland on May 21, 1987; Bayer's enrofloxacin product for poultry was first registered in Switzerland on October 10, 1991. Revised Joint Stipulation No. 78

Exhibits

1623. Fluoroquinolone resistance rates in Denmark are low because relatively little Baytril is being used in Denmark. B-454

1624. Fluoroquinolone-resistant *Campylobacter* rates in retail samples may be lower than those assessed in flocks, owing to a low persistence. B-454

1625. More than 50% of sporadic cases of Campylobacter enteritis are linked to eating or handling poultry. B-288, p. 1

1626. In Denmark, the public health burden associated with the 4161 registered cases of campylobacteriosis in 1999 comprised more than 41,000 days of illness, 820 hospitalizations (and approximately 3800 bed days), and 1800 general practitioner consultations. B-561, p. 7

1627. In Neimann's case control study of campylobacteriosis in Denmark in 1999, among cases treated with antimicrobials (mainly fluoroquinolones) a 5 days longer duration of illness seemed to be associated with a ciprofloxacin resistant infection, compared to a ciprofloxacin susceptible infection. B-561, p. 191, and p. 200

1628. In Neimann's case control study, consumption of undercooked poultry was identified as a risk factor for acquiring campylobacteriosis. B-561, p. 50

1629. Goodman and Gillman's The Pharmacological Basis of Therapeutics, 9th Edition, recommends ciprofloxacin or ofloxacin as a treatment for Campylobacter enteritis. B-656, p. 2-3

1630. Fluoroquinolones are the therapy of choice for human enteric infections caused by foodborne *Campylobacter jejuni*. B-147, p. 1

1631. Human to human transmission of *Campylobacter jejuni* is almost nonexistent. B-147, p. 2

1632. By far the largest contributor to individual or sporadic cases of campylobacteriosis is the consumption of improperly cooked or improperly handled poultry which may be associated with more than 60% of the cases. B-147, p. 2

1633. In a quantitative risk assessment, a lack of data and adequate scientific studies at critical points in the processing steps that precede the final retail product would introduce a large degree of uncertainty into a comprehensive model entailing all steps of production. B-147, p. 2

1634. Years of research have demonstrated that the selective pressure of fluoroquinolones, even delivered in a controlled therapeutic capacity, leads to the emergence of resistant organisms. B-147, p. 11

1635. Of 442 human strains of *Campylobacter jejuni* investigated by Clow, 77% of these strains came from genotypes common to human and chicken strains of *Campylobacter jejuni*. B-250, p. 3

1636. In Australia, where there is a relatively low use of fluoroquinolones in humans and animals ciprofloxacin resistant Campylobacter are uncommon. B-255, p. 2

1637. In an experiment of 50 4-day old *Campylobacter* free broilers were divided into 4 groups: one group was challenged with *Campylobacter jejuni* and not treated with enrofloxacin; one group was challenged with *Campylobacter jejuni* and treated with 25 ppm enrofloxacin via drinking water for 5 consecutive days; one group was challenged with *Campylobacter jejuni*

and treated with 50 ppm enrofloxacin via drinking water for 5 consecutive days; and, one group was not challenged with *Campylobacter* or treated with enrofloxacin. Nearly all the isolates from the 2 enrofloxacin treated groups showed MICs of > 32 μg/ml. *Campylobacter* isolates from the group challenged with *Campylobacter* but not treated with enrofloxacin were sensitive to ciprofloxacin during the entire experiment. A-190, p. 1

1638. Fluoroquinolone treatment does not eradicate *Campylobacter* in broilers. A-190, p. 2

1639. Fluoroquinolone-resistant *Campylobacter jejuni* develops very quickly in fluoroquinolone treated broilers. A-190, p. 2

1640. In Zhang's experiment, fluoroquinolone-resistant *Campylobacter* isolates from broilers showed the PFGE pattern identical to the inoculated fluoroquinolone sensitive strains which indicates that fluoroquinolone isolates did not come from environmental contamination but evolved from the original fluoroquinolone sensitive *Campylobacter jejuni* inoculated. A-190, p. 2

1641. In 1996, 7,598,000,000 broilers were raised in the United States. A-158, p. 2

1642. In 1997, 7,764,000,000 broilers were raised in the United States. A-158, p. 2

1643. In 1998, 7,934,000,000 broilers were raised in the United States. A-158, p. 2

1644. In 1999, 8,146,000,000 broilers were raised in the United States. A-158, p. 2

1645. In 2000, 8,263,000,000 broilers were raised in the United States. A-158, p. 2

1646. In 2001, the projected 2002 number of broilers were raised in the United States was 8,650,000,000. A-158, p. 2

1647. Per capita poultry consumption in the United States (in pounds) was 69.8 in 1995; 70.8 in 1996; 71.8 in 1997; 72.4 in 1999; 77.6 in 1999; and, 81.7 in 2000. A-101, p. 1

1648. Gaudreau studied the antimicrobial susceptibility in Campylobacter jejuni strains isolated in Quebec, Canada in 1995-1997 compared to Campylobacter jejuni strains isolated in 1992-1993 and 1985-1986 and found that 13.9% were resistant to nalidixic acid in 1995-1997 while 4.7% were resistant to nalidixic acid in 1992-1993 and 0% were resistant to nalidixic acid in 1985-1986, and 12.7% were resistant to ciprofloxacin in 1995-1997 while 3.5% were resistant to ciprofloxacin in 1992-1993 and 0% were resistant to ciprofloxacin in 1985-1986. G-239

1649. In a case-control study conducted in Norway in 1989-1990, poultry consumption of poultry produced in Denmark or Sweden was strongly associated with *Campylobacter* illness whereas poultry consumption of poultry produced in Norway was not. G-334

1650. There is a relatively low prevalence of *Campylobacter* in Norwegian broiler chicken flocks. G-334

1651. During the period of time that Belgian poultry was withdrawn from market in June 1999 due to the dioxin crisis, no other events that could explain the decline in Campylobacter numbers were known to have occurred. G-672, p. 3

1652. In June 1999, the decline in the number of Campylobacter infections in Belgium by 40% was due to the withdrawal of Belgian poultry from the market. G-672, p. 4

1653. In a double blind placebo-controlled study of the use of norfloxacin or a placebo to treat traveler's diarrhea, norfloxacin was found to reduce the duration of diarrhea. The difference in duration of diarrhea among those treated with norfloxacin and those treated with placebo was 1.8 days vs. 5.0 days (P < .01). G-399

1654. In a study of retail meat in the United States, 44% yielded *Campylobacter* and 24% of the *Campylobacter* isolated were resistant to fluoroquinolones. In all, 11% of the chickens tested yielded fluoroquinolone-resistant *Campylobacter*. G-541

1655. An industry summary of fluoroquinolone use indicates that from August 1995 to March 1998, approximately 1.1 percent of broilers were treated with fluoroquinolones. A-192

1656. An industry summary of fluoroquinolone use indicates that from August 1995 to March 1998, approximately 3.7 percent of breeders were treated with fluoroquinolones. A-192

1657. The Sanford Guide to Antimicrobial Therapy, 2000, lists ciprofloxacin, norfloxacin and azithromycin as suggested primary regimens for *Campylobacter jejuni* infection. G-244

1658. The Sanford Guide to Antimicrobial Therapy, 2000, lists ciprofloxacin and norfloxacin as suggested primary regimens for the empiric treatment of severe diarrhea associated with gastroenteritis. G-244

1659. In the Netherlands, among human *Campylobacter* isolates, no ciprofloxacin resistance was found during 1982 to 1983 or 1985. The percentage of resistant isolates increased to 8% during 1987 to 1988 and to 11% during 1989. Ciprofloxacin resistance among *Campylobacter* isolates from poultry products closely paralleled that found among human isolates. No resistance was found in poultry isolates from 1982 to 1983; the percentage of resistant isolates increased to 8.4% during 1987 to 1988 and to 14% during 1989. G-586, p. 2

1660. Eberhart-Phillips' case-control study of risk factors for campylobacteriosis in New Zealand found the strongest associations for food exposures were with recent consumption of chicken, particularly raw or undercooked chicken, or chicken prepared at a sit-down restaurant. Barbecued chicken and fried chicken were positively associated with disease, while consumption of baked or roasted chicken seemed to be protective, as was chicken purchased frozen. G-182, p. 3

1661. Eberhart-Phillips' case-control study of risk factors for campylobacteriosis in New Zealand confirmed a leading role for poultry in human *Campylobacter* infections. The combined population attributable risk percentage for the chicken related variables in the multivariate model exceed 50% suggesting that consumption of chicken lies behind more cases of campylobacteriosis in New Zealand than all other risk factors combined. G-182, p. 4

1662. Ciprofloxacin, a fluoroquinolone, is active against all the recognized bacterial causes of gastroenteritis. G-172, p. 1

1663. In a randomized control trial, Dryden demonstrated that a 5-day course of therapy with oral ciprofloxacin reduces the duration of diarrhea and other symptoms in patients with severe acute community-acquired gastroenteritis. G-172, p. 4

1664. In Rodrigues' case-control study, consumption of chicken in a restaurant was identified as a risk factor for intestinal infection with *Campylobacter jejuni*. Rodrigues' study was conducted in England and enrolled 229 cases and 229 controls matched on age, sex, and general practitioner practice. G-1711

1665. Deming's case-control study identified eating fully cooked chicken and eating chicken reported to be raw or undercooked as risk factors for *Campylobacter* enteritis. Deming's study was conducted at the University of Georgia during the fall and winter quarters of the 1983-1984 academic year. Deming enrolled 45 case-control pairs matched on age, sex, and residence. G-162

1666. Consumption of chicken was associated with more than a doubling of the risk of *Campylobacter jejuni/coli* enteritis in Harris' case-control study; the consumption of raw or rare chicken was even more strongly associated with *Campylobacter* infection. Harris' study was conducted between April 1982 and September 1983 in Washington State and enrolled 218 cases and 526 controls. G-268

1667. In 2001, there were 8.6 billion broilers raised in the United States. Bayer Narrative Statement, p. 3

1668. Large chilling tanks are used to cool the birds after evisceration, creating the potential to further cross-contaminate carcasses with various bacteria. Bayer's Narrative Statement, p. 4

1669. *Campylobacter* are commensal organisms in poultry. Bayer's Narrative Statement, p. 4

1670. To the extent that poultry is a source of campylobacteriosis in humans, there is no reason to believe that poultry could not also be a source of fluoroquinolone-resistant infections in humans. Bayer's Narrative Statement, p. 5

1671. The use of enrofloxacin in chickens and turkeys exert selection pressure that leads to fluoroquinolone-resistant *Campylobacter*. Bayer's Narrative Statement, p. 11
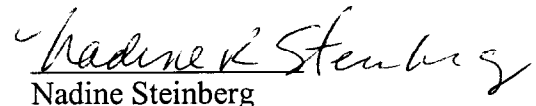
1672. Chickens that harbor *Campylobacter* quickly develop fluoroquinolone-resistant *Campylobacter* once a fluoroquinolone is administered in laboratory conditions. Bayer's Narrative Statement, p. 12

1673. Bayer does not dispute the fact that fluoroquinolone-resistant *Campylobacter* spp. from chickens and turkeys can be transferred to humans. Bayer Narrative Statement, p. 16
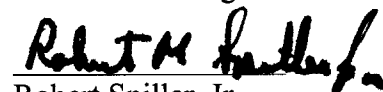
1674. Approximately 1.6% of all broilers raised in the United States in 1999 were treated with Baytril. Bayer's response to CVM's Interrogatory No. 2

1675. Approximately 4% of all turkeys raised annually in the United States are treated with Baytril. Bayer's response to CVM's Interrogatory No. 2
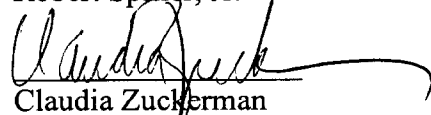
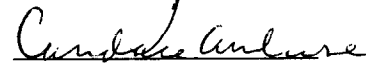
Respectfully submitted, this 17th day of March by:

Nadine Steinberg

Robert Spiller, Jr.

Claudia Zuckerman

Candace Ambrose
Counsel for Veterinary Medicine

CERTIFICATE OF SERVICE

I hereby certify that an original and one copy of the foregoing Center for Veterinary Medicine's Proposed Findings of Fact was hand delivered this 17th day of March, 2003, 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 Center for Veterinary Medicine's Proposed Findings of Fact has been hand delivered and e-mailed, this 17th day of March, 2003, to:
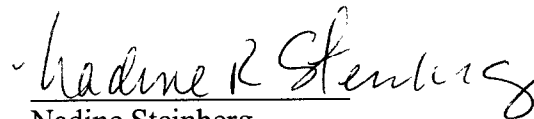
The Office of the Administrative Law Judge
Food and Drug Administration
Room 9-57, HF-3
5600 Fishers Lane
Rockville, MD 20857

I also certify that a copy of the Center for Veterinary Medicine's Proposed Findings of Fact was e-mailed and mailed by First Class U.S. mail, this 17th day of March, 2003, to:

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Dated: 3|17|03

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